University of Southampton

Faculty of Medicine

Outcome after aneurysmal subarachnoid haemorrhage

by

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Abstract

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Aneurysmal subarachnoid haemorrhage (aSAH) is a devastating form of stroke associated with significant morbidity and mortality. It affects younger people than other stroke types and consequently has a disproportionately high socio-economic impact. Survivors often suffer a range of disabling symptoms including physical and cognitive deficits. These deficits lead to a reduced quality of life and impair ability to return to work following aSAH. In order to develop treatments to improve outcome after aSAH we need to better understand the mechanisms that underlie neurological injury. At present the calcium channel blocker, nimodipine which was developed in the 1980s, is the only licensed treatment to improve outcome following aSAH. Despite multiple trials there have been no major developments to improve outcome following haemorrhage for over 30 years and it is our lack of understanding of the pathophysiology of neurological injury that, at least in part, explains this lack of therapeutic innovation.

This thesis focusses on understanding outcome after aSAH and has three main aims. First, to explore the burden of hidden disability by studying cognitive, auditory, headache and fatigue outcomes in the long-term following aSAH including the implications for quality of life and employment. Second, to improve outcome prediction following aSAH by including additional variables and advanced statistical learning methods. Third, to use a genetic approach to provide insight into the mechanisms underlying neurological injury following aSAH.

The results reported in this thesis are that cognitive and auditory deficits, in addition to persistent headache and fatigue, are significantly more common in aSAH survivors compared to a control population. Auditory deficits are most in keeping with a central auditory processing disorder rather than a peripheral deficit and are closely linked to impaired cognition following aSAH. Cognitive deficits, persistent headache and fatigue all significantly contribute to unemployment following aSAH.

In addition to the well-known clinical variables that predict outcome after aSAH, C-reactive protein (CRP) is an independent predictor of outcome following aSAH. The incorporation of CRP

and the use of advanced predictive modelling methodology such as support vector machines improves outcome prediction following aSAH. Despite a statistical improvement these new models are unlikely to have major clinical impact and require validation in an external cohort.

To identify genetic contribution to outcome after SAH, first a candidate gene approach was used. This identified that haptoglobin, a haemoglobin scavenging molecule, is implicated in outcome after aSAH, although the evidence is not conclusive. Genetic variation in NRF2, a transcription factor regulating haemoglobin scavenging, inflammation and oxidative injury, is also associated with outcome after aSAH. These findings provide insight into the pathophysiology of neurological injury following aSAH but are limited by the candidate nature of the analyses. A subsequent unbiased genome-wide analysis provides novel evidence for a role of the sphingosine-1-phosphate signalling pathway in neurological injury and outcome following aSAH.

This thesis concludes that aSAH leads to widespread long-term neurological symptoms which impact quality of life and employment. The inclusion of additional clinically available predictors and use of advanced statistical learning methods can improve outcome prediction following aSAH. However, the improvements are small and unlikely to influence clinical practice. Ultimately to improve outcome we need to better understand the mechanisms which drive neurological injury. Using both candidate and genome-wide analysis a number of mechanisms including haemoglobin scavenging, inflammation, oxidative injury and sphingolipid signalling have been implicated in outcome. These pathways may act as therapeutic targets to improve outcome after aSAH and warrant further study.

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Research Thesis: Declaration of Authorship

Print name: Ben Gaastra

Title of thesis: Outcome after aneurysmal subarachnoid haemorrhage

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Parts of this work have been published as:-

- Gaastra B, Ewbank F, Tapper W, Bulters D, Galea I. Long-Term Cognitive Outcome following Aneurysmal Subarachnoid Haemorrhage. *Journal of Stroke and Cerebrovascular Diseases*. 2022/01/01/ 2022;31(1):106184. doi:10.1016/j.jstrokecerebrovasdis.2021.
- Gaastra B, Ashokumar M, Bulters D, Campbell N, Galea I. Auditory outcome following aneurysmal subarachnoid haemorrhage. *J Neurol Sci*. Dec 30 2021;434:120125. doi:10.1016/j.jns.2021.120125
- Gaastra B, Carmichael H, Galea I, Bulters D. Duration and characteristics of persistent headache following aneurysmal subarachnoid hemorrhage. *Headache*. Nov 25 2022;doi:10.1111/head.14418
- Gaastra B, Carmichael H, Galea I, Bulters D. Long-term fatigue following aneurysmal subarachnoid haemorrhage and the impact on employment. *Eur J Neurol*. Aug 30 2022;doi:10.1111/ene.15533

- Gaastra B, Barron P, Newitt L, et al. CRP (C-Reactive Protein) in Outcome Prediction After Subarachnoid Hemorrhage and the Role of Machine Learning. *Stroke*. Oct 2021;52(10):3276-3285. doi:10.1161/STROKEAHA.120.030950
- Bulters D, Gaastra B, Zolnourian A, et al. Haemoglobin scavenging in intracranial bleeding: biology and clinical implications. *Nat Rev Neurol*. Jul 2018;14(7):416-432. doi:10.1038/s41582-018-0020-0
- Gaastra B, Glazier J, Bulters D, Galea I. Corrigendum to "Haptoglobin Genotype and Outcome after Subarachnoid Haemorrhage: New Insights from a Meta-Analysis". *Oxid Med Cell Longev*. 2018;2018:9105120. doi:10.1155/2018/9105120
- Gaastra B, Ren D, Alexander S, et al. Haptoglobin genotype and aneurysmal subarachnoid hemorrhage: Individual patient data analysis. *Neurology*. Apr 2019;92(18):e2150-e2164. doi:10.1212/WNL.0000000000007397
- Gaastra B, Duncan P, Bakker MK, et al. Genetic variation in NFE2L2 is associated with outcome following aneurysmal subarachnoid haemorrhage. *Eur J Neurol*. Sep 23 2022;doi:10.1111/ene.15571
- Gaastra B, Alexander S, Bakker MK, et al. Genome-Wide Association Study of Clinical Outcome After Aneurysmal Subarachnoid Haemorrhage: Protocol. *Transl Stroke Res*. Jan 06 2022;doi:10.1007/s12975-021-00978-2
- Gaastra B, Alexander S, Bakker MK, et al. A Genome-Wide Association Study of Outcome After Aneurysmal Subarachnoid Haemorrhage: Discovery Analysis. *Transl Stroke Res*. Oct 20 2022;doi:10.1007/s12975-022-01095-4
- Gaastra B, Ren D, Alexander S, et al. Evidence-based interconversion of the Glasgow Outcome and modified Rankin scales: pitfalls and best practices. *J Stroke Cerebrovasc Dis*. Dec 2022;31(12):106845. doi:10.1016/j.jstrokecerebrovasdis.2022.106845

Signature: .....Date:.....

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Abbreviations

SAH ..... Subarachnoid haemorrhage

aSAH..... Aneurysmal subarachnoid haemorrhage

CRP..... C-reactive protein

CSF ..... Cerebrospinal fluid

DCI ..... Delayed cerebral ischemia

mRS..... Modified Rankin scale

GOS ..... Glasgow outcome scale

SAHOT..... Subarachnoid haemorrhage specific outcome tool

SAHIT ..... Subarachnoid Haemorrhage International Trialists'

AUC ..... Area under the receiver operator characteristics curve

WFNS ..... World Federation of Neurological Surgeons

GCS..... Glasgow coma score

SVM..... Support vector machine

OR ..... Odds ratio

CI ..... Confidence interval

HATCH..... Haemoglobin After intraCranial Haemorrhage

NRF2 ..... Nuclear factor erythroid 2-related factor 2 (protein)

NFE2L2 ..... Human gene encoding NRF2

sQTL ..... Splicing quantitative trait loci

GWAS..... Genome-wide association study

SPNS2..... Sphingolipid transporter 2

S1P Spingosine-1-phosphate

S1PR..... Spingosine-1-phosphate receptor

PPM1A ..... Protein phosphatase 1A

LRP1B..... LDL receptor related protein 1B

ZNF423..... Zinc finger protein 423

Chapter 1 Introduction

1.1 Diagnosis and management of aneurysmal subarachnoid haemorrhage

Spontaneous subarachnoid haemorrhage (SAH) represents 5% of strokes[1] and has an incidence of around 9 per 100 000 person years[2]. Aneurysmal subarachnoid haemorrhage (aSAH) is the most common cause of spontaneous SAH representing about 85% of cases[3]. aSAH is caused by rupture of a cerebral artery aneurysm in the subarachnoid space leading to haemorrhage into the cerebrospinal fluid (CSF) and surrounding compartments. Other causes of spontaneous SAH include non-aneurysmal perimesencephalic SAH and bleeding from other vascular anomalies such as arteriovenous malformations and dural arteriovenous fistulae.

Patients with spontaneous SAH most commonly present with sudden onset headache. More severe presentations include focal neurological deficits and reduced consciousness. Diagnosis is made using a non-contrast CT head followed by a lumbar puncture if the CT is inconclusive and clinical suspicion remains. Once SAH has been confirmed the underlying cause of the bleed is investigated using dedicated vascular imaging. If an aneurysmal source is identified the next phase of management is to secure the aneurysm to prevent re-bleeding either by endovascular coiling or surgical clipping.

The aneurysm treatment modality (endovascular versus surgical) depends on a number of factors including clinical condition of the patient, imaging appearances and specific features of the aneurysm. When both surgical and endovascular options are possible, endovascular treatment is usually preferred as it is a less invasive procedure and is associated with reduced morbidity and mortality[4]. Re-bleeding from an aneurysm is associated with high mortality and occurs in up to 23% of cases within 72 hours[5] hence guidelines recommend securing the aneurysm as soon as possible unless the patient is deemed to have a particularly poor prognosis. In such cases patients may undergo a period of prognostication to assess neurological recovery before treatment of the aneurysm is considered[6,7].

Once the aneurysm is secured the patient is closely monitored for neurological deterioration or complications associated with aSAH until well enough for discharge. A range of complications can occur including hydrocephalus, electrolyte abnormalities and delayed cerebral ischemia (DCI). DCI occurs in up to 28% of patients[8] and is characterised by a neurological deterioration in the days to weeks following aSAH not attributable to other causes[9]. Oral nimodipine, a calcium-channel

antagonist, is the only pharmacological treatment to reduce the incidence of DCI and poor outcome following aSAH[10,11]. Should DCI occur there are no specific treatments although induced hypertension is a commonly used strategy[12].

1.2 Outcome following aSAH

aSAH is associated with significant morbidity, mortality and socioeconomic cost. Despite representing only 5% of all strokes, aSAH is responsible for over 25% of life years lost due to stroke[13]. Mortality has gradually improved over time[14], however, one in five patients die within the first year[15] and survivors often suffer persistent disabling symptoms which impair ability to return to work[16]. Neurological morbidity in survivors and loss of productive employment in addition to the fact that aSAH affects younger individuals than other stroke types[13] means that it has the greatest socioeconomic burden of any stroke form. The lifetime cost of aSAH is over twice the average for stroke[17] with the annual economic burden in the United Kingdom estimated at £510 million[18].

Outcome following aSAH is most commonly assessed using the modified Rankin (mRS)[19,20] or Glasgow Outcome Scales (GOS)[21]. Both scales assess functional recovery with the mRS ranging from 0 (no disability) to 6 (death) and the GOS from 1 (death) to 5 (good recovery). Neither scale was specifically designed to assess outcome after aSAH with the mRS designed primarily in the context of ischemic stroke[20] and the GOS in the context of head injury[21]. A recent European study assessing outcome at 1 year using the mRS reported that 21.8% of patients died, 10.4% lived in a dependent state (mRS 3-5) and 58.3% were independent (mRS 0-2)[15] which is consistent with previous studies[22]. In addition to the physical neurological deficits identified by the mRS/GOS, survivors of aSAH frequently report a wide range of less immediately obvious, yet equally disabling, sequelae. These hidden disabilities include cognitive, psychological and auditory deficits in addition to fatigue and mood disturbance[23-25]. These sequelae have a significant impact on aSAH survivors, especially independent individuals who are not preoccupied with severe physical deficits, affecting quality of life and return to work following haemorrhage[23]. Our appreciation of the burden of hidden disability following aSAH is increasing but further work is required to understand the broad range of symptoms and to improve recognition and management.

1.3 Neurological injury following aSAH

Ultimately neurological injury caused by aSAH is the driving force behind outcome[8,26]. Neurological injury following aSAH can be considered in two phases: an early brain injury which

occurs in the first 72 hours of haemorrhage and a subsequent delayed brain injury, including DCI, which develops over days to weeks.

Early brain injury is initiated at the time of aSAH by a rapid rise in intracranial pressure and associated reduction in cerebral blood flow caused by the haemorrhage. This leads to global cerebral ischemia followed by cell death via apoptosis/necrosis causing cerebral oedema and blood brain barrier disruption[27]. The harmful pathways activated by early brain injury together with the presence of blood and its breakdown products in the CSF lead to delayed brain injury which is characterised by cerebral vasospasm, inflammation, oxidative injury, microthrombosis and cortical spreading depression[28-31](Figure 1).

Blood and its breakdown products released into the subarachnoid space at time of aSAH are thought to play a key role in the pathology of neurological injury. Haemoglobin is directly neurotoxic and prolonged intrathecal exposure in mice has been shown to generate the same pattern of neurological injury as observed in aSAH[32]. A natural scavenging system exists within the brain to clear blood and its breakdown products but it is easily overwhelmed allowing neurotoxic products to accumulate[33]. Blood breakdown products have been implicated in several of the pathological mechanisms seen during delayed brain injury. Haem can activate both the TLR4[34] and NLRP3[35] inflammatory signalling pathways leading to upregulation of inflammatory cytokines such as IL-1β[36] and can generate reactive oxygen species leading to oxidative injury[37,38]. In addition, haemoglobin has been shown to cause cerebral artery vasospasm in both animals and humans through depletion of the vasodilator nitric oxide[39,40]. Nitric oxide depletion has also been implicated in cerebral vessel microthrombosis[41] and cortical spreading depression[42].

Although blood breakdown products and a number of pathological processes have been implicated in neurological injury following aSAH the majority of evidence is derived from animal studies and the significance in humans is unclear. In addition, other processes may be involved in outcome and need to be identified if we are to better understand the pathophysiology of aSAH.

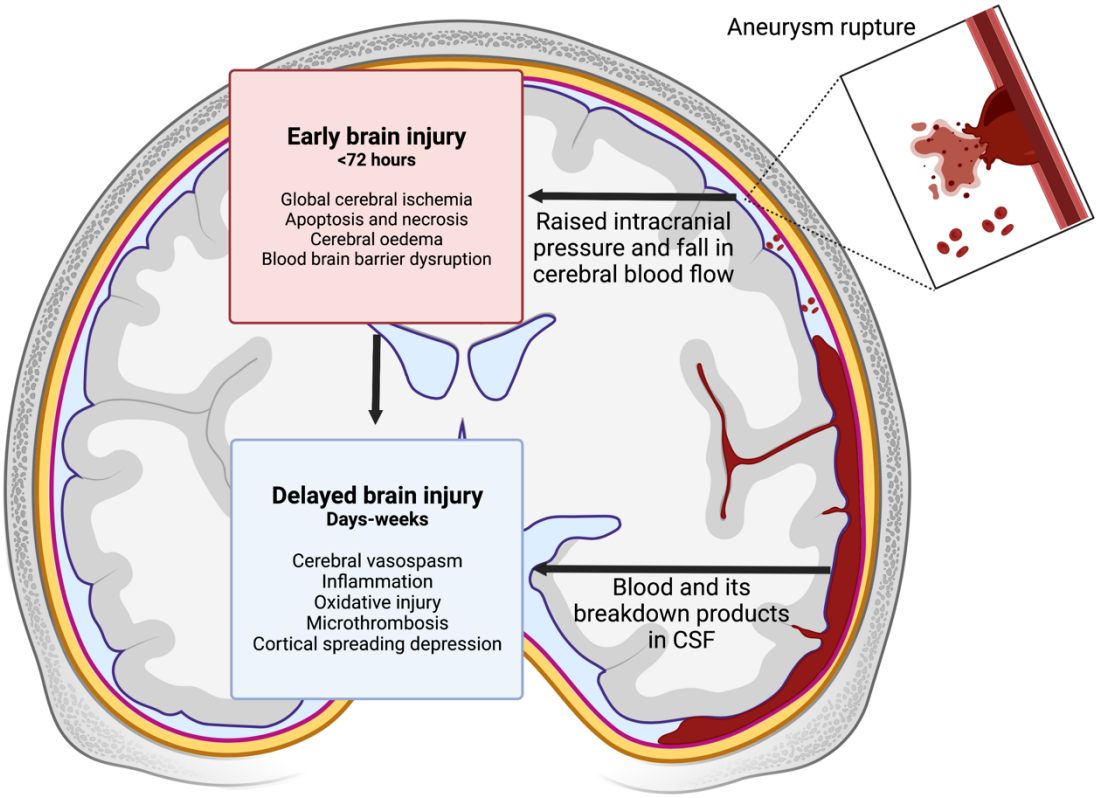

Figure 1. Mechanisms of neurological injury which occur during early and delayed brain injury following aSAH. Created with BioRender.com.

1.4 Improving outcome after aSAH

Oral nimodipine is the only pharmacological treatment to improve outcome after aSAH. A meta-analysis of four trials (n=853) identified a reduction in relative risk of death or poor outcome of 0.67 (95% confidence interval (CI) 0.55-0.81) compared to placebo, although the results depend primarily on a single centre trial conducted over 30 years ago[10,11]. In the UK nimodipine is routinely administered to patients with aSAH on admission and continued for 21 days.

Despite multiple clinical trials (including clazosentan[43], magnesium sulphate[44], simvastatin[45] and trilazad[46]) no other medications have been identified to improve outcome[28]. The most promising current therapies include tranexamic acid which was shown in a prospective randomised trial of 505 patients to reduce the risk of re-bleeding after aSAH but did not affect outcome[47]. In addition, the phosphodiesterase-3 inhibitor cilostazol which has anti-platelet and vasodilatory effects has been shown in a meta-analysis to reduce the risk of poor outcome (defined as mRS 3-6) by 0.40 (95% CI 0.25-0.62) however this was only based on a small sample size of 543 patients and further investigation into its efficacy is required[48].

Delayed brain injury following aSAH is a compelling therapeutic target as it occurs during a window of time when patients are engaged with healthcare and treatment could be initiated to alleviate or prevent neurological injury. It is likely that our lack of understanding of the pathophysiology of neurological injury following aSAH has so far hindered the development of treatments to improve outcome. We urgently need to address this gap in our knowledge if we are to identify new treatments to improve outcome.

1.5 Aims and structure of thesis

In this thesis I explore outcome following aSAH.

The specific aims are to:

A. Better understand the burden of hidden disability by studying cognitive, auditory, headache and fatigue outcomes in the long-term following aSAH including the implications for quality of life and employment
B. Improve outcome prediction following aSAH using additional clinically available predictive variables and advanced statistical learning methods
C. Provide insight into the mechanisms underlying neurological injury following aSAH using both candidate gene and genome wide analyses

The following 12 publications comprising this thesis have been grouped thematically into four chapters:

Chapter 2: The burden of hidden disability following aSAH

1. Long-term cognitive outcome following aneurysmal subarachnoid haemorrhage
   Journal of Stroke and Cerebrovascular Diseases
2. Auditory outcome following aneurysmal subarachnoid haemorrhage
   Journal of the Neurological Sciences
3. Duration and characteristics of persistent headache following aneurysmal subarachnoid haemorrhage
   Headache
4. Long-term fatigue following aneurysmal subarachnoid haemorrhage and the impact on employment
   European Journal of Neurology

Chapter 3: Outcome prediction following aSAH

5. CRP (C-reactive protein) in outcome prediction after subarachnoid haemorrhage and the role of machine learning
   Stroke

Chapter 4: Haemoglobin scavenging after aSAH: candidate gene studies

6. Haemoglobin scavenging in intracranial bleeding: biology and clinical implications
   Nature Reviews Neurology
7. Haptoglobin genotype and outcome after subarachnoid haemorrhage
   Oxidative Medicine and Cellular Longevity
8. Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis

Neurology

9. Genetic variation in NFE2L2 is associated with outcome following aneurysmal subarachnoid haemorrhage
   European Journal of Neurology

Chapter 5: Genome-wide analysis of outcome following aSAH

10. Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol
    Translational Stroke Research
11. A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis
    Translational Stroke Research
12. Evidence-based interconversion of the Glasgow Outcome and modified Rankin scales: pitfalls and best practices
    Journal of Stroke and Cerebrovascular Diseases

The commentary accompanying each chapter summarises the included publications and contextualises them in current research. Within the commentary the publications are highlighted in bold. A full copy of each publication along with statements of contribution are included in Appendix A and B.

Chapter 2 The burden of hidden disability following aSAH

The majority of clinical trials assess outcome following aSAH using the mRS or GOS which categorise patients according to physical disability. There is, however, a growing body of evidence that a wide range of other deficits, not detected by these traditional scales, play an important role in outcome following aSAH. Compared to obvious physical deficits these hidden disabilities often require direct questioning or specific assessment tools to elicit and are therefore frequently overlooked in clinical assessment. The scale and impact of these hidden disabilities should, however, not be underestimated. During the development of a subarachnoid haemorrhage specific outcome tool, SAHOT, Pace *et al.* together with a panel of aSAH patients created a 56-item outcome score including cognitive, physical, behavioural and psychological sequelae, the majority of which represent hidden disabilities that would not be detected by the mRS or GOS[49].

Although there is very little literature on the nature and significance of hidden disability after aSAH cognition has been extensively studied and emphasises the importance of these deficits. In a recent review of 65 studies published since 2010 as many as 70% of survivors experience cognitive deficits following aSAH[24] with the most frequently impaired domains including memory, executive function and language[23]. Even patients who have regained independence following aSAH and would commonly be considered as 'good' outcome according to the mRS/GOS, suffer cognitive deficits which significantly impact activities of daily living, quality of life and ability to return to work[23]. Other sequelae including mood disorders, fatigue and auditory deficits are also common following aSAH and have been reported in up to 32%, 67% and 23% of cases respectively[25,50]. If the consequences of all hidden disabilities are comparable to cognition the potential impact on outcome is highly significant.

Despite the frequency and potential implications of hidden disabilities, our understanding is limited especially in relation to the range of symptoms, the long term consequences and how they impact functional outcomes such as return to work and quality of life[51]. This chapter includes four publications which use the UK Biobank to further explore the burden of hidden disability following aSAH. The UK Biobank is a large biomedical database with detailed phenotype information on over half a million UK participants aged 40-69 at time of recruitment in 2006-2010[52]. The major advantages of using the UK Biobank to study hidden disability after aSAH are: (1) the database includes a large cohort of aSAH cases with long-term follow up, and (2) the participants have undergone a wide range of outcome assessments meaning multiple hidden disabilities can be assessed. All four publications compare the frequency of a hidden disability following aSAH to a

matched control cohort and explore the burden of the respective disability. Sample sizes differ between studies as data availability in the UK Biobank varies between the different disabilities studied.

2.1 Cognitive deficits following aSAH (Publication 1)

Although cognition is the most widely studied of these disabilities following aSAH, the majority of publications only assess cognitive deficits in the first year following haemorrhage[24]. Consequently, our understanding of the long-term frequency of cognitive deficits and its implications is limited. In this study, using cognitive outcome data on 884 aSAH cases at a mean follow up of over 10 years, I showed that although reasoning, prospective, visual and verbal memory did not differ, psychomotor reaction time was significantly slower following aSAH compared to controls (controls: 589±138ms, aSAH: 569±121ms, $t=3.84$, $p<0.001$). This suggests that although the majority of cognitive domains normalise by 10 years following aSAH, psychomotor deficits persist. I also showed a significant difference in employment status (controls: 94.0%, aSAH: 82.8%; $\chi^2=116.8$, $p<0.001$) with aSAH cases more likely to be unemployed or unable to work due to sickness or disability. Using regression-based mediation analysis I demonstrated that a significant proportion (6.59%) of the effect of aSAH on employment status was mediated by psychomotor cognitive deficits. I concluded that cognitive disability persists in the long-term following aSAH and continues to significantly impact patients as demonstrated by its ongoing contribution to unemployment.

2.2 Auditory outcomes following aSAH (Publication 2)

There is a growing body of evidence supporting a significant burden of hearing impairment following aSAH. Up to 23% of patients report new onset hearing difficulty following aSAH and a central auditory processing disorder, rather than a peripheral deficit, is thought to underlie this impairment[25,53]. Central auditory processing disorder arises due to pathology within the central nervous system rather than within the auditory nerve or outer/middle/inner ear. Individuals classically present with hearing difficulty particularly in the presence of background noise despite normal pure tone audiograms. Other central networks such as cognition modulate the central auditory nervous system and can therefore contribute to central auditory processing disorder[54]. Identifying the origin of hearing impairment is important as central and peripheral origin deficits require different management strategies.

In this publication, using auditory outcome data on 270 patients assessed with a speech-in-noise test, I confirmed the presence of significant hearing impairment following aSAH compared to controls at a mean follow up of 106 months (aSAH: -6.88, controls: -7.38, t=3.05, p=0.002). Using regression-based mediation analysis I also showed that cognitive deficits as assessed by psychomotor reaction time mediate a significant proportion (9.8%) of the effect of aSAH on hearing impairment. Pure tone audiometry was not performed in the UK Biobank and consequently the contribution of peripheral hearing loss to the hearing impairment identified following aSAH cannot be assessed. However, speech-in-noise test deficits compounded by the significant role for cognition identified in this study strongly suggest that central auditory processing disorder is integral to hearing impairment after aSAH. Hearing impairment is known to impact quality of life[55] and this publication emphasises the prevalence of this disability following aSAH. As central auditory processing disorder appears to play a key role, survivors of aSAH with hearing impairment should be tested for both central and peripheral deficits as this has implications for treatment.

2.3 Headache following aSAH (Publication 3)

Although headache is the most common presenting symptom, the long-term burden of headache following aSAH is not well defined. In a single study of 93 patients 40.9% reported burdensome headache at a mean follow up of 32.6 months. Beyond this the frequency, phenotype and prognosis of headache is not known.

In this publication, using data from 864 aSAH patients, I showed that headache impacting quality of life is significantly more common following aSAH compared to controls at a median follow up of 7.5 years (aSAH: 29.9%, controls: 19.3%; χ^2=45.5, p<0.001). Headache frequency significantly reduced over time (Spearman Rank correlation coefficient = -0.71, p=0.028) affecting 50% in the first year and falling to 28% a decade later. In addition, migrainous features including those consistent with cortical spreading depression were consistently more common following aSAH compared to controls, although this was not statistically significant. This publication highlights that significant headache, impacting quality of life, persists in the long-term following aSAH but it gradually improves over time. The dominance of migrainous symptoms suggests that anti-migraine treatments may be beneficial in the management of this symptom.

2.4 Fatigue following aSAH (Publication 4)

Fatigue is frequently reported by survivors of aSAH but like many of the hidden disabilities there is limited literature and very little is known about the long-term prognosis and impact of this

symptom[56]. In this publication, in a cohort of 829 patients, I showed that significant fatigue is more frequent following aSAH compared to controls at a mean follow up of 10 years (aSAH: 18.7%, controls: 13.7%; χ^2=13.0, p<0.001). Much like headache following aSAH, fatigue significantly improves over time (Spearman Rank correlation coefficient = -0.62, p=0.004) affecting 19.6% in the first year and decreasing to 11.1% after a decade. Using causal mediation analysis I demonstrated that fatigue has a highly significant impact on employment status mediating 24% of the effect of aSAH on employment. Of all the hidden disabilities discussed in this chapter fatigue has by far the greatest impact on employment emphasising the importance of identifying and managing this symptom to improve outcome.

The UK Biobank is a powerful database to assess the impact of aSAH, however, there are a number of limitations which must be considered when interpreting these results. The UK Biobank is biased towards more motivated, better outcome individuals as inclusion requires attendance at multiple detailed assessment centre visits. This is a characteristic of the UK Biobank dataset but is not necessarily a major limitation, especially in the study of hidden disability. Anecdotally hidden disability is more commonly reported by better outcome individuals, most likely because poorer outcome individuals are preoccupied by other major deficits such as significant physical disability. Therefore, the selection bias in the UK Biobank is towards the individuals most likely to benefit from greater insight into hidden disability. Additionally, the majority of individuals in the UK Biobank only have outcome assessments at a single time point making the assessment of change over time more difficult. Future studies of hidden disability should include a broad range of outcome metrics, measured at multiple time points in good and poor aSAH survivors to better assess the overall burden of these deficits.

The publications included in this chapter provide multiple novel insights into the burden of hidden disability after aSAH including the long-term impact on patients. In order to improve management of these symptoms they must first be identified and disease specific outcome tools such as SAHOT[49] could be used to aid recognition. Once they have been identified management strategies can be put in place to improve outcome and are discussed in Chapter 6.

Chapter 3 Outcome prediction following aSAH

The ability to accurately predict outcome following aSAH particularly early in the clinical course using commonly available variables has a number of potential benefits including guiding treatment strategy and resource allocation. This is particularly true for the challenging patient cohort who present in poor clinical state where decisions need to be made about the appropriateness of aneurysm treatment and duration of active management[57]. Accurate prognostic information is also useful in setting expectations and counselling patients and their relatives following aSAH.

A number of outcome prediction tools have been developed which predominantly use logistic regression, a generalised linear model, to predict mortality or dichotomised functional outcome (assessed by the mRS or GOS) at between 3 and 12 months following aSAH[58]. The best available model in the Subarachnoid Haemorrhage International Trialists' (SAHIT) prediction tool which was developed and validated in 10936 and 3355 patients respectively[59]. The full SAHIT model uses commonly available demographic, clinical and imaging variables (age, World Federation of Neurological Surgeons [WFNS] score, previous hypertension, size and location of aneurysm, treatment modality, Fisher grade) to predict dichotomised clinical outcome as assessed by the GOS (GOS 1-3 versus 4-5) at 3 months post-haemorrhage. The externally validated model achieved good discrimination with a maximum area under the receiver operator characteristics curve (AUC) of 0.81[59].

Despite methodologically rigorous design and good model performance the uptake of SAHIT and other outcome prediction tools in clinical practice is limited. The most likely reason for the lack of uptake of these prediction tools is that they only offer marginal benefits over bedside assessment tools such as the WFNS score and Glasgow Coma Scale (GCS) which assess severity of clinical presentation[60,61]. The additional complexity of implementing these more advanced multivariable prediction models may therefore outweigh the benefit of improved model performance. Further improvements in outcome prediction models are required if they are to be incorporated into clinical practice.

The two main methodologies to further improve outcome prediction tools are to: (1) identify and include additional predictive variables, and/or (2) use more advanced statistical learning methods to develop outcome tools such as machine learning.

The SAHIT prediction tool achieves an R^2 statistics ranging from 22-31% which means that up to 78% of the observed variation in clinical outcome is not explained by the clinical, demographic

and imaging variables included in the model. Additional unknown variables therefore play a significant role in outcome variability and their inclusion in models has the potential to boost performance. A number of variables including blood, CSF and imaging biomarkers could be explored to improve outcome prediction. Routine blood tests are perhaps the most readily available of these investigations. Preliminary work within our research group suggested that blood inflammatory biomarkers were predictive of outcome and consequently I chose to focus on the acute phase reactant C-reactive protein (CRP). CRP is a cheap and routinely available blood test frequently performed early in the course of a patient's admission. Some studies have suggested it is predictive of outcome after aSAH but are underpowered to definitively show this[62-64].

Advanced statistical learning methods such as machine learning are being increasingly applied in medicine[65]. These methods may be superior to classical statistical learning methods such as logistic regression as they are better able to deal with multi-dimensional data and non-linear relationships[66,67]. Although first applied to the field of aSAH in 1998[68] there has been limited application of these methods until recently. Prior to the submission of **Publication 5** only relatively simple decision tree methodology had been applied to outcome prediction following aSAH with similar performance to logistic regression[68-71].

In **Publication 5** I aimed to improve the prediction of dichotomised clinical outcome following aSAH (mRS 0-2 versus 3-6) by exploring both the additional variable CRP and by applying advanced statistical learning methodology including machine learning. Firstly, using logistic regression and the same predictors as the SAHIT prognostic model I confirmed that CRP is an independent predictor of outcome following aSAH. I showed that a prediction model developed using logistic regression performs significantly better when trained on the SAHIT predictors plus CRP compared to the SAHIT predictors alone ($p<0.001$) (Figure 2). The logistic regression model including CRP achieved an R^2 statistic of 27% which is comparable to the SAHIT prediction tool[59] with CRP explaining 4.5% of the variation in outcome. I also explored the benefits of machine learning methodology and showed that although the ensemble method random forest is not superior to logistic regression a support vector machine (SVM) performs superiorly to the comparable logistic regression model whether trained on the SAHIT predictors plus CRP ($p<0.001$) or the SAHIT predictors alone ($p=0.004$) (Figure 2).

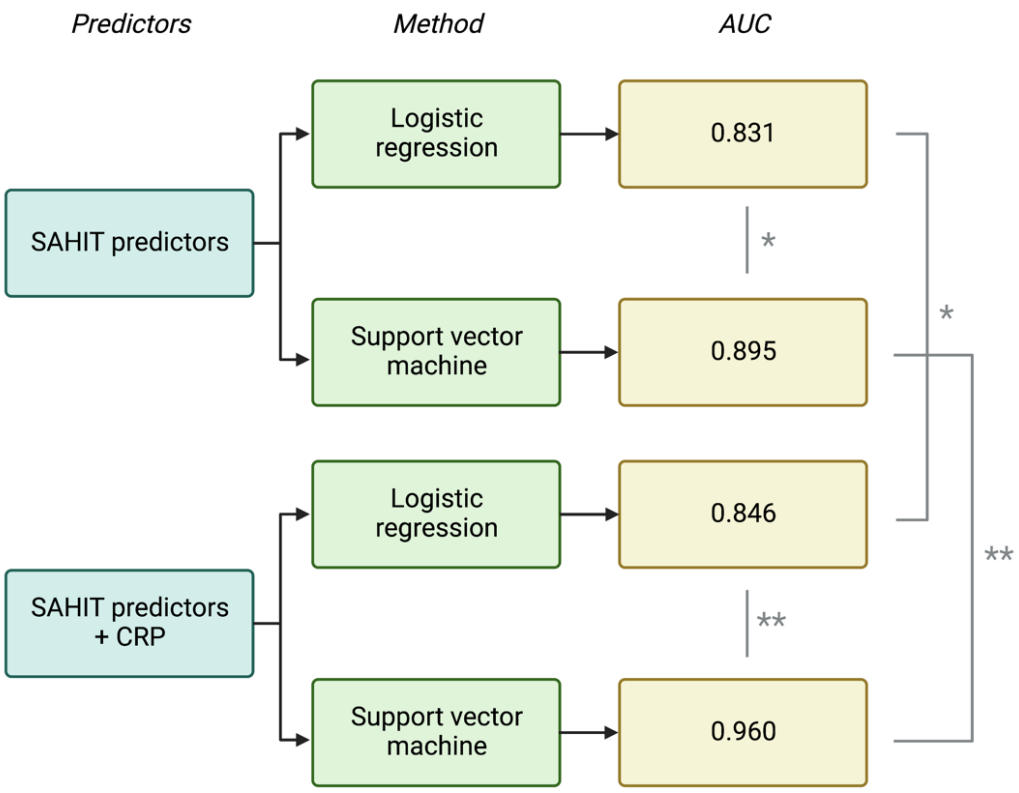

Figure 2. AUCs for logistic regression and SVMs trained on SAHIT predictors and SAHIT predictors + CRP. SAHIT predictors: age, WFNS score, previous hypertension, size and location of aneurysm, treatment modality, Fisher grade. *p<0.05 **p<0.001. Created with BioRender.com.

The addition of CRP to the SAHIT predictors although statistically significant only results in modest improvements in model performance and consequently is unlikely to influence daily clinical practice. The machine learning method random forest failed to improve outcome prediction compared to logistic regression, however, the SVM showed a significant improvement in model performance demonstrating the potential benefits of these advanced methods. The results of my study have not been externally validated and this is essential to confirm the benefits of this methodology. This is particularly important as the SVMs show the greatest error rates during internal 10-fold cross validation (up to 20%) suggesting overfitting which may inflate the AUC.

In recent publications the evidence for a benefit of machine learning over classical statistical learning methods in outcome prediction following aSAH is conflicting. Savarraj *et al.* demonstrated improvements in model performance using a range of machine learning methods including artificial neural networks whereas other groups have failed to show improvements using similar methodology[72,73]. SVMs have been shown to be particularly powerful when applied to medical problems[74] and Yu *et al.* show excellent performance using an SVM achieving AUC 0.94

when predicting risk of death after aSAH[75]. Dengler *et al.*, however, explored a range of machine learning methods to predict dichotomised clinical outcome following aSAH and the SVM did not perform superiorly to classical learning methods[60]. This emphasises the importance of external validation of **Publication 5**.

Clinicians frequently rely on traditional bedside assessment tools such as GCS and WFNS when making clinical decisions about patient management early during admission even though studies have demonstrated that poor grade individuals can recover with good physical and cognitive function[76]. Both CRP and machine learning methods can be used to improve outcome prediction, however, unless significant improvement in model performance is seen and validated advanced outcome prediction tools like these are unlikely to make a significant impact on clinical practice.

Chapter 4 Haemoglobin scavenging following aSAH: candidate gene studies

At the time of aneurysm rupture blood is released into the subarachnoid space and red blood cell lysis releases haemoglobin. Haemoglobin has been demonstrated to be neurotoxic both *in vitro* and *in vivo*[32] and is thought to play an integral role in the pathophysiology of delayed brain injury[3,28]. A haemoglobin scavenging system exists in the brain which acts to clear the neurotoxic stimulus although its significance to outcome following aSAH in humans is unknown.

Publication 6 is a review of haemoglobin scavenging after intracranial haemorrhage. The neurotoxic effects of haemoglobin are summarised including direct neurotoxicity, oxidative injury, inflammation, nitric oxide depletion and induction of cerebral oedema. The haemoglobin scavenging pathway is introduced (Figure 3). In brief, following aSAH haemoglobin released by the lysis of extravasated red blood cells can either be: (1) bound by haptoglobin and then taken up into macrophages via CD163 where it is broken down releasing haem which is subsequently degraded to generate iron by the action of haem oxygenases, or (2) further degraded in the extracellular space releasing haem which binds haemopexin and is transported into macrophages via CD91 where is similarly degraded to release iron. I then summarise the limited evidence supporting an integral role for haemoglobin and its scavenging pathway in neurological injury and outcome after aSAH in humans.

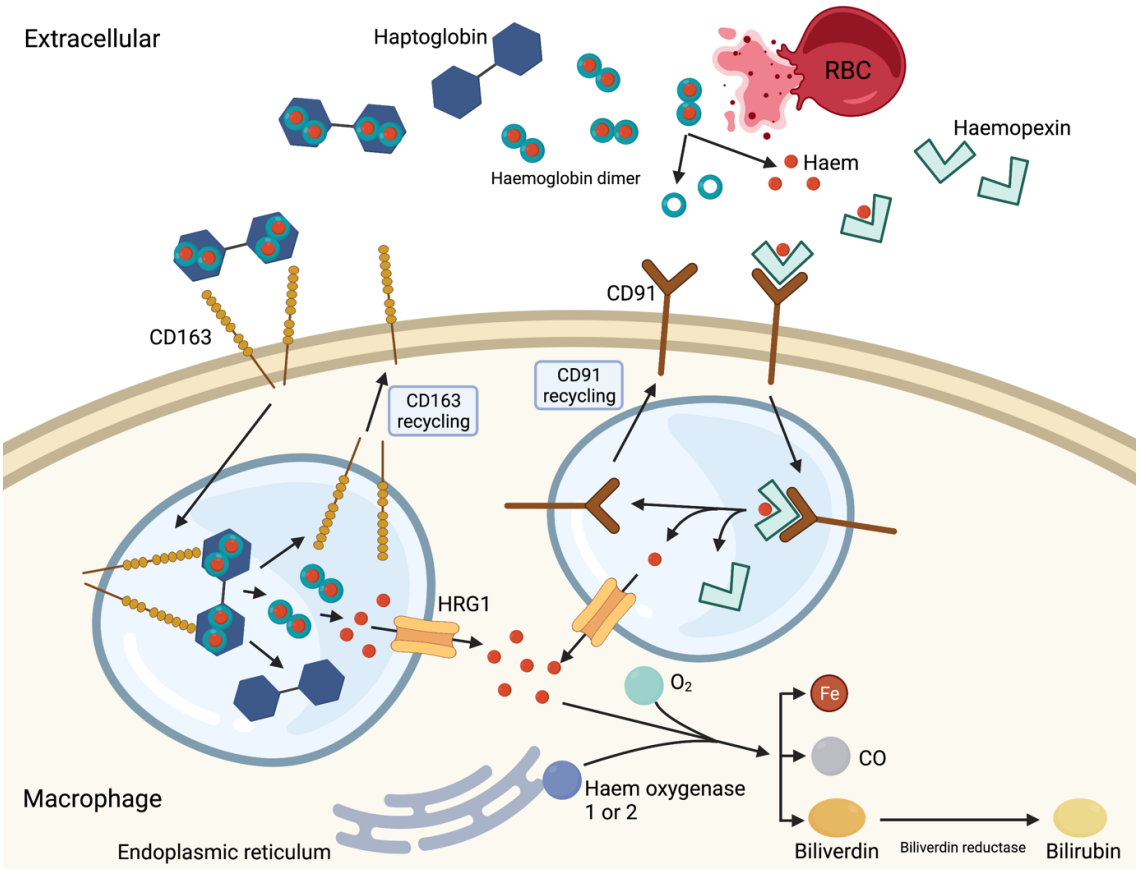

Figure 3. Summary of haemoglobin scavenging cascade. See text for details. RBHC: red blood cell; CO: carbon monoxide; Fe: iron; HRG1: haem-responsive gene 1 protein homologue. Created with BioRender.com.

With the remaining publications included in this chapter (**Publications 7-9**) I aim to provide insight into the importance of the haemoglobin scavenging pathway for outcome in humans following aSAH through a series of candidate gene studies.

Haptoglobin is the most studied component of the haemoglobin scavenging pathway following aSAH. Haptoglobin is an acute phase glycoprotein which binds and clears free haemoglobin via CD163[77]. Haptoglobin also has anti-inflammatory[78] and anti-oxidant properties[79]. Two haptoglobin alleles exist (Hp1 and Hp2) and individuals can express one of three haptoglobin polymers (Hp1-1, Hp 2-1 and Hp2-2) depending on their genotype. The functional differences between haptoglobin polymers and potential impact on outcome is not well understood. In a mouse model of SAH the Hp2-2 polymer was associated with vasospasm, increased CSF inflammatory markers and worse neurological deficits compared to Hp1-1[80]. In humans there is a growing body of evidence that haptoglobin genotype influences outcome after aSAH[81-86]. This supports a key role for haptoglobin in the pathophysiology of neurological injury. There are a number of possible mechanisms by which haptoglobin genotype could influence outcome after aSAH including effects on haptoglobin expression, function or haptoglobin-haemoglobin complex size. Hp1-1 has higher expression in

serum than other subtypes[87] and has been reported to have superior anti-oxidant[88] and anti-inflammatory effects[89]. Hp1 is also smaller than Hp2 which may promote clearance via the glymphatic system[90], however the larger size of Hp2-haemoglobin complexes may impair their ability to enter the brain parenchyma and subsequent neurotoxicity[91].

Publication 7 is a meta-analysis of the effect of haptoglobin genotype on outcome after aSAH including data on 533 patients from six published studies. In this study I showed that Hp2-2 was associated with worse short-term outcomes (defined as cerebral vasospasm and/or delayed cerebral ischemia during the inpatient period) compared to both Hp2-1 (OR 1.90 (95% CI 1.11-3.25), p=0.02) and Hp1-1 (OR 2.37 (95% CI 1.12-5.04), p=0.02). Hp2-1 grouped with Hp1-1 rather than Hp2-2 in its effect on short term outcome suggesting it is the Hp1 allele that is protective rather than a dose dependent harmful effect of Hp2. There was, however, no difference in long-term outcomes (defined by dichotomised mRS/GOS at 1-3 months post-haemorrhage) between haptoglobin genotypes. I concluded that the Hp2 allele was associated with worse short-term but not long-term outcomes. The effect on short-term outcome is supported by a recent small Egyptian study (n=50) which demonstrates that the Hp2 allele is associated with increased risk of cerebral vasospasm and delayed cerebral ischemia following aSAH, although in this study the Hp2-1 genotype appeared to group with Hp2-2 rather than Hp1-1[92]. My meta-analysis has a number of limitations including heterogenous outcome definitions, small individual study sample sizes and relatively short follow up periods for long-term outcome. I was also not able to control for covariates known to influence outcome after aSAH such as WFNS score.

In order to address these limitations and further explore the role of haptoglobin genotype on outcome I went on to perform an individual patient level data meta-analysis (**Publication 8**). To facilitate sample recruitment for this study I co-founded the Haemoglobin After intraCranial Haemorrhage (HATCH) consortium, an international consortium focussing on improving outcome after brain haemorrhage. Through the consortium I identified individual patient level data on 939 aSAH cases from 11 studies (5 published and 6 unpublished). The primary outcome was mRS/GOS dichotomised into good (mRS 0-2, GOS 4-5) and poor (mRS 3-6, GOS 1-3) in the first year following haemorrhage. The secondary outcomes were the presence of DCI, vasospasm or cerebral infarction during inpatient admission. I controlled for covariates known to influence outcome after aSAH including age, Fisher grade, WFNS or Hunt and Hess score and treatment modality[26]. There was no significant difference between haptoglobin genotypes for either the primary or secondary outcomes. This was not consistent with the previous meta-analysis which showed an association between Hp2-2 and poor short-term outcome.

Although there may simply be no effect of haptoglobin genotype on outcome there are a number of possible explanations why this study may have missed a significant effect. Although this was the largest sample size of any analysis of the effect of Hp genotype on outcome it was only powered to detect an estimated effect size of OR 1.6 and may therefore have missed a smaller yet significant effect. In addition, like many studies of outcome after aSAH this study used the mRS/GOS to assess outcome which as demonstrated in Chapter 2 are not sensitive enough to detect subtle yet clinically significant outcomes including hidden disability. Outcome metrics such as the SAHOT may be better placed to identify the influence of haptoglobin genotype on outcome after aSAH[49]. Finally, outcome was still assessed at a relatively short follow period with the majority assessed within the first 3 months and it may take longer for the effects of haptoglobin to be revealed. Prior studies have shown improvements in mRS in 19% of patients between 12 and 36 months post-aSAH[93].

In order to explore whether the effect of haptoglobin genotype on outcome after aSAH requires longer follow up time periods, our group performed a further analysis (on which I am a co-author) in a separate cohort of 1299 aSAH patients with a median follow up of 18 months[91]. We showed that the Hp2 allele (comparing Hp2-2 and Hp1-1) was associated with favourable long-term outcome (mRS 0-1) in high volume (Fisher 3+4) but not in low volume (Fisher 1+2) aSAH at follow up times beyond 2 years. No difference was identified when Hp2-1 was compared to Hp2-2 or Hp1-1. We also showed that the effect on outcome was not associated with haptoglobin expression level. Finally, we identified that haemoglobin concentration did not exceed the maximum binding capacity of haptoglobin in low volume but did in high volume aSAH. Consequently, haptoglobin genotype may only impact outcome when it becomes saturated leaving free haemoglobin in the CSF. We concluded that Hp genotype may influence long-term outcome following high-volume aSAH in a mechanism unrelated to expression level. The effect is only seen beyond 2 years which may suggest other factors obscure the effect of haptoglobin genotype at shorter follow up times as seen in the individual patient level data analysis.

This result, however, conflicts with another recent study (of which I am a co-author) in a Korean population where we report that the Hp2 allele was associated with worse functional (n=336, classified as mRS 3-6) and cognitive outcomes (n=292, assessed by the Mini-Mental State Examination) at both short (6 month) and long-term follow up (mean around 3 years)[94]. In this study when the effect of the haptoglobin allele was analysed according to dichotomised Fisher grade, in comparison to the previous study, the Hp2 allele was associated with worse outcomes in high blood volume (Fisher 3+4) patients. Like in the original meta-analysis (**Publication 7**) Hp2-1 groups with Hp1-1 rather than Hp2-2 for both short- and long-term outcome. Although this study includes a smaller sample size than any of the prior analyses it adds novelty by including cognitive

outcomes and by showing that lower expression of Hp1 in Hp2-1 individuals is associated with worse outcome at 6 months. This again suggests it is the presence of Hp1 that improves outcome rather than a negative effect of Hp2. A number of factors may explain the differing effect of the haptoglobin allele on long-term outcome seen in this analysis compared to the previous study including use of a different definition of good/poor functional outcome and differing distributions of haptoglobin genotype.

In summary, although the protocolised individual patient level data meta-analysis did not identify any difference between haptoglobin genotype and outcome, subsequent studies have shown conflicting results. If present, the relationship between haptoglobin genotype and outcome is likely to be complex although it is possible no relationship exists. This may be because haptoglobin levels are too low in the CSF following aSAH for genotype to significantly impact outcome[95]. Further studies including an updated individual patient level data analysis are required to clarify the effect of haptoglobin genotype on outcome.

Publication 9 is a candidate gene study assessing the impact of genetic variation in NFE2L2 on outcome after aSAH. Nuclear factor erythroid 2-related factor 2 (NRF2, encoded in humans by the NFE2L2 gene) is a transcription factor which controls expression of a number of anti-inflammatory[96] and anti-oxidant[97] genes in addition to components of the haemoglobin scavenging cascade including haem-oxygenase 1[97] and haptoglobin[98] (Figure 3). NRF2 has the potential to have a significant impact on outcome after aSAH as not only does it protect against the neurotoxicity of haemoglobin it also upregulates its scavenging pathway. NRF2 has been implicated in the pathophysiology of neurological injury in a number of animal SAH models[99,100] but there is no evidence for its importance in humans. In **Publication 9** I identified in both discovery (n=1007) and validation (n=466) cohorts that the intronic SNP rs10183914 is associated with outcome following aSAH. The minor T allele is associated with a 1.33-fold (95% CI 1.12-1.58) increased risk of poor outcome following aSAH (p_{meta}=0.001). *In silico* functional analysis suggests it has a regulatory effect on NRF2 by influencing transcription factor binding and significantly reducing the intron-excision ratio ($p_{sQTL}=1.3x10^{-7}$) potentially reducing expression of functional NRF2. This study builds on previous animal evidence by demonstrating an important role for NRF2 in outcome after aSAH in humans. This provides further insight into the pathophysiology of neurological injury following aSAH emphasising the importance of oxidative stress, inflammation and haemoglobin scavenging after aSAH.

The candidate gene studies included in this chapter highlight the importance of haemoglobin and its scavenging pathways in the pathophysiology of outcome after aSAH specifically in humans. Importantly they have implications for treatment which will be discussed in Chapter 6.

Chapter 5 Genome-wide analysis of outcome following aSAH

In addition to NFE2L2 and haptoglobin, a number of other genes have been implicated in the pathophysiology of outcome following aSAH including endothelial nitric oxide synthase[101], apolipoprotein E[102], brain-derived neurotrophic factor[103] and genes associated with inflammation[104] and fibrinolysis[105]. All these genes have been identified using a candidate gene approach which requires *a priori* knowledge of the potential pathways influencing outcome after aSAH. As our understanding of these pathways is incomplete this focussed approach may overlook significant contributors and is unlikely to provide novel insight into the mechanisms governing outcome.

I am therefore undertaking a genome-wide association study (GWAS) of outcome after aSAH which addresses these limitations by exploring the breadth of the human genome and has the potential to radically advance our understanding of outcome. The aim of this study is to identify genes associated with clinical outcome and target future research including the development of treatments to improve outcome after aSAH. Genome-wide analyses have provided novel insight into aneurysm formation[106] but have never been used to study outcome after aSAH. The GWAS is designed in two stages (discovery and validation) and the protocol was published in 2022 (**Publication 10**).

5.1 Discovery analysis (Publication 11)

For the discovery analysis multiple retrospective cohorts were identified from two sources: 1) the HATCH consortium, and 2) the UK Biobank. Individual genetic variants were tested for association with dichotomised clinical outcome using multivariable logistic regression under an additive model controlling for key covariates (age and genetic ancestry). Genetic variants were tested within each separate cohort and then combined using a fixed effects meta-analysis. Genetic variants which achieved a threshold of suggestive significance ($p<1x10^{-4}$) will be taken forward for validation in the second stage of the study. The primary outcome was the mRS or GOS dichotomised into good and poor outcome within one year of aSAH.

A challenge of combining multiple retrospective datasets using different outcome metrics (mRS or GOS) is defining comparable dichotomisation thresholds for each scale. Although studies often pool these two scales for analysis of outcome there is no evidence as to which dichotomisation threshold shows the strongest agreement between the two scales. In order to address this, I

analysed 3474 paired mRS and GOS recordings from individuals with haemorrhagic stroke (**Publication 12**). The cohort consisted of 496 aSAH individuals from the haptoglobin individual patient level data meta-analysis (**Publication 8**), 499 patients with intraventricular haemorrhage recruited to the CLEAR III trial[107] and 500 intracerebral haemorrhage patients from the MISTIE III trial[108]. I confirmed that the mRS and GOS are strongly correlated (Spearman's rank correlation coefficient -0.90, $p<0.001$). Using Cohen's kappa coefficient I identified the strongest agreement between the two scoring systems occurs when mRS 0-2 and GOS 4-5 are used to define good outcome. Finally, I provided a method of score interconversion so that the ordinal nature of the scales could be preserved if required and showed that where possible conversion of mRS to GOS is superior to the reverse direction as assessed by the two-sample Kolmogorov-Smirnov test. Using the results of **Publication 11** for the GWAS I defined good outcome as mRS 0-2/GOS 4-5 and poor outcome as mRS 3-6/GOS 1-3.

The UK Biobank does not include data on the mRS or GOS and consequently I used cognition, as measured by psychomotor reaction time, which I demonstrated in **Publication 1** differs significantly between cases and controls in the UK Biobank, to define outcome. aSAH cases from the UK Biobank were ranked from fastest to slowest and then dichotomised into good (faster) and poor (slower) outcome. The threshold for dichotomisation was set in order to generate an equivalent proportion of good/poor outcome individuals to the HATCH dataset. This method is supported by the finding that the mRS and cognition are highly correlated after aSAH[109]. As the outcome assessment metric differed between the UK Biobank and HATCH datasets the GWAS was performed both in the HATCH dataset alone and the whole dataset (HATCH + UK Biobank).

A total of 2489 samples were identified for inclusion in the discovery analysis from seven datasets. Six datasets were identified from the HATCH consortium (n=1685) and 804 individuals from the UK Biobank. Analysis of the HATCH dataset identified 403 genetic variants from 97 independent loci associated with clinical outcome ($p<1x10^{-4}$). Including all seven datasets (n=2489) 406 genetic variants from 85 independent loci were associated with clinical outcome ($p<1x10^{-4}$). In this second analysis a single variant, rs12949158, achieved genome wide significance ($p=4.29x10^{-8}$). Including both analyses a total of 157 unique independent genetic loci were identified for validation in the second stage of the study.

The rs12949158 variant which achieved genome-wide significance is an intronic variant in the sphingolipid transporter 2 (SPNS2) gene, a component of the spingosine-1-phosphate (S1P) signalling pathway. The alternate A allele was associated with an increased risk of poor outcome with an odds ratio of 2.15 (95% CI 1.63-2.82). The rs12949158 genotype was only available in two datasets (n=833) as the other datasets did not use genotyping platforms which included the

variant and the available variants did not allow imputation at sufficient quality to pass quality control. In addition, the majority of the effect was driven by the UK Biobank which uses the alternative outcome metric, cognition. This finding therefore requires validation in the second stage of the study.

Unpublished protein-protein interaction analysis using NDEx Integrated Query[110], BioGRID[111], Cytoscape[112] and PathLinker[113,114] within the discovery data supports the importance of the genome-wide significant finding in SPNS2. Proteins mapped to genetic variants which achieved $p<1x10^{-5}$ in the HATCH dataset GWAS (i.e. when the UK Biobank was excluded) were assessed for interaction with SPNS2. Identifying interactions between SPNS2 and other significant proteins when the UK Biobank is excluded has the potential to cross validate the SPNS2 finding. Two mapped genes highlighted by analysis in the HATCH dataset, protein phosphatase 1A (PPM1A) and LDL receptor related protein 1B (LRP1B), were robustly linked to SPNS2 within 3 edges (Figure 4) supporting the significance of the SPNS2 finding.

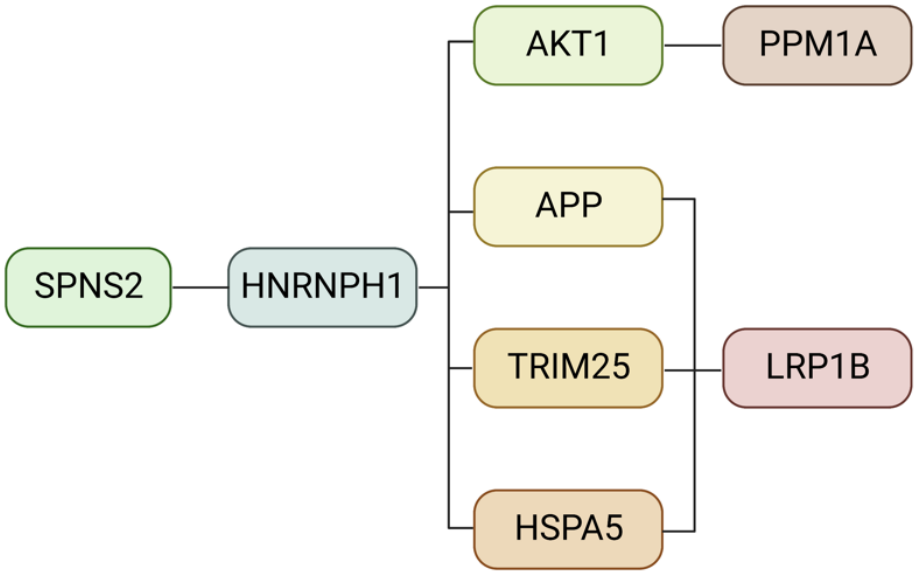

Figure 4. Protein-protein interactions linking SPNS2 to LRP1B and PPM1A. HNRNPH1: heterogeneous nuclear ribonucleoprotein H1. AKT1: AKT serine/threonine kinase 1. APP: amyloid beta precursor protein. TRIM25: tripartite motif containing 25. HSPA5: heat shock protein family A member 5. Created with BioRender.com.

In addition to the supporting protein-protein interaction evidence for SPNS2, the S1P signalling pathway could theoretically influence outcome after aSAH. S1P is synthesised intracellularly by the phosphorylation of sphingosine by sphingosine kinase[115]. S1P is transported to the extracellular compartment by SPNS2[116] and signals via binding one of five S1P receptor subtypes ($S1PR_{1-5}$)[117]. S1PR activation by S1P has been linked to neurological injury following stroke causing

microglial activation, neuronal death, inflammation and blood-brain barrier disruption[118,119] (Figure 5).

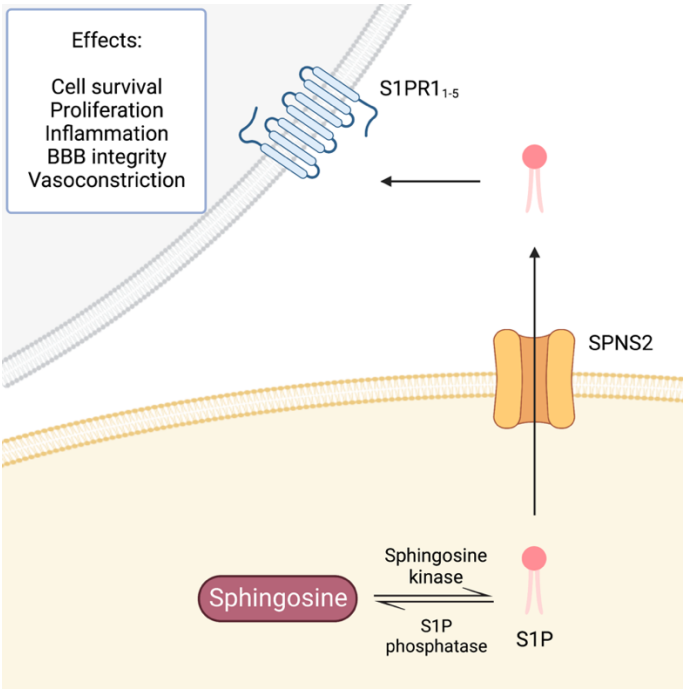

Figure 5. Summary of S1P signalling in the brain. Created with BioRender.com.

Studies have shown in humans following aSAH that S1P levels are raised in the CSF with higher levels associated with larger haemorrhage volume[120] and worse neurological outcome[120]. S1P has been shown to cause cerebral vasoconstriction in both canine and murine basilar arteries both *in vitro* and *in vivo* via $S1PR_3$ [121,122]. This effect on cerebral vasculature may represent a mechanism by which S1P signalling potentially influences outcome after aSAH, with elevated S1P levels leading to vasoconstriction, ischemia and neurological injury. These studies suggest that S1P signalling plays a potentially important role in neurological injury following aSAH, supporting the present finding of SPNS2's role in influencing outcome after aSAH.

Unpublished *in silico* functional analysis strongly suggests that the rs12949158 variant has a regulatory effect. It is located in intron 8 of SPNS2 within a candidate cis-Regulatory Element (EH38E1842415) [123] with evidence of significant DNase sensitivity (DNase max Z-score 3.94) and histone modification (H3K27ac max Z-score 4.09) in adult brain. A Z-score >1.64 corresponds to the 95th percentile of a one-tailed test and is considered as "high". Regulatory function is also supported by a Regulome DB categorical score of 4 based on evidence of transcription factor binding and DNase sensitivity [124]. When annotated against the 15-state chromatin model rs12949158 demonstrates enhancer activity specifically in the brain[125,126]. rs12949158 has a CADD score of 12.18 meaning it is predicted to be in the top 10% most deleterious substitutions in the human genome[127]. rs12949158 is situated within the transcription factor binding motif for zinc

finger protein 423 (ZNF423) and the risk A allele increases transcription factor binding affinity from a LOGOD of -3.4 to 7.6 ($P = 5.5x10^{-13}$)[128]. This suggests a mechanism by which rs12949158 could exert an effect on outcome. Increased binding affinity of ZNF423 may lead to upregulation of SPNS2, a subsequent increase in S1P in the extracellular compartment and greater neurological injury through mechanisms including inflammation, vasoconstriction, blood-brain barrier disruption and neuronal death (Figure 6). Further *in vitro* and *in vivo* studies are required to assess the functional effect of this variant.

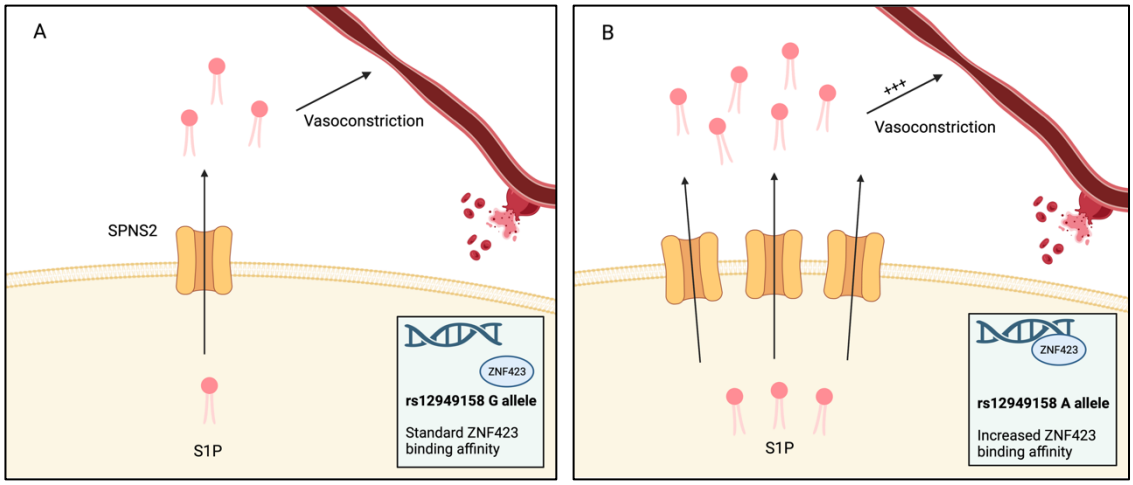

Figure 6. Illustration of potential mechanism by which rs129049158 could influence outcome after aSAH. A: S1P is transported into the extracellular space by SPNS2 where it can cause cerebral artery vasoconstriction via $S1PR_3$. B: The alternate A allele is associated with increased ZNF423 transcription factor binding affinity leading to upregulation of SPNS2, an increase in S1P transported into the extracellular space and increased cerebral artery vasoconstriction. Created with BioRender.com.

Overall, although rs12949158 like the other variants identified in the discovery analysis requires validation in the second stage of the study internal validation using protein-protein interactions and a plausible biological effect combined with relevant functional evidence highlights the S1P signalling pathway as potential integral to outcome after aSAH. It also showcases the benefit of a genome-wide approach to the analysis of outcome by highlighting a relatively understudied pathway in aSAH.

5.2 Validation analysis

All variants achieving suggestive significance including rs12949158 will be taken forward for validation. Recruitment for the validation study is ongoing with a target sample size of at least 2500.

Chapter 6 Therapeutic implications and conclusion

6.1 Therapeutic implications

As outlined in **Publications 1-4** and by recent studies using traditional assessment tools such as the mRS[15,129] outcomes remain poor following aSAH. Although the most effective way to improve outcome is to prevent or reduce neurological injury following aSAH other methods include managing the sequelae to reduce the long-term impact on survivors. The ultimate aim of my work is to improve outcome after aSAH and this thesis highlights a number of potential therapeutic strategies to achieve this goal.

In **Publications 1-4** I highlight the significant burden of hidden disability in the long-term following aSAH and its impact on patients including impairing quality of life and return to work. The very nature of these hidden symptoms is that they are not immediately obvious and may be overlooked. The first step in their management is recognising them in survivors of aSAH and **Publications 1-4** call attention to the prevalence of hidden disabilities. Tools such as the aSAH specific outcome tool, SAHOT[49], may help clinicians to recognise these symptoms following aSAH. Although no specific management strategies exist for hidden disability once recognised a number of potential treatment and support strategies could be considered.

Fatigue not only decreases quality of life but also has the greatest impact of the disabilities highlighted in **Publications 1-4** on employment following aSAH. Impaired return to work is common feature of aSAH with as many as 65% of survivors unable to return to their previous work[130]. Strategies to manage fatigue have not been specifically explored in aSAH. In the context of stroke although no drugs have been shown to improve fatigue[131], non-pharmacological treatments have been shown to improve symptoms such as Community Health Management and Cognitive Behavioural Therapy[132]. These strategies could be translated to aSAH with a view to improving fatigue and its consequences such as unemployment. Likewise, there are no specific treatments to manage cognitive deficits following aSAH. In stroke there is evolving evidence for a role of both cognitive training and pharmacological therapies to improve cognition and these could also be explored in aSAH[133,134].

The management of persistent headache following aSAH is limited[135]. Routine analgesia including paracetamol and opioids does not significantly improve headache following aSAH[136]. A number of other treatments have been trialled without success including magnesium which reduced pain scores but was not clinically significant[137,138]. **In Publication 3** by demonstrating that aura and prodrome are more common in headache following aSAH I implicate cortical spreading

depression, as seen in migraine, in the pathophysiology of persistent headache. This has potential implications for management in that anti-migraine therapy, particularly treatments which target cortical spreading depression, may be beneficial following aSAH.

Publication 2 demonstrates significant auditory deficits following aSAH and identifies that cognitive deficits significantly contribute to hearing impairment. This supports the previous finding that central auditory processing disorder plays an integral role in the pathophysiology of hearing imapirment[25]. This has significant implications for treatment. Peripheral hearing loss is managed with hearing aids while central origin deficits may need both audiological (such as assistive listening devices)[54] and cognitive therapies[139]. This emphasises the importance of assessing for both central and peripheral hearing loss in addition to cognitive deficits in patients who report hearing loss after aSAH in order to tailor treatment effectively. We are currently undertaking a clinical study in patients who report hearing loss following aSAH and have normal peripheral hearing. The study aims to assess the benefit of an assistive listening device following aSAH and should this be successful we will aim to introduce this therapy to improve auditory outcome after aSAH.

In **Publication 5** I demonstrate potential improvements in outcome prediction following aSAH using machine learning methodology and by including CRP in the prediction model. These findings require validation and we have secured the SAHIT dataset to validate my findings. Although, as I discuss in Chapter 3, the uptake of complex outcome prediction tools is limited in clinical practice there is a potential role for them in the management of patients with aSAH. At present the decision to secure a ruptured aneurysm is guided by a patient's clinical state (e.g. assessed by WFNS) with poor grade individuals often monitored for neurological improvement before aneurysm treatment is performed. This potentially delays treatment increasing the risk of re-bleed in this cohort of patients[57]. 'Poor grade' individuals can, however, have favourable outcomes with as many as 41.4% of WFNS 4-5 individuals moderately disabled or better at 6-12 months following haemorrhage[140] suggesting that early aneurysm treatment may be reasonable in this group. Outcome prediction tools such as the models presented in **Publication 5** perform superiorly to assessment of clinical state (e.g. WFNS) and may therefore be useful for decision making in worse performing individuals. Outcome prediction tools could also be used to highlight patients at risk of deterioration who may benefit from intensive monitoring and early intervention for example on an intensive care in order to minimise neurological injury following aSAH. Finally, outcome prediction tools may not only be useful in clinical practice, they could also be used to improve patient stratification in clinical trials, reducing required sample sizes and improving trial efficiency.

Rather than managing the consequences of aSAH, the most effective way to improve outcome is to prevent or reduce neurological injury following aSAH. As neurological injury following aSAH consists of early and delayed phases, of which the latter develops days to weeks after haemorrhage when patients are frequently admitted to hospital, there is a therapeutic window in which treatments could be administered to reduce neurological injury. At present the only treatment to improve outcome after aSAH is nimodipine[11] and despite multiple clinical trials[3] no new treatments have been identified for over 30 years. It is likely that our incomplete understanding of the pathophysiological mechanisms underlying outcome contributes to this lack of therapeutic progress. Improving our understanding of the mechanisms may help target the development of the desperately needed new treatments to improve outcome.

In this thesis using both candidate and gene and genome-wide analyses I highlight the haemoglobin scavenging and S1P signalling pathways as important following aSAH which has therapeutic implications.

Although the exact impact of haptoglobin genotype on outcome is unknown the genetic analyses presented in Chapter 4 (**Publications 7 and 8**) support a role for haptoglobin in outcome after aSAH. Haptoglobin levels in the CSF can be depleted following aSAH due to scavenging of haemoglobin and it is therefore a tempting therapeutic target to reduce neurological injury. The potential therapeutic benefits of haptoglobin have been demonstrated in a number of animal models. Haptoglobin overexpression in mice has been shown to be neuroprotective in an intracerebral haemorrhage model[98]. In a sheep model of SAH intrathecal administration of haptoglobin reduced cerebral artery vasospasm, preserved nitric oxide signalling and prevents translocation of haemoglobin into the brain parenchyma[39]. We have also demonstrated in a mouse model of SAH that administration of intrathecal haptoglobin improved neurological outcome by reducing diffusion of haemoglobin into the brain parenchyma and small vessel vasospasm[141]. Haptoglobin has been shown to reduce the risk of post-operative kidney injury following cardiac surgery in humans[142] and a small open-label uncontrolled clinical study of intrathecal haptoglobin treatment following aSAH conducted in 1979 reported improvement in cerebral vasospasm[143]. The growing body of evidence from both animal and genetic studies supports further investigation of haptoglobin as a treatment to improve outcome after aSAH. As the exact role of haptoglobin genotype is not clear, further investigation is required to inform which haptoglobin polymer would be most efficacious.

NRF2 upregulates multiple components of the haemoglobin scavenging system in addition to other neuroprotective molecules including antioxidants. In view of these widespread protective effects its upregulation is therefore another appealing therapeutic strategy[144]. NRF2 activators

such as sulforaphane have been shown in animal models of SAH to decrease neurological injury and improve outcome[99,145]. **Publication 9** demonstrates the importance of NRF2 in humans following aSAH and the results of a multicentre randomised double-blinded placebo controlled trial of sulforaphane after aSAH are eagerly awaited[146]

Although the results of the discovery GWAS (**Publication 12**) require validation they highlight S1P signalling as a potential therapeutic target following aSAH. A number of S1PR modulators (fingolimod, siponimod and ozanimod) are already licensed for the treatment of multiple sclerosis[147]. In a trial of 22 patients with acute ischemic stroke fingolimod treatment was associated with reduced neurological injury and improved functional outcome[148]. Fingolimod has also been shown to reduce neurological injury and improve outcome in a trial of 23 patients with intracerebral haemorrhage[149]. This beneficial effect in other stroke forms suggests that S1PR modulation may also be neuroprotective in aSAH and is supported by evidence in rodent SAH models where fingolimod has been demonstrated to reduce neurological injury and improve outcome[150,151]. If the findings of **Publication 12** are validated, this will highlight the importance of S1P signalling after aSAH and support trials of S1PR modulators to improve outcome. S1PR modulators act primarily by functional antagonism at S1PRs with different modulators targeting different receptors. This has implications for choice of S1PR modulator in aSAH. In aSAH S1P signalling within the CSF has been shown to causes cerebral artery vasoconstriction in animals via $S1PR_3$[121] (Figure 5). In the brain parenchyma both $S1PR_1$ and $S1PR_3$ have been implicated in neurological injury[152-154]. As fingolimod is the only modulator to target bother $S1PR_1$ and $S1PR_3$ it may be the most appropriate to trial in aSAH. Beyond the choice of S1PR modulator there are a number of further factors to consider before trials of S1PR modulators can be conducted in aSAH. Most importantly, S1PRs can cause first dose cardiac side effects including bradycardia and as aSAH can have cardiac manifestations (cardiomyopathy, ischemia, arrhythmias) the safety profile of such drugs needs to be evaluated in aSAH patients.

6.2 Conclusion

aSAH leads to a significant burden of neurological disability following aSAH with long term implications for quality of life and employment. Although current outcome prediction tools can be improved by incorporating additional variables such as CRP and utilising machine learning they are unlikely to influence clinical practice, which relies on simpler bedside prediction tools. Although strategies to recognise and manage the range of disability following aSAH will improve outcome, ultimately, we need to better understand the mechanisms which underlie neurological injury so we can develop treatments to prevent or reduce this injury. Using candidate gene and genome wide analyses I highlight the importance of haemoglobin scavenging and S1P signalling in

outcome after aSAH. These pathways represent potential therapeutic targets and future studies are required to investigate whether they can be manipulated to improve outcome after aSAH.

Appendix A Core publications

A.1 Summary

Each publication is presented along with its citation, a detailed description of my specific contribution and the summarised version of my contribution signed by all co-authors (Appendix B).

A.2 Chapter 2

A.2.1 Publication 1: Long-term cognitive outcome following aneurysmal subarachnoid haemorrhage

Citation: Gaastra B, Ewbank F, Tapper W, Bulters D, Galea I. Long-Term Cognitive Outcome following Aneurysmal Subarachnoid Haemorrhage. *Journal of Stroke and Cerebrovascular Diseases*. 2022/01/01/ 2022;31(1):106184. doi:10.1016/j.jstrokecerebrovasdis.2021.106184

This study is based on data from the UK Biobank. I curated the data and performed all the analyses. I authored the manuscript with input from the co-authors after completion of the first draft.

Specific contribution: Primary role in study design, data curation and analysis. Authored first draft of manuscript.

Long-Term Cognitive Outcome following Aneurysmal Subarachnoid Haemorrhage

Ben Gaastra, M.R.C.S.,[a,c] Frederick Ewbank, B.M.B.S.,[a] William Tapper, Ph.D.,[b] Diederik Bulters, F.R.C.S.,[a] and Ian Galea, Ph.D.[c]

Objectives: Survivors of aneurysmal subarachnoid haemorrhage (aSAH) frequently suffer from cognitive dysfunction. The aim of this study was to assess, in a large sample size with long term follow-up, the characteristics of cognitive dysfunction following aSAH and explore whether cognitive deficits mediate employment outcome. *Materials and methods:* In this retrospective case-controlled study, aSAH survivors (n = 884) were identified from the UK Biobank and compared to matched controls (n = 3536). Controls were propensity score matched according to age, sex, Townsend deprivation score, educational status and relevant medications known to influence cognition. Cognitive outcomes and employment status were compared between cases and controls using group comparison and cross-tabulation tests. A regression-based mediation analysis was performed to assess whether cognitive deficits mediate employment status following aSAH. *Results:* Psychomotor reaction time and employment status significantly differed between aSAH cases and controls with slower reaction times ($p < 0.001$) and more unemployment or inability to work due to illness ($p < 0.001$) in the aSAH cohort at a mean follow-up of 125 months. Psychomotor slowing was estimated to mediate a significant proportion (6.59%) of the effect of aSAH on employment status. *Conclusions:* Psychomotor reaction time and employment status differed significantly between aSAH cases and control matched individuals in the UK Biobank. Psychomotor slowing following aSAH had a discernible impact on employment status. Psychomotor reaction time and employment status are practical to acquire and can be used as surrogate measures of outcome in future studies of aSAH survivors.

Key Words: Subarachnoid haemorrhage—Outcome—Cognition—Employment

Introduction

Aneurysmal subarachnoid haemorrhage (aSAH) is a devastating form of stroke associated with significant morbidity and mortality. Survivors of aSAH frequently suffer from cognitive impairment,[1,2] however the prevalence, characteristics and impact of the cognitive dysfunction needs further study.

The majority of clinical trials following aSAH use traditional outcome scales,[3] including the modified Rankin score (mRS)[4] and the Glasgow Outcome Scale (GOS).[5] These scales have their limitations since they are relatively insensitive to hidden disabilities such as cognitive outcome. For example, in one study half the patients with a mRS of zero (no symptoms) after aSAH exhibited significant cognitive impairment when evaluated neuropsychologically.[6] In other studies, of those previously in full or part-time employment, 40–50% were unemployed at one to three years after aSAH.[7,8] A large number of studies have examined cognitive deficits after aSAH,[1,2,9] but more work is needed to determine whether they persist in the very long-term, which domains are most affected and how these relate to return to employment. In particular, the mediation of employment status by cognitive dysfunction has not been studied.

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The UK Biobank is a large-scale biomedical database. Participants underwent detailed cognitive testing and their employment status was recorded during their assessment visits. Within the UK Biobank cohort there are a subset of participants who have suffered aSAH. This makes it the largest database of long-term aSAH cognitive outcomes and the ideal tool to answer some of the outstanding questions about cognitive outcome after aSAH.

The aim of this study was to explore cognitive dysfunction in a larger sample size with longer follow-up compared to other studies, as is possible in the UK Biobank. We hypothesized that: (1) cognitive measures and employment status differ between aSAH cases and controls (2) cognitive deficits mediate employment outcome following aSAH.

Methods

This research has been conducted using data from the UK Biobank, a major biomedical database.[10] Study design consisted of a retrospective case-controlled study, as part of application ID 49305. The study was performed under National Research Ethics Committee Approval 16/NW/0274 and institutional approval (ERGO 49253). The study has been reported in accordance with the STROBE statement for case-control studies.[11] This study uses information on 502 490 participants, with informed consent, recruited in the UK between 2006 and 2010.

Outcome measures

In order to assess cognitive outcome five cognitive tests were included as they have been shown in previous studies to detect cognitive impairment in the context of neurological disease in the UK Biobank.[12] All tests were administered on a touch screen at assessment centre visits.

Cognition was measured in the majority of participants (98%) using two tests. The "reaction time" test (data field 20023) asked participants to press a button as soon as matching pairs of symbols were presented and therefore measured psychomotor reaction time. Visual memory was assessed using a pairs matching test (data field 399) during which three or six pairs of matching symbol cards were displayed to participants for three seconds, then turned face down, and participants were asked to identify matched pairs.

After UK Biobank recruitment started, three other tests were added in succession, and consequently these tests were done in less than 40% of participants. A "fluid intelligence" test (data field 20016) measured verbal and numeric reasoning. Prospective memory (data field 20018) was assessed by asking participants at the start of the assessment to memorize a certain shape from an array of shapes, and after the whole assessment, they were asked to recall the shape. Working memory was also assessed by the "numeric memory" test (data field 4282) during which participants were asked to repeat in reverse a string of numbers presented to them.

All cognitive tests report a continuous score apart from prospective memory which was dichotomised into correct on first attempt (good outcome) or not (poor outcome). The UK Biobank website and prior publications[12] provide more detail on these cognitive tests. Employment status (data field 6142) was dichotomised into good and poor outcome, with poor outcome defined as "unable to work because of sickness or disability" or "unemployed". For the aSAH cohort, the outcome measure was taken at the first available follow-up assessment following diagnosis of aSAH; most patients only had one assessment. Hence there is only one outcome datapoint for each aSAH patient in this study. For the control cohorts the outcome measure was taken at first assessment in the UK Biobank.

aSAH cohort

aSAH cases were identified from the UK Biobank using ICD-9 (data field 41271) and ICD-10 codes (data field 41270) from hospital inpatient data; read code information from primary care data (data field 42040); and self-reported medical conditions (data field 20002) reported at baseline or subsequent assessment centre visits. Cases were excluded if there was significant evidence that they were not actually aneurysmal in nature and likely to have been miscoded. This was defined using ICD-9 and ICD-10 codes from hospital inpatient and primary care data. Cases with a traumatic event code within 30 days of diagnosis of aSAH were excluded (Supplementary Table 1 for inclusion and exclusion codes). Cases were cross-checked against the algorithmically generated subarachnoid haemorrhage diagnosis (data field 42012) and first occurrence database for ICD-10 code I60 (data field 131360). Cases were included if there was data available in at least one outcome measure subsequent to the date of diagnosis.

Control cohorts

To allow for comparison of aSAH patients and controls four separate matched control populations were generated. Propensity score matching was performed with a nearest neighbour method and a case: control ratio of 1:4. All four control populations were matched to the aSAH cohort according to the following variables, known to influence outcome and cognition following aSAH: age at time of outcome assessment in the UK Biobank (data field 21003), sex (data field 31), Townsend deprivation score[13] (data field 189), and education status dichotomised into individuals holding a college or university degree at time of initial assessment in the UK Biobank or not (data field 6138). Sex and education status were treated as binary, age and Townsend deprivation score were treated as continuous variables.

Three of the control populations were additionally matched for the presence of medications which have been shown in a detailed study to influence verbal-numerical reasoning, memory and reaction time in the UK Biobank.[14] This

is of particular relevance as these three cognitive domains are directly tested by the cognitive outcome measures used in this study. Medications were categorised according to therapeutic subgroup (for example beta blocking agents) for the purpose of this study. Three binary medication variables were created to indicate, for each individual in the UK Biobank, whether they were taking a medication within a therapeutic subgroup which influences reasoning, visual memory and psychomotor reaction time. Each of these three medication variables was used in addition to the above four covariates to generate three matched control cohorts, to study the corresponding cognitive outcomes (reasoning, visual memory and psychomotor reaction time). The fourth control cohort was not matched for medications influencing cognition to allow analysis of other outcome measures which have not been shown to be affected by medications in the UK Biobank. To generate the matched populations, individuals with missing variables were excluded from the analysis.

Statistical analysis

In order to identify which outcome measures differ between aSAH and control cohorts the raw outcome scores were compared. A *t*-test was performed for continuous outcome measures and a chi-squared test for binary outcome measures. The six outcome measures (five cognitive tests and employment status) were considered separately and compared to the relevant matched control population (see Table 1). Individuals with missing outcome data were excluded from the analysis for the outcome of interest.

In order to assess whether cognitive deficits following aSAH influence employment status a regression-based mediation analysis was performed using PROCESS.[15] For this analysis aSAH case versus control status was used as the independent variable, employment status was used as the dependent variable and any significant cognitive measure considered as the mediator. The relevant control population matched to the cognitive measure of interest was used. Significance of the indirect effect was tested using 5000 bootstrapped samples. The proportion of the mediator effect on employment status was calculated using the method described by VanderWeele.[16]

Analyses were performed in statistical software *R* (version 3.6.2, *R* Foundation for Statistical Computing) and SPSS Statistics (version 27.0, IBM Corporation). A p value of < 0.05 was considered significant with Bonferroni correction for multiple testing where appropriate.

Results

Patients

888 aSAH patients were identified from the UK Biobank. Four aSAH patients were excluded from the matching process due to missing data regarding educational status meaning 884 were used in the final analysis (see Fig. 1 for flow chart of inclusion of patients; see Table 2 for demographics of aSAH patients). The mean follow-up time at first assessment following aSAH was 125 months (range 12 days−662 months).

501 609 individuals were available in the UK Biobank to generate the matched control cohorts. 5260 participants were excluded from the potential control pool due to missing data on age ($n = 1$), Townsend deprivation score ($n = 623$) and educational status ($n = 4636$). Four matched control populations ($n = 3536$) were generated with the average standard mean difference after matching across all variables < 0.04.

Outcome data availability

Three of the cognitive outcome measures (reasoning, prospective memory and working memory) were introduced late after recruitment had started in the UK Biobank, and therefore data for these variables was available in less than half the participants. Missingness analysis did not reveal any systematic differences between aSAH and controls (Supplementary Tables 2 and 3).

Comparison of aSAH cases versus controls

aSAH patients had significant psychomotor slowing in comparison to the matched control cohort (589 ms (standard deviation ± 138 ms) versus 569 ms (standard deviation ± 121 ms); $t = 3.84$, $p < 0.001$). No other significant difference was identified between the aSAH cohort and the relevant matched control cohort for the other four

Table 1. *Control cohort used for each outcome measure in analysis. Data on medications influencing prospective and numeric memory test scores was not available. Data on medications influencing reasoning, visual memory and psychomotor reaction time within UK Biobank was from Nevado-Holgado et al. (see text).*

Control cohort	Outcome measure
Not matched for medications	6142-employment status 20018-prospective memory 4282-numeric memory (working memory)
Matched for medications influencing psychomotor reaction time	20023-reaction time (psychomotor reaction time)
Matched for medications influencing visual memory	399-visual memory
Matched for medications influencing reasoning	20016-fluid intelligence (reasoning)

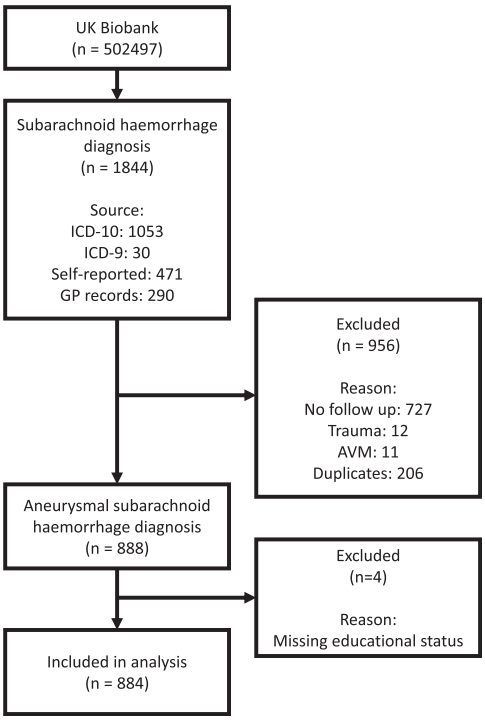

Fig. 1. *Flow diagram for identification of aSAH patients from the UK Biobank.*

cognitive tests (see Table 3). For outcomes with missing data (reasoning, prospective memory and working memory), the complete case analysis was followed by multiple imputation, with similar results ($p > 0.2$). In another sensitivity analysis, control populations were additionally matched for neurological/psychiatric diagnoses; results were similar (Supplementary Table 4).

A significant difference in employment status between the aSAH and matched control cohort was identified (82.8 versus 94.0%; $\chi^2 = 116.8$, $p < 0.001$), with aSAH patients more likely to be unemployed or unable to work because of sickness or disability.

Mediation analysis

Mediation analysis was performed to assess whether the psychomotor slowing had a discernible impact on employment status following aSAH (see Fig. 2). The control cohort matched for medications influencing psychomotor reaction time was used. The indirect effect of aSAH on employment status mediated by psychomotor slowing was significant, with an odds ratio of 1.05 (95% confidence intervals 1.02−1.08). The odds ratio for the direct effect of aSAH on employment status was 3.09 (95% confidence intervals 2.45−3.89). The proportion of the effect of aSAH on employment status mediated by psychomotor slowing was estimated to be 6.59%.

Discussion

In this study we demonstrate that psychomotor reaction time and employment status differ significantly between aSAH survivors and matched control individuals in the UK Biobank. This study had a relatively long follow-up time of just over 10 years and shows that the cognitive impact of aSAH in survivors is long-lasting. This study is significantly larger than previous cognitive outcome studies with 884 cases, most prior studies have fewer than 200 patients.[1,2] Findings here support the use of psychomotor reaction time and employment status as alternative measures of outcome in future studies of aSAH survivors. Both types of outcome data are practical to collect; the psychomotor reaction time test is based on 12 rounds of the card-game 'Snap', and a variety of online and digital versions are available. The results are clinically relevant and will help clinicians to advise aSAH survivors

Table 2. *Demographics of aSAH patients included in study. SD: Standard deviation. IQR: Interquartile range. GP: General practitioner. ICD: International Classification of Diseases.*

Sample size	884
Age at time of follow-up	
Mean (± SD) years	58 (± 7)
Range	40−74
Sex	F: 524 (59%) M: 360 (41%)
Source in UK Biobank	
ICD-10	543
ICD-9	25
Self-reported	266
GP records	50
Time to follow-up	
Mean (± SD) months	125 (± 119)
Length of stay	
Median (IQR) days	7 (2−14)
Missing	333
Townsend deprivation score	
Mean (± SD)	- 1.003 (± 3.23)
Education status	
College or university degree (good outcome) (%)	233 (26%)
Medication status	
Medications influencing psychomotor reaction time test scores (%)	697 (79%)
Medications influencing visual memory test scores (%)	634 (72%)
Medications influencing reasoning test scores (%)	668 (76%)
Presence of neurological or psychiatric diagnosis other than aSAH (%)	493 (56%)

and their relatives regarding cognition and employment outcomes in the long term. As psychomotor reaction time deficits mediate poor employment outcomes future studies should consider methods to optimise this cognitive domain in the long term as it may be beneficial in promoting quality of life and return to work following aSAH.

Changes in employment status following aSAH have been described previously with up to 50% of individuals either delayed or unable to return to their normal work following aSAH.[2] There is, however, minimal information on how psychomotor reaction time changes following aSAH and its impact on the patient's life. One small retrospective study of 58 patients following aSAH demonstrated longer reaction times[17] and another showed a trend towards slower reaction times following aSAH.[18] We demonstrate that psychomotor slowing has a discernible impact on employment after aSAH. It was surprising to be able to detect this effect in view of the multifactorial nature of the ability to remain in employment; this emphasises that cognitive deficits following aSAH are

Table 3. *Comparison of aSAH cases and relevant matched populations with regard to outcome measure. * indicates* $p < 0.05$.

Outcome	aSAH cohort (n = 884)	Matched: no medications (n = 3536)	Matched: reasoning medications (n = 3536)	Matched: visual memory medications (n = 3536)	Matched: psychomotor reaction time medications (n = 3536)	p value for comparison of SAH cohort and relevant matched population
Psychomotor reaction time	589 (± 138)				569 (± 121)	< 0.001*
At first assessment						
Mean (± SD)						
Visual memory						
3 pair trial	0.60 (± 1.19)			0.55 (± 1.13)		0.262
6 pair trial	4.35 (± 3.62)			4.34 (± 3.66)		0.889
Mean (± SD)						
Prospective memory						
Correct on first attempt (good outcome) (%)	207 (72.4%)	942 (74.1%)				0.597
Reasoning Mean (± SD)	5.64 (± 2.06)		5.89 (± 2.12)			0.06
Numeric memory						
Mean (± SD)	6.31 (± 1.63)	6.31 (± 1.86)				0.969
Employment status						
Good outcome (%)	732 (82.8%)	3325 (94.0%)				< 0.001*

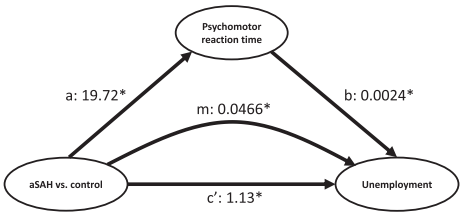

Fig. 2. Mediation analysis. *Effect sizes are as follows (a): the unstandardized regression coefficient for the association between the independent variable (aSAH and control) and the mediator (psychomotor reaction time); (b): the logit for the association between the mediator and the dependent variable (unemployment); (c'): the logit for the direct effect of the independent variable on the dependent variable; (m): the logit for the indirect effect of the independent variable on the dependent variable, via the mediator. For the analysis aSAH cases and controls were coded as 1 and 0, respectively; and with regard to employment good outcome and poor outcome were coded as 0 and 1, respectively. * indicates* $p < 0.001$.

clinically meaningful, with effects extending to employment.

Deficits in multiple domains of cognitive function have been demonstrated following aSAH including visual and verbal memory,[6,19,20] yet we could only demonstrate dysfunction in psychomotor reaction time, and not in other domains including visual memory, reasoning, prospective memory and working memory. Two factors may contribute to this finding.

Firstly, for reasoning, prospective memory and working memory scores, there was a high percentage of missing data in the UK Biobank as a whole, and therefore within the aSAH and control cohorts (Supplementary Table 2). This is because these three tests were not performed during most assessment visits at the UK Biobank; all three tests were introduced in the last two years of recruitment and working memory was subsequently removed due to assessment time constraints. The proportion of missing data was similar across aSAH cohorts, control cohorts and the UK Biobank as a whole (Supplementary Table 2). There was no evidence that participants with missing cognitive outcome data performed disproportionally poorly in other cognitive domains to suggest difficulty experienced during testing (Supplementary Table 3).

Secondly, the mean follow-up time following aSAH in the UK Biobank was 125 months. Cognitive outcome studies after aSAH are usually of much shorter duration, around 12 months, with the majority under 60 months.[2] Follow-up time in the UK Biobank was therefore significantly longer than that of the previous studies. Longer follow-up may allow for recovery of cognitive deficits explaining why no significant differences in certain cognitive domains were identified in this study. In keeping with this explanation, one study demonstrated significant improvements in motor, psychomotor, verbal and visual memory, executive function and intelligence between three and 12 months following aSAH.[21] On the other hand, the same study showed that motor function did not recover to the normative mean by 12 months post-aSAH,[21] which may explain why psychomotor reaction time is the only cognitive test to demonstrate a significant difference at a mean follow-up of 125 months. The follow-up time within the UK Biobank cohort was not a significant predictor of psychomotor reaction time (Supplementary Results), which may indicate that some irreversible residual disability in certain cognitive domains occurs after SAH, even if some improvement occurs in the first few years.

The presence of hydrocephalus has been shown to be a significant predictor of outcome following aSAH in some[22] but not other studies.[6,23] A sensitivity analysis was performed to assess whether the presence of hydrocephalus following aSAH, as defined using ICD-9 and ICD-10 codes, influenced cognitive and employment outcomes, in our cohort. Hydrocephalus was not a significant predictor of psychomotor reaction time ($p = 0.384$) or employment status ($p = 0.977$), controlling for age, Townsend score, sex, relevant medications, education status and time to follow-up.

This study has a number of strengths and limitations. It has a large sample size, long follow-up and employment data. Case ascertainment was more detailed than the algorithmically generated SAH diagnosis field already available in the UK Biobank (data field 42012).[24] Employment status was dichotomised into good and poor outcome, with poor outcome defined as "unable to work because of sickness or disability" or "unemployed". The UK Biobank data does not specify the reason for inability to return to work. Granular detail regarding the precise reason for the inability to return to work might have delivered additional insight into the frequency and nature of neurological deficits linked to the inability to return to work. Future studies of employment should include more detailed assessment. The World Federation of Neurological Surgeons (WFNS) score is a marker of early brain injury following aSAH and a strong predictor of outcome,[25] but it was not available and could not be controlled for in this analysis. However, since length of stay in hospital is strongly associated with WFNS score,[26] we considered

Appendix A

using it as a surrogate marker of the WFNS score (n = 551). Length of stay was not a significant predictor of psychomotor reaction time (p = 0.071) or employment status (p = 0.053), controlling for age, Townsend score, sex, relevant medications, education status and time to follow-up. This is not unexpected as recruitment to the UK Biobank was likely to be biased towards aSAH survivors with a short length of stay and good outcome, due to the need to attend multiple detailed assessments. It should also be noted that aSAH cases who died in the acute phase of their illness would not have been recruited to the UK Biobank study. Hence the findings of this study are most transferable to aSAH survivors who have recovered sufficiently to participate in the UK Biobank study. In the aSAH cohort the year of haemorrhage ranges from 1953 to 2016, and there have been significant changes in the management of aSAH over this time period.[27] As improvement in management over this period may have influenced outcome, a sensitivity analysis was performed. Year of aSAH was not a significant predictor of psychomotor reaction time (p = 0.612) or employment status (p = 0.687). Finally, in the majority of aSAH cases cognitive measures and employment status were only assessed at a single time point, so it was not possible to assess change over time. Future studies to assess the change in these outcome measures over time would provide further insight.

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Declaration of Competing Interest

None.

CRediT authorship contribution statement

Ben Gaastra: Conceptualization, Visualization, Validation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Frederick Ewbank:** Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **William Tapper:** Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **Diederik Bulters:** Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **Ian Galea:** Conceptualization, Visualization, Writing – original draft, Writing – review & editing.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jstrokecerebrovasdis.2021.106184.

References

1. Al-Khindi T, Macdonald RL, Schweizer TA. Cognitive and functional outcome after aneurysmal subarachnoid hemorrhage. Stroke 2010;41:e519-e536. https://doi.org/10.1161/STROKEAHA.110.581975. 2010/07/01.
2. Nussbaum ES, Mikoff N, Paranjape GS. Cognitive deficits among patients surviving aneurysmal subarachnoid hemorrhage. A contemporary systematic review. Br J Neurosurg 2020:1-18. https://doi.org/10.1080/02688697.2020.1859462. 20201221.
3. Pace A, Mitchell S, Casselden E, et al. A subarachnoid haemorrhage-specific outcome tool. Brain 2018. https://doi.org/10.1093/brain/awy003. 2018/02/01.
4. Farrell B, Godwin J, Richards S, et al. The United Kingdom transient ischaemic attack (UK-TIA) aspirin trial: final results. J Neurol Neurosurg Psychiatry 1991;54:1044-1054.
5. Jennett B, Bond M. Assessment of outcome after severe brain damage. Lancet 1975;1:480-484.
6. Kreiter KT, Copeland D, Bernardini GL, et al. Predictors of cognitive dysfunction after subarachnoid hemorrhage. Stroke 2002;33:200-208. https://doi.org/10.1161/hs0102.101080.
7. Quinn AC, Bhargava D, Al-Tamimi YZ, et al. Self-perceived health status following aneurysmal subarachnoid haemorrhage: a cohort study. BMJ Open 2014;4:e003932. https://doi.org/10.1136/bmjopen-2013-003932. 2014/04/03.
8. Passier PE, Visser-Meily JM, Rinkel GJ, et al. Life satisfaction and return to work after aneurysmal subarachnoid hemorrhage. J Stroke Cerebrovasc Dis 2011;20:324-329. https://doi.org/10.1016/j.jstrokecerebrovasdis.2010.02.001. 20100724.
9. Passier PE, Visser-Meily JM, van Zandvoort MJ, et al. Prevalence and determinants of cognitive complaints after aneurysmal subarachnoid hemorrhage. Cerebrovasc Dis 2010;29:557-563. https://doi.org/10.1159/000306642. 20100408.
10. Sudlow C, Gallacher J, Allen N, et al. UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med 2015;12:e1001779. https://doi.org/10.1371/journal.pmed.1001779. 2015/03/31.
11. von Elm E, Altman DG, Egger M, et al. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol 2008;61:344-349. https://doi.org/10.1016/j.jclinepi.2007.11.008.
12. Cullen B, Smith DJ, Deary IJ, et al. The cognitive footprint' of psychiatric and neurological conditions: cross-sectional study in the UK Biobank cohort. Acta Psychiatr Scand 2017;135:593-605. https://doi.org/10.1111/acps.12733. 2017/04/07.
13. by Mackenbach JP, Townsend P, Phillimore P, Beattie A. Health and deprivation: inequality and the North by, 10. Croom Helm Ltd.; 1988. p. 206-207.

14. Nevado-Holgado AJ, Kim CH, Winchester L, et al. Commonly prescribed drugs associate with cognitive function: a cross-sectional study in UK Biobank. BMJ Open 2016;6:e012177. https://doi.org/10.1136/bmjopen-2016-012177. 2016/11/30.
15. Hayes AF. Introduction to mediation, moderation, and conditional process analysis: a regression-based approach. 2nd ed. New York, USA: Guilford Press; 2018. ISBN 9781462534654.
16. VanderWeele TJ. Mediation analysis: a practitioner's guide. Annu Rev Public Health 2016;37:17-32. https://doi.org/10.1146/annurev-publhealth-032315-021402. 2015/11/30.
17. Hütter BO, Gilsbach JM, Kreitschmann I. Quality of life and cognitive deficits after subarachnoid haemorrhage. Br J Neurosurg 1995;9:465-475. https://doi.org/10.1080/02688699550041106.
18. da Costa L, Shah-Basak PP, Dunkley BT, et al. Visual working memory encoding and recognition in good outcome aneurysmal subarachnoid patients. Front Neurol 2018;9:494. https://doi.org/10.3389/fneur.2018.00494. 20180626.
19. Hillis AE, Anderson N, Sampath P, et al. Cognitive impairments after surgical repair of ruptured and unruptured aneurysms. J Neurol Neurosurg Psychiatry 2000;69:608-615. https://doi.org/10.1136/jnnp.69.5.608.
20. Mayer SA, Kreiter KT, Copeland D, et al. Global and domain-specific cognitive impairment and outcome after subarachnoid hemorrhage. Neurology 2002;59:1750-1758. https://doi.org/10.1212/01.wnl.0000035748.91128.c2. 2002/12/11.
21. Haug T, Sorteberg A, Sorteberg W, et al. Cognitive outcome after aneurysmal subarachnoid hemorrhage: time course of recovery and relationship to clinical, radiological, and management parameters. Neurosurgery 2007;60:649-656. https://doi.org/10.1227/01.NEU.0000255414.70807.A0. discussion 656-647.
22. Stienen MN, Smoll NR, Weisshaupt R, et al. Delayed cerebral ischemia predicts neurocognitive impairment following aneurysmal subarachnoid hemorrhage. World Neurosurg 2014;82:e599-e605. https://doi.org/10.1016/j.wneu.2014.05.011. 20140515.
23. Springer MV, Schmidt JM, Wartenberg KE, et al. Predictors of global cognitive impairment 1 year after subarachnoid hemorrhage. Neurosurgery 2009;65:1043-1050. https://doi.org/10.1227/01.NEU.0000359317.15269.20. discussion 1050-1041.
24. Rannikmäe K, Ngoh K, Bush K, et al. Accuracy of identifying incident stroke cases from linked health care data in UK Biobank. Neurology 2020;95:e697-e707. https://doi.org/10.1212/WNL.0000000000009924. 2020/07/02.
25. Jaja BNR, Saposnik G, Lingsma HF, et al. Development and validation of outcome prediction models for aneurysmal subarachnoid haemorrhage: the SAHIT multinational cohort study. BMJ 2018;360:j5745. https://doi.org/10.1136/bmj.j5745.
26. Yousef K, Crago E, Fisher A, et al. 3: grading scales in subarachnoid hemorrhage: which scale to control for when studying outcomes. Crit Care Med 2019;47:2. https://doi.org/10.1097/01.ccm.0000550795.32974.8f.
27. Lovelock CE, Rinkel GJ, Rothwell PM. Time trends in outcome of subarachnoid hemorrhage: population-based study and systematic review. Neurology 2010;74:1494-1501. https://doi.org/10.1212/WNL.0b013e3181dd42b3. 20100407.

A.2.2 Publication 2: Auditory outcome following aneurysmal subarachnoid haemorrhage

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This study is based on data from the UK Biobank. I curated the data and performed all the analyses. I authored the manuscript with input from the co-authors after completion of the first draft.

Specific contribution: Primary role in study design, data curation and analysis. Authored first draft of manuscript.

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Auditory outcome following aneurysmal subarachnoid haemorrhage

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ABSTRACT

Auditory deficits are increasingly recognised following aneurysmal subarachnoid haemorrhage (aSAH) and are thought to be of central rather than peripheral origin. Central hearing impairment, also known as auditory processing disorder (APD), often coexists with cognitive deficits and it is thought that APD has both auditory and cognitive elements. The aim of this study was to assess auditory outcome following aSAH and its relationship with cognition. A retrospective case-controlled study design was employed with aSAH cases and matched controls identified from the UK Biobank. Auditory and cognitive outcomes were assessed using the digit triplet test (DTT) and a test of psychomotor reaction time, respectively. Best DTT score was compared between cases and controls using the *t*-test. A regression-based mediation analysis was performed to assess whether cognition mediated auditory outcome. 270 aSAH patients with auditory outcomes were identified with an average follow-up of 106 months. A matched control cohort of 1080 individuals was also identified. The aSAH cohort had significantly impaired best DTT scores compared to matched controls ($p = 0.002$). Cognition significantly mediated auditory outcome following aSAH, accounting for 9.8% of the hearing impairment after aSAH. In conclusion significant hearing impairment follows aSAH. The deficit is bilateral and non-progressive. There is a link with cognitive deficit, pointing to a central rather than peripheral source, in keeping with an auditory processing disorder. All aSAH patients should be asked about hearing difficulty at follow-up and when present it should be investigated with peripheral and central auditory assessments, as well as cognitive tests.

1. Introduction

Aneurysmal subarachnoid haemorrhage (aSAH) accounts for 5% of all strokes with a case fatality of over 20% [1]. It has a disproportionately high socio-economic burden as it affects younger people than other stroke forms, with most survivors suffering significant neurological sequelae leading to decreased productivity, unemployment and cost to the economy [2]. The morbidity of aSAH includes functional [3], cognitive [4], psychological [5,6] and auditory [7] deficits.

Auditory deficits significantly affect quality of life [8] and are relatively understudied in aSAH. A recent retrospective study involving 212 patients, suggested that 20% of aSAH patients presented with self-reported hearing difficulty post-aSAH [9]. While developing an aSAH specific outcome tool our group found that 21.4% of patients reported new onset hearing difficulty following aSAH [10], corroborating this finding. In a detailed case-control study ($n = 41$), we assessed aSAH patients identifying peripheral hearing loss and/or auditory processing disorder, using pure tone audiometry and a speech-in-noise test. Twenty-three percent of aSAH patients reported new onset hearing difficulty post-aSAH. Some peripheral hearing loss was present after aSAH, but the most striking finding was an auditory processing disorder [7].

The origin of hearing difficulty may be peripheral (if the cause resides within the auditory nerve or the outer/middle/inner ear) or central (when the pathology is within the central nervous system); the latter is known as auditory processing disorder (APD) or a central auditory processing disorder (CAPD) [11]. Individuals with APD characteristically present with listening difficulties (especially greater difficulty with hearing in background noise) yet the pure tone audiogram is normal. The pathology underlying APD may include afferent or efferent pathways of the central auditory nervous system (CANS), as well as other central networks that interact with the CANS ('top down' modulation),

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such as cognition [11]. For this reason, APD may co-exist with cognitive dysfunction. After aSAH, several pathological processes may damage peripheral and central auditory systems such as ischemia, inflammation, oxidative stress and iron deposition. In a study of 21 aSAH patients using magnetic susceptibility mapping of the auditory cortex we showed that all aSAH patients had detectable iron signal in the auditory cortex. This was more striking in those who experienced hearing difficulty, suggesting that the hearing difficulty found in aSAH patients may be mechanistically linked to iron deposition in the auditory cortex [7].

Cognitive deficits are common following aSAH influencing activities of daily living, quality of life and return to work [4]. Multiple domains of cognition are affected following aSAH including psychomotor, executive, visual and verbal memory/function [12]. Cognition is a key modulator of the CANS with cognitive deficits often found alongside APD [13]; it is thought that APD has both auditory and cognitive components [11]. However, no studies have yet simultaneously assessed cognition and auditory processing in aSAH patients to understand the relationship between the two. This has implications for management of APD after aSAH as patients may require both audiological and cognitive strategies. APD often co-exists with cognitive dysfunction [11] and this emphasises the importance of a combined approach when investigating and managing hearing difficulties reported by aSAH patients [7].

We recognised that further research looking at hearing and cognition in aSAH is needed and this would require larger sample sizes. The UK Biobank contains a cohort of aSAH patients and has detailed cognitive and auditory data on a subset of these individuals. The design, data collection and utility has been described in detail elsewhere [14]. We have demonstrated cognitive deficits in UK Biobank participants following aSAH with significant psychomotor deficits following aSAH compared to matched controls[18].

The aims of this study were to: (1) compare auditory outcomes of individuals following aSAH and controls in the UK Biobank to identify evidence of hearing impairment following aSAH (i.e. in a larger sample); (2) assess the relationship between cognitive deficits and hearing impairment after aSAH.

2. Material and methods

This retrospective case-controlled study was conducted using the UK Biobank Resource under application ID 49305, under national REC approval 16/NW/0274 and institutional approval (ERGO 49253). Reporting is in accordance with the STROBE statement for case-controlled studies [15]. The study uses information on 502,490 participants aged 40–69 recruited in the UK with informed consent between 2006 and 2010.

2.1. Auditory outcome

Auditory outcome was assessed in the UK Biobank with a speech-in-noise test, the digit triplet test (DTT) [16], the details of which have been published elsewhere [17]. Briefly, fifteen sets of three monosyllabic digits (e.g. 3-6-2) were presented with each ear tested separately. The digit triplets were presented in a background of noise shaped to match the spectrum of the speech stimuli. Noise levels varied adaptively after each triplet to estimate the signal to noise ratio (SNR) for 50% correct recognition of the three digits. The recognition threshold was taken as the mean SNR for the last eight triplets [16]. The DTT identifies hearing impairment with a view to onward referral for more comprehensive audiological assessment but does not differentiate between peripheral and central origin. The DTT is the only auditory measure done as part of the UK Biobank data collection. A higher (more positive) score represents worse hearing and is reported for each ear (data fields 20019 and 20021). The DTT score was considered as a continuous variable. In this study the best performing ear was used for the analysis in keeping with prior studies using the DTT in the UK Biobank [17].

2.2. Cognitive outcome

Cognitive outcome was assessed using a psychomotor reaction time test (data field 20023) where individuals press a button as soon as matching symbols are displayed on a digital screen. Other cognitive tests were available in the UK Biobank, but aSAH cases and controls significantly differed in psychomotor reaction time only [18].

2.3. aSAH cohort

aSAH cases were identified from the UK Biobank using hospital inpatient data (data fields 41271 and 41270), read code information from primary care data (data field 42040), and self-reported medical conditions (data field 20002) recorded at baseline or subsequent assessment centre visits (see Supplementary Table 1 for ICD 9 and 10 inclusion codes).

For this study cases were included if the DTT was performed subsequent to the date of diagnosis of aSAH. Cases were excluded if there was evidence that the subarachnoid haemorrhage was non-aneurysmal in nature, such as trauma within 30 days of diagnosis of subarachnoid haemorrhage (see Supplementary Table 1 for ICD 9 and 10 exclusion codes).

2.4. Control cohort

A single matched control cohort with DTT recorded at baseline assessment centre visit was identified from the UK Biobank using propensity score matching with a nearest neighbour method and a case: control ratio of 1: 4. The control population was matched according to variables known to influence auditory and cognitive outcomes in the UK Biobank:

1. Age at time of follow-up (data field 21003)
2. Townsend deprivation score (data field 189)
3. Sex (data field 31)
4. Education status dichotomised into individuals holding a college or university degree at time of assessment in the UK Biobank or not (data filed 6138)
5. Presence of medications known to influence psychomotor reaction time in the UK Biobank [19]
6. History of working in a noisy environment dichotomised into present or absent (data field 4825)
7. History of listening to loud music dichotomised into present or absent (data field 4836)

All data was considered as binary for the matching process apart from age and Townsend deprivation score which were considered as continuous. Individuals with missing data were excluded from the pool of individuals available for matching.

2.5. Primary analysis

t-test was used to compare the best DTT scores between cases and controls. In view of the large sample size, the central limit theorem applies allowing for parametric tests of the mean.

2.6. Sensitivity analyses

Both mean and worst DTT scores were compared between cases and controls with the *t*-test. Left and right ear DTT scores were also compared within case and control populations to assess for right ear advantage, which has been described in dichotic hearing tests though not typically seen on monaural hearing tests [20].

A further analysis was performed additionally matching the control population for ethnicity (data field 21000) which has been proposed to influence DTT scores in the UK Biobank [17]. This was conducted as a

sensitivity analysis as there is missing ethnicity data in the aSAH cohort limiting sample size.

Time to follow-up in the UK Biobank is not standardised. A sensitivity analysis was therefore performed to assess whether time to follow-up in the aSAH cohort was a significant predictor of auditory outcome using linear regression with best DTT score as the dependent variable, controlling for the same seven variables as listed above.

In order to assess whether auditory outcome changes over time, the subset of post-aSAH and control participants with DTT assessments at two timepoints were compared to look for change in best DTT score using the paired samples Wilcoxon test. In the aSAH cohort both DTT assessments were after the aSAH.

2.7. Mediation analysis

To investigate whether cognition mediates auditory outcome, as assessed by the DTT, a regression-based mediation analysis was performed using PROCESS [21]. For this analysis aSAH status was considered as the independent variable, best DTT as the dependent variable and psychomotor reaction time as the mediator. Significance of the indirect effect of cognition on auditory outcome was tested using 5000 bootstrapped samples and 95% confidence intervals. Percent mediation was calculated as the indirect effect over total effect.

Analyses were performed in statistical software R (version 3.6.2, R Foundation for Statistical Computing) and SPSS Statistics (version 27.0, IBM Corporation). A p value of <0.05 was considered significant.

3. Results

888 aSAH patients were identified from the UK Biobank, of which 270 individuals had auditory outcome data. See Fig. 1 for flow chart of inclusion of patients and Table 1 for demographics and data availability of aSAH patients. The mean follow-up time was 106 months (range 1–493 months) with 95% of aSAH patients being followed up after 1 year.

160,385 individuals were available in the UK Biobank with auditory outcome data to generate the matched control population. A single matched control population was generated ($n = 1080$) with a standardised mean difference < 0.035 for all variables.

3.1. Hearing impairment after aSAH

The aSAH cohort had significantly impaired best DTT scores compared to the matched controls (aSAH: −6.88, control: −7.38, $t = 3.05$, $p = 0.002$). In multivariable linear regression analysis time to follow-up was not a significant predictor of best DTT score in the aSAH cohort ($p = 0.42$).

In the sensitivity analyses both mean and worst DTT scores were significantly impaired between the aSAH and control cohorts (mean aSAH: −6.10, mean control −6.63, $t = 3.20$, $p = 0.001$; worst aSAH: −5.31, worst control: −5.89, $t = 3.05$, $p = 0.002$). Compared to the control cohort additionally matched for ethnicity the aSAH had significantly impaired best DTT scores (aSAH: −6.94, control: −7.25, $t = 2.03$, $p = 0.043$).

There was no difference in DTT scores between right and left ear in either the aSAH (left: −6.06, right: −6.13, $t = -0.43$, $p = 0.655$) or control cohort (left: −6.66, right: −6.61, $t = 0.78$, $p = 0.433$) suggesting that both ears deteriorate equally following aSAH as opposed to this being a focal unilateral change.

3.2. Hearing impairment after aSAH is fixed at late follow up

Twenty post-aSAH patients and 124 control participants had DTT assessments at two timepoints. In the aSAH cohort these time points were both following aSAH. The average interval between DTT assessment was 71 months for the aSAH cohort and 86 months for the control

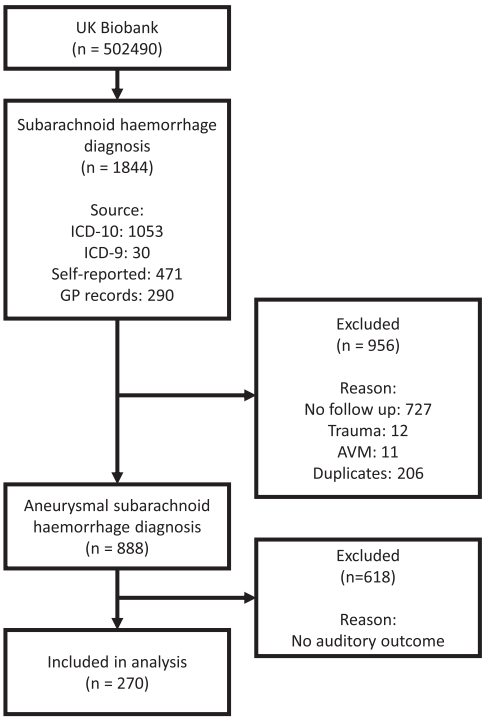

Fig. 1. Flow diagram for identification of aSAH patients from the UK Biobank.

Table 1
Demographics of aSAH patients included in study.

Sample size	270
Age at time of follow-up	
Mean (±SD) years	58 (±7)
Range	40–74
Sex	F: 169 (63%)
	M: 101 (37%)
Source in UK Biobank	
ICD-10	200
ICD-9	0
Self-reported	55
GP records	15
Time to follow-up	
Mean (±SD) months	106 (±95)
Length of stay	
Median (IQR) days	6 (2−13)
Missing	70
Townsend deprivation score	
Mean (±SD)	−1.05 (±2.94)
Education status	
College or university degree (good outcome) (%)	85 (31%)
Medication status	
Medications influencing psychomotor speed test scores (%)	206 (76%)
History of working in a noisy environment (%)	55 (20%)
History of listening to loud music (%)	27 (10%)

SD: standard deviation. IQR: interquartile range. GP: general practitioner. ICD: International Classification of Diseases.

cohort. There was a significant deterioration in best DTT score in both the aSAH (mean first DTT: −7.90, mean second DTT: −6.28, $p = 0.001$) and the control cohort (mean first DTT: −7.53, mean second DTT: −6.31, $p < 0.001$). There was no significant difference in change in DTT score over time between the aSAH and control cohorts ($p = 0.97$).

3.3. Cognition and hearing

Psychomotor reaction time was significantly slowed in the aSAH compared to the control cohort (aSAH: 589 ms, control: 566 ms, $t = 2.22$, $p = 0.027$). In the mediation analysis the odds ratio of the direct effect of case/control status on best DTT score was 0.46 (95% confidence interval 0.21–0.71). The indirect effect mediated by psychomotor reaction time was significant with an effect size of 0.05 (95% confidence interval 0.009–0.096) (see Fig. 2). Cognition significantly mediated auditory outcome following aSAH, accounting for 9.8% of the hearing impairment after aSAH.

aSAH patients with severe auditory and cognitive deficits were identified if their DTT score and psychomotor reaction time were greater than two standard deviations above the mean of the control cohort (Table 2). Of the 21 aSAH patients with very poor auditory outcome, only two patients (10%) had pronounced cognitive issues as measured with psychomotor reaction time.

4. Discussion

In this large study of auditory outcome following aSAH, we confirm the presence of significant hearing impairment following aSAH, compared to matched controls, at an average follow-up of 106 months. We also demonstrate that cognitive deficits following aSAH, as measured by psychomotor reaction time, mediate a significant proportion of this hearing impairment.

Although the DTT is unable to differentiate between central and peripheral hearing impairment the fact that cognitive deficits mediate a significant proportion supports the previous findings that central origin hearing impairment plays an integral role in hearing deficits following aSAH [7]. The addition of PTA and brainstem auditory evoked responses would provide information on the relative contributions of peripheral and central hearing impairment following aSAH, however, it is not available in the UK Biobank. This is a limitation of this retrospective study. PTA and brainstem auditory evoked responses should be included in future prospective studies to further investigate the role of central and peripheral components to hearing impairment following aSAH. As stated earlier, the DTT identifies hearing impairment with the view of onward referral for more comprehensive audiological assessment. In addition to PTA, tests of APD and other more 'real world' measures including roving speech in multi-speaker babble, localisation and tracking of speech using a 180 or 360 degree rig, as well as a test of listening effort should be considered.

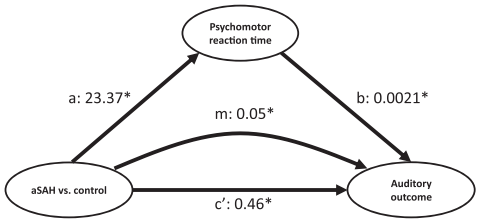

Fig. 2. Mediation analysis. Effect sizes (regression coefficients, β) reported for a: dependent variable on mediator; b: mediator on dependent variable, controlling for independent variable; c': independent variable on dependent variable; m: indirect effect of the independent variable on the dependent variable, via the mediator. For the analysis aSAH case and control were coded as 1 and 0 respectively. * indicates $p < 0.05$.

Table 2
Frequency of severe cognitive and auditory deficits in the aSAH cohort.

	Best DTT normal (n = 249)	Best DTT >2 SD from mean of controls (n = 21) (DTT score > −4.07)
Psychomotor reaction time normal	222 (89%)	19 (90%)
Psychomotor reaction time normal >2 SD from mean of controls (reaction time > 792 ms)	22 (9%)	2 (10%)
Missing	5 (2%)	0 (0%)

SD: standard deviation, DTT: digit triplet test.

Cognitive deficits mediate a significant proportion of hearing impairment following aSAH with psychomotor reaction time significantly slower in the aSAH cohort compared to controls. However, the prevalence of cognitive impairment in aSAH patients with pronounced hearing difficulty was relatively low (10%, and quite similar to patients without pronounced hearing difficulty, see Table 2), suggesting that although cognition is an important contributor, other mechanisms play an integral role in hearing impairment after aSAH. A number of pathophysiological mechanisms have been proposed to underlie auditory deficits following aSAH including ischemia secondary to vasospasm [22] and more globalised pathology such as iron deposition in the cortex secondary to blood breakdown [7]. In this study we demonstrate that both ears deteriorate equally following aSAH supporting the theory that a more generalised process such as iron deposition is likely to be integral to hearing impairment following aSAH. Iron deposition can lead to both central and peripheral hearing deficits [23] and we are not able to differentiate between the two in this study.

Our previous study using both PTA and the Bamford-Kowal-Bench (BKB) speech-in-noise test demonstrated the presence of APD or central hearing impairment following aSAH [7]. The BKB test used had a fixed signal-to-noise ratio compared to an adaptive signal-to-noise ratio as in the DTT used here. The adaptive signal-to-noise ratio is thought to be more reflective of the real world auditory environment and consequently the results of this study confirm previous findings in a situation more comparable to real life emphasising the implications of these deficits to patients following aSAH.

A number of cognitive domains have been associated with APD including memory, language executive function and fluid reasoning [11,24]. In this study we have used a measure of psychomotor reaction time as the cognitive outcome as this is the only cognitive domain in the UK Biobank which has been shown to differ between aSAH cases and controls [18]. Although this cognitive measure has been shown to correlate with deterioration in DTT [25], it may not be the most sensitive cognitive assessment to identify a significant relationship between cognitive deficits and hearing impairment following aSAH. Alternative cognitive tests may demonstrate a stronger association between cognition and auditory outcomes following aSAH and future studies should consider a comprehensive battery of cognitive assessments covering a range of cognitive domains to further explore this.

The World Federation of Neurological Surgeons (WFNS) grade is the strongest known predictor of clinical outcome following aSAH [26]. WFNS is not available in the UK Biobank, however, a subset of patients have length of stay (LOS) data which is strongly associated with WFNS [27]. When LOS is included as a predictor of best DTT score using linear regression including the other covariates controlled for above it is not a significant predictor of auditory outcome ($p = 0.315$). This is in keeping with our previous study which did not identify WFNS as a significant predictor of BKB or PTA [7]. Consequently, the lack of WFNS data is unlikely to influence the outcome of this study. Hearing aid use is available in the UK Biobank and 7 individuals in the aSAH cohort reported using a hearing aid (2.6%). When hearing aid use is included in a linear regression controlling for all other covariates it is not a significant

predictor of auditory outcome ($p = 0.525$) and therefore unlikely to influence the results of this analysis.

Repeat DTT scores post-aSAH are only available for a small subset of the aSAH patients in the UK Biobank and demonstrate a deterioration in DTT over time following aSAH. This deterioration is not limited to the aSAH cohort with the same deterioration identified in the control cohort. The auditory deficit after aSAH therefore appears fixed, without recovery or progression, at late follow up (90% of aSAH cases with repeat DTT had both tests more than 1 year after aSAH). This contrasts with Kang who reported that 5 out of 8 cases of hearing impairment following aSAH completely normalised at 3–6 months [28]. This difference is likely explained by the much longer interval between DTT assessments in our aSAH cohort (mean 71 months) suggesting that the progressive deterioration is not a result of aSAH but rather more likely associated with aging (presbycusis). An age-related deterioration has been shown previously, specifically in speech-in-noise tests such as the DTT [29]. Future studies should include serial auditory assessments to allow more detailed evaluation of how auditory outcomes change over time.

4.1. Clinical implications

Hearing impairment has been shown to significantly impair quality of life [8]. The results of this study emphasise that aSAH patients should be asked about hearing difficulty during follow-up and those reporting hearing difficulty should be assessed using tests of both peripheral and central hearing, in addition to cognitive assessments.

APD after aSAH may require both audiological (assistive listening devices, listening environment modification, auditory training and compensatory strategies) [11] and cognitive rehabilitation. In addition, hearing aids should be considered for those identified with peripheral hearing loss. APD often co-exists with cognitive dysfunction [11] and this emphasises the importance of a combined approach when investigating and managing hearing difficulties reported by aSAH patients [7].

Cognitive assessment is essential given cognitive deficits contribute to hearing impairment post-aSAH. This is important given cognitive training can improve auditory outcomes and quality of life [30], and therefore should be considered in the management of hearing impairment. Additionally, cognitive assessment in conjunction with audiological assessment is beneficial as both cognitive dysfunction and hearing impairment may be over diagnosed when tested in isolation, resulting in less appropriate interventions [11].

5. Conclusion

Significant hearing impairment follows aSAH. The deficit is bilateral and non-progressive. There is a link with cognitive deficit, pointing to a central rather than peripheral source, in keeping with an auditory processing disorder. All aSAH patients should be asked about hearing difficulty at follow-up and when present it should be investigated with peripheral and central auditory assessments, as well as cognitive tests.

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Availability of data and material

The data that support the findings of this study are available from the UK Biobank (https://www.ukbiobank.ac.uk) by application.

Authors' contributions

IG and NC conceived the study. All authors contributed to the study design. The first draft of the manuscript was written by BG, IG and NC, all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approvals

The study was performed under National Research Ethics Committee Approval 16/NW/0274 and institutional approval (ERGO 49253).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

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Conflict of interest

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2021.120125.

References

[1] B. Schatlo, et al., Incidence and outcome of aneurysmal subarachnoid hemorrhage: the Swiss study on subarachnoid hemorrhage (Swiss SOS) (in eng), Stroke 52 (1) (Jan 2021) 344–347, https://doi.org/10.1161/STROKEAHA.120.029538.
[2] O. Rivero-Arias, A. Gray, J. Wolstenholme, Burden of disease and costs of aneurysmal subarachnoid haemorrhage (aSAH) in the United Kingdom (in eng), Cost Eff Resour Alloc 8 (Apr 2010) 6, https://doi.org/10.1186/1478-7547-8-6.
[3] Y. Takemoto, Y. Hasegawa, A. Hashiguchi, K. Moroki, H. Tokuda, A. Mukasa, Predictors for functional outcome in patients with aneurysmal subarachnoid hemorrhage who completed in-hospital rehabilitation in a single institution (in eng), J. Stroke Cerebrovasc. Dis. 28 (7) (Jul 2019) 1943–1950, https://doi.org/10.1016/j.jstrokecerebrovasdis.2019.03.042.
[4] T. Al-Khindi, R.L. Macdonald, T.A. Schweizer, Cognitive and functional outcome after aneurysmal subarachnoid hemorrhage (in eng), Stroke 41 (8) (Aug 2010) e519–e536, https://doi.org/10.1161/STROKEAHA.110.581975.
[5] B.O. Hütter, I. Kreitschmann-Andermahr, Subarachnoid hemorrhage as a psychological trauma (in eng), J. Neurosurg. 120 (4) (Apr 2014) 923–930, https://doi.org/10.3171/2013.11.JNS121552.
[6] J.M. Visser-Meily, M.L. Rhebergen, G.J. Rinkel, M.J. van Zandvoort, M.W. Post, Long-term health-related quality of life after aneurysmal subarachnoid hemorrhage: relationship with psychological symptoms and personality characteristics (in eng), Stroke 40 (4) (Apr 2009) 1526–1529, https://doi.org/10.1161/STROKEAHA.108.531277.
[7] N. Campbell, et al., Hearing impairment after subarachnoid hemorrhage (in eng), Ann Clin Transl Neurol 6 (3) (Mar 2019) 420–430, https://doi.org/10.1002/acn3.714.
[8] E.M. Chia, J.J. Wang, E. Rochtchina, R.R. Cumming, P. Newall, P. Mitchell, Hearing impairment and health-related quality of life: the Blue Mountains Hearing Study (in eng), Ear Hear. 28 (2) (Apr 2007) 187–195, https://doi.org/10.1097/AUD.0b013e31803126b6.
[9] E.M. Vos, P. Greebe, J.M.A. Visser-Meily, G.J.E. Rinkel, M.D.I. Vergouwen, Subjective hearing impairment after subarachnoid haemorrhage: prevalence and risk factors (in eng), J. Neurol. Sci. 372 (Jan 2017) 184–186, https://doi.org/10.1016/j.jns.2016.11.062.
[10] A. Pace, et al., A subarachnoid haemorrhage-specific outcome tool (in eng), Brain (Feb 2018), https://doi.org/10.1093/brain/awy003.

Appendix A

[11] British Society of Audiology Position Statement and Practice Guidance Auditory Processing Disorder (APD). http://www.thebsa.org.uk/wp-content/uploads/2018/09/Position-Statement-and-Practice-Guidance-APD-2018.pdf, 2018.
[12] T. Haug, A. Sorteberg, W. Sorteberg, K.F. Lindegaard, T. Lundar, A. Finset, Cognitive outcome after aneurysmal subarachnoid hemorrhage: time course of recovery and relationship to clinical, radiological, and management parameters (in eng), Neurosurgery 60 (4) (Apr 2007) 649–656. discussion 656–7, https://doi.org/10.1227/01.NEU.0000255414.70807.A0.
[13] S.E. Fulton, J.J. Lister, A.L. Bush, J.D. Edwards, R. Andel, Mechanisms of the hearing-cognition relationship (in eng), Semin. Hear. 36 (3) (Aug 2015) 140–149, https://doi.org/10.1055/s-0035-1555117.
[14] C. Sudlow, et al., UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age (in eng), PLoS Med. 12 (3) (Mar 2015), e1001779, https://doi.org/10.1371/journal.pmed.1001779.
[15] E. von Elm, et al., The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies (in eng), J. Clin. Epidemiol. 61 (4) (Apr 2008) 344–349, https://doi.org/10.1016/j.jclinepi.2007.11.008.
[16] C. Smits, T.S. Kapteyn, T. Houtgast, Development and validation of an automatic speech-in-noise screening test by telephone (in eng), Int. J. Audiol. 43 (1) (Jan 2004) 15–28, https://doi.org/10.1080/14992020400050004.
[17] P. Dawes, et al., Hearing in middle age: a population snapshot of 40- to 69-year olds in the United Kingdom (in eng), Ear Hear 35 (3) (May–Jun 2014), https://doi.org/10.1097/AUD.0000000000000010. pp. e44–51.
[18] B. Gaastra, F. Ewbank, W. Tapper, D. Bulters, I. Galea, Long-term cognitive outcome following aneurysmal subarachnoid Haemorrhage, J. Stroke Cerebrovasc. Dis. 31 (1) (2022), https://doi.org/10.1016/j.jstrokecerebrovasdis.2021.106184, p. 106184, 2022/01/01/.
[19] A.J. Nevado-Holgado, C.H. Kim, L. Winchester, J. Gallacher, S. Lovestone, Commonly prescribed drugs associate with cognitive function: a cross-sectional study in UK Biobank (in eng), BMJ Open 6 (11) (Nov. 2016), https://doi.org/10.1136/bmjopen-2016-012177. p. e012177.
[20] J. Jerger, J. Martin, Hemispheric asymmetry of the right ear advantage in dichotic listening (in eng), Hear. Res. 198 (1–2) (Dec 2004) 125–136, https://doi.org/10.1016/j.heares.2004.07.019.
[21] A.F. Hayes, T.D. Little, Introduction to Mediation, Moderation, and Conditional Process Analysis: A Regression-Based Approach. http://www.dawsonera.com/depp/reader/protected/external/AbstractView/S9781462534678.
[22] E. Ponzetto, et al., Partly reversible central auditory dysfunction induced by cerebral vasospasm after subarachnoid hemorrhage (in eng), J. Neurosurg. 119 (5) (Nov 2013) 1125–1128, https://doi.org/10.3171/2013.7.JNS13674.
[23] A. Yoo, J. Jou, J.D. Klopfenstein, J.C. Kattah, Focused neuro-otological review of superficial siderosis of the central nervous system (in eng), Front. Neurol. 9 (2018) 358, https://doi.org/10.3389/fneur.2018.00358.
[24] S. Cameron, H. Glyde, H. Dillon, A. King, K. Gillies, Results from a National Central Auditory Processing Disorder Service: a real-world assessment of diagnostic practices and remediation for central auditory processing disorder (in eng), Semin. Hear. 36 (4) (Nov 2015) 216–236, https://doi.org/10.1055/s-0035-1564457.
[25] D.R. Moore, et al., Relation between speech-in-noise threshold, hearing loss and cognition from 40-69 years of age (in eng), PLoS One 9 (9) (2014), e107720, https://doi.org/10.1371/journal.pone.0107720.
[26] B.N.R. Jaja, et al., Development and validation of outcome prediction models for aneurysmal subarachnoid haemorrhage: the SAHIT multinational cohort study, BMJ 360 (Jan 18, 2018), https://doi.org/10.1136/bmj.j5745 p. j5745.
[27] K. Yousef, E. Crago, A. Fisher, K. Mahmoud, T. Lagattuta, M. Hravnak, 3: Grading scales in subarachnoid hemorrhage: which scale to control for when studying outcomes, Crit. Care Med. 47 (1) (2019) 2, https://doi.org/10.1097/01.ccm.0000550795.32974.8f.
[28] S. Kang, Hearing loss after surgery of ruptured cerebral aneurysm (in eng), Br. J. Neurosurg. 15 (3) (Jun 2001) 258–263, https://doi.org/10.1080/02688690175035371 9.
[29] A. Heidari, A. Moossavi, F. Yadegari, E. Bakhshi, M. Ahadi, Effects of age on speech-in-noise identification: subjective ratings of hearing difficulties and encoding of fundamental frequency in older adults (in eng), J Audiol Otol 22 (3) (Jun 2018) 134–139, https://doi.org/10.7874/jao.2017.00304.
[30] J.L. O'Brien, J.J. Lister, B.A. Fausto, G.K. Clifton, J.D. Edwards, Cognitive training enhances auditory attention efficiency in older adults (in eng), Front. Aging Neurosci. 9 (2017) 322, https://doi.org/10.3389/fnagi.2017.00322.

A.2.3 Publication 3: Duration and characteristics of persistent headache following aneurysmal subarachnoid hemorrhage

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This study is based on data from the UK Biobank. The study was originally conceived as a research project for Harry Carmichael as part of his medical degree, which I co-supervised. I subsequently further developed the concept, curated the data and performed all the analyses included in this manuscript. Alongside Harry Carmichael I authored the manuscript with input from the co-authors after completion of the first draft.

Specific contribution: Primary role in study design, data curation and analysis. Authored first draft of manuscript with Harry Carmichael.

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RESEARCH SUBMISSIONS

Duration and characteristics of persistent headache following aneurysmal subarachnoid hemorrhage

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Abstract

Objective: To assess the long-term frequency, prognosis, and phenotype of persistent headache following aneurysmal subarachnoid hemorrhage (aSAH).

Background: Very little is known about long-term headache following aSAH with no studies looking beyond 3 years.

Methods: Retrospective analysis comparing aSAH cases to matched controls in the UK Biobank, a prospective cohort study. Headache frequency and phenotype were compared using group comparison tests. The relationship between headache frequency and time was assessed using correlation analysis.

Results: Headache was more frequent following aSAH (aSAH: 258/864 [29.9%] vs. controls: 666/3456 [19.3%], $\chi^2 = 45.5$, $p < 0.001$) at a median follow-up of 7.5 years. Headache frequency decreased over time ($R_S = -0.71$, $p = 0.028$), affecting 29/58 (50%) patients in the first year and reducing to 13/47 (28%) patients 10 years later. Headache frequency was not related to aSAH severity ($z = 0.249$, $p = 0.803$), treatment ($z = 0.583$, $p = 0.560$), or hydrocephalus ($z = -1.244$, $p = 0.214$). There was a consistently higher frequency of migrainous features following aSAH compared to controls, although this did not reach statistical significance.

Conclusions: Persistent headache is more frequent following aSAH compared to controls in the long term and the prevalence reduces gradually over time. The increased frequency of migrainous features suggests that selected patients with post-aSAH headache may benefit from migraine treatment.

KEYWORDS
headache, migraine, outcome, subarachnoid hemorrhage

INTRODUCTION

Aneurysmal subarachnoid hemorrhage (aSAH) is a rare but devastating form of stroke which most commonly presents with headache. The International Classification of Headache Disorders, 3rd edition (ICHD-3)[1] classifies aSAH-related headache as either acute (ICHD-3 6.2.2: Acute headache attributed to non-traumatic subarachnoid hemorrhage) or chronic (ICHD-3 6.2.4.2: Persistent headache

Abbreviations: aSAH, aneurysmal subarachnoid hemorrhage; CSD, cortical spreading depression; ICHD-3, International Classification of Headache Disorders, 3rd edition; QoL, quality of life; WFNS, World Federation of Neurological Surgeons.

Ian Galea and Diederik Bulters are joint senior authors.

attributed to past non-traumatic subarachnoid hemorrhage, which occurs when the headache persists for >3 months).

In the only cohort study of persistent headache after aSAH (n = 217), the ictal headache improved continuously over a 12-month period.[2] Two further cross-sectional studies reported that 32.3% (n = 405) and 40.9% (n = 93) of aSAH cases had headache at 12 and 32.6 months, respectively, with significant negative impact on quality of life (QoL).[3,4] Beyond these studies, the phenotype, chronicity, and prognosis of persistent headache following aSAH remain poorly defined.

The pathophysiological mechanisms underlying persistent headache following aSAH are not well understood. Potential mechanisms contributing to headache include direct stretch and chemical irritation of the meninges by blood, a neuro-inflammatory response,[5] and vascular hyperreactivity including vasospasm.[6] Cortical spreading depression (CSD) is linked to migraine aura[7] and has been demonstrated following aSAH.[8] As CSD has been implicated in both migraine and aSAH, with evidence that it may play a role in initiating headache,[7] we hypothesized that persistent headache following aSAH may have migrainous features.

The aims of this study were to:

1. Compare the frequency of persistent headache between aSAH and controls at long-term follow-up
2. Assess whether and how post-aSAH headache resolves over time
3. Establish the prevalence of migrainous features in the phenotype of persistent headache following aSAH.

METHODS

This was a retrospective analysis of cases and matched controls using data from the UK Biobank, a prospective cohort study (application ID 49305). The UK Biobank is a major biomedical database[9] which recruited 502,497 participants aged 40–69 with informed written consent between 2006 and 2010. Participants attend an initial assessment center visit and were then followed up in person at assessment centers and using multiple online questionnaires. The UK Biobank also obtains data from regular health care record searches dating from before recruitment to the present day. The study is reported according to the STROBE statement and has UK national REC (16/NW/0274) and institutional (University of Southapmton, ERGO 49253) ethical approval.

aSAH and control individuals

aSAH cases were identified from the UK Biobank using ICD-9 (data field 41271) and ICD-10 (data field 41270) codes, self-reported medical conditions (data field 20002), and primary care data (data field 42040). Consequently, the aSAH could have occurred before or after initial recruitment to the UK Biobank. Individuals were excluded if the subarachnoid hemorrhage was non-aneurysmal in nature or if they had any traumatic injury within 30 days before/after diagnosis of aSAH (see Table S1 in the Supporting Information for inclusion/exclusion codes). Individuals were included if they had data available on headache outcome (see below).

A single matched control population was generated from the UK Biobank using propensity score matching with a nearest neighbor method and a case:control ratio of 1:4. Control individuals were matched according to variables known to influence headache following aSAH: sex and age at time of follow-up. Individuals missing headache outcome, age, or sex data were excluded from matching.

Headache outcome

The question "In the last month have you experienced headache that interfered with usual activity?" (data field 6159, Table S2) was used to assess headache frequency at the first assessment center visit following diagnosis of aSAH. Individuals who answered "yes" were subsequently asked whether they had headache for greater than 3 months (data field 3799).

Migrainous features were assessed in a subset of the above participants who had completed a separate online follow-up questionnaire focusing on pain (Table S2). Migraine-type headache was defined as the presence of at least two of the following features: unilateral, throbbing, and moderate-to-severe and worse on exertion (data fields 120058-61), plus at least one feature among nausea, photophobia, and phonophobia (data fields 120062-64). Individual migrainous features were dichotomized with the feature considered present if reported for either "half the time or more" or "less than half the time," and absent if the feature was "rarely" or "never" present. Aura was defined as the presence of spreading visual or sensory symptoms preceding or near headache onset (data fields 120065-68), and prodrome was defined as tiredness, yawning, concentration problems, changes in mood or appetite, irritability, neck stiffness, light or sound sensitivity before or near the onset of headaches (data field 120069). All headache outcomes were considered as binary variables for analysis.

Statistical analyses

Descriptive statistics of the cohorts were reported using percentages, means (± standard deviation), and medians with interquartile range. The mean was reported when there was no evidence of significant outliers on a histogram; otherwise, the median was reported. Two-tailed hypothesis testing was performed. The chi-square test was used to compare binary outcomes and Spearman's rank correlation coefficient used to assess the relationship between headache frequency and time. A p value of <0.05 was considered significant with Bonferroni correction for multiple testing applied separately to headache frequency and phenotype. All analyses were performed in R (R Foundation for Statistical Computing).

RESULTS

A total of 869 aSAH cases were identified of which 864 had headache frequency data available, and were matched with 3456 control individuals (see Figure 1 and Table S3 for demographics).

Headache frequency

More aSAH cases reported headache in the last month that interfered with usual activities compared to the matched control cohort (aSAH: 258/864 [29.9%] vs. controls: 666/3456 [19.3%], $\chi^2 = 45.5$, $p < 0.001$; Table 1A) at a median follow-up of 7.5 years. Of these individuals who reported headache, significantly more aSAH cases reported that the headache lasted for more than 3 months (aSAH: 159/252 [63.1%] vs. controls: 302/642 [47.0%], $\chi^2 = 18.0$, $p < 0.001$; Table 1A). Within the aSAH cases, length of stay (a surrogate marker[10] of the World Federation of Neurological Surgeons' [WFNS] grade which is not available in the UK Biobank), aneurysm treatment (endovascular/surgical), and hydrocephalus (a known cause of post-ictal headache) were not significant predictors of headache following aSAH in logistic regression modeling ($z = 0.249$, $p = 0.803$; $z = 0.583$, $p = 0.560$; and $z = -1.244$, $p = 0.214$, respectively).

Headache time course

When patients were binned into annual groups according to time of follow-up after aSAH the frequency of headache negatively correlated with time, $R_S = -0.71$ ($p = 0.028$; Figure 2A and Table S4), decreasing

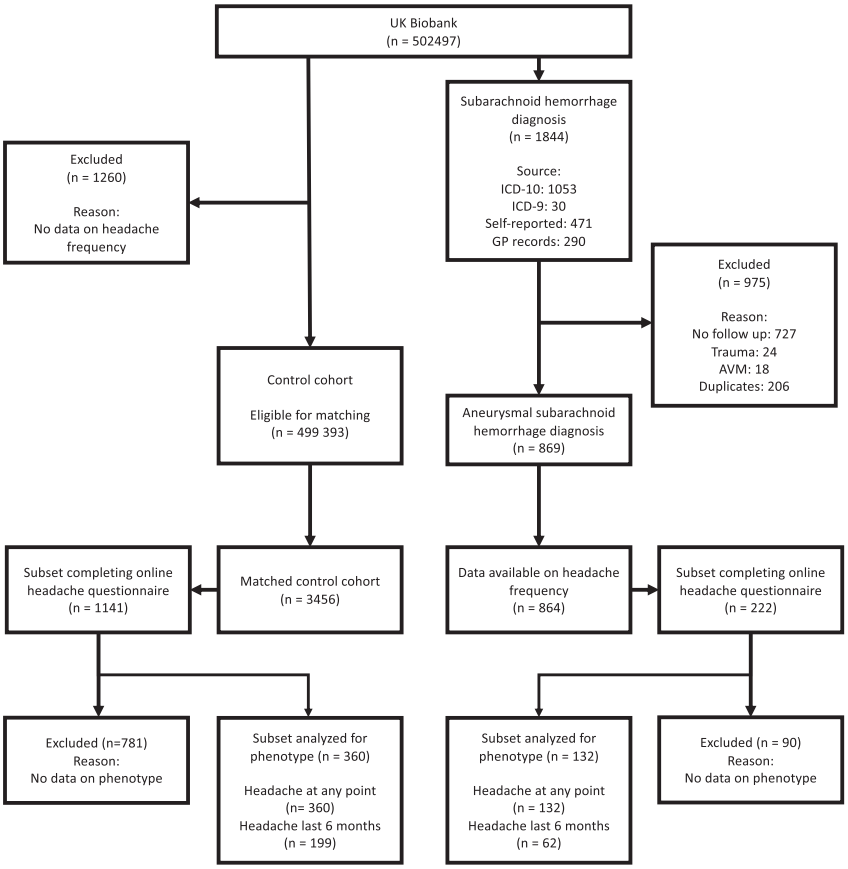


FIGURE 1 Flow chart for inclusion of aneurysmal subarachnoid hemorrhage and control cohorts from the UK Biobank. Mean standard difference following matching was zero. AVM, arteriovenous malformation.

TABLE 1 Comparison of frequency of headache, migraine, aura, and prodrome between aSAH and control cohorts.

		aSAH cohort ($n = 864$)	Control cohort ($n = 3456$)	χ^2	p value
Headache frequency	**A. In the whole study population**				
	Headache in last month that interfered with your usual activities (data field 6159)	258/864 (29.9%)	666/3456 (19.3%)	45.5	<0.001*
	Headache for > 3 months (data field 3799)	159/252 (63.1%)[a]	302/642 (47.0%)[a]	18.0	<0.001*
Headache phenotype	**B. In those reporting a headache at any time**				
	Migraine-type headache	84/132 (63.6%)	199/359 (55.4%)	2.34	0.127
	C. In those reporting a headache in the last 6 months				
	Migraine-type headache	42/62 (68%)	118/199 (59.3%)	1.09	0.297
	Aura	31/62 (50%)	86/199 (43.2%)	0.63	0.428
	Prodrome	43/62 (69%)	128/198 (64.6%)[b]	0.28	0.597
	Prodrome or aura	48/62 (77%)	146/199 (73.4%)	0.22	0.637
	D. In those reporting a migraine-type headache in the last 6 months				
	Aura	25/42 (59%)	65/118 (55.1%)	0.10	0.751
	Prodrome	34/42 (81%)	86/117 (73.5%)[b]	0.57	0.451
	Prodrome or aura	37/42 (88%)	96/118 (81.4%)	0.58	0.446

Note: Migraine type headache was defined as the presence of at least two of the three features of unilateral, throbbing, and moderate-to-severe and worse on exertion, plus at least one feature among nausea, photophobia, and phonophobia. Aura was defined as the presence of spreading visual or sensory symptoms preceding or near headache onset, and prodrome was defined as tiredness, yawning, concentration problems, changes in mood or appetite, irritability, neck stiffness, light or sound sensitivity before or near the onset of headaches. Bonferroni correction was applied separately to headache frequency and phenotype domains with the p value threshold of significance as <0.025 (0.05/2) and $<6.25\times10^{-3}$ (0.05/8), respectively.

[a]There were 6 aSAH cases and 24 control individuals with missing data for determining prodrome status.

[b]1 individual was missing data on prodrome.

*$p < 0.02$.

from 29/58 (50%) in the first year to 13/47 (28%) 10 years later. The mean age at follow-up of aSAH cases increased form 55.9 years to 59.3 years over these 10 annual groups. Twenty-one individuals with post-aSAH headache had data on headache frequency at two time-points. Headache resolved in 9/21 (43%) individuals (Figure 2B); the median time to recovery of headache was 149 months from aSAH.

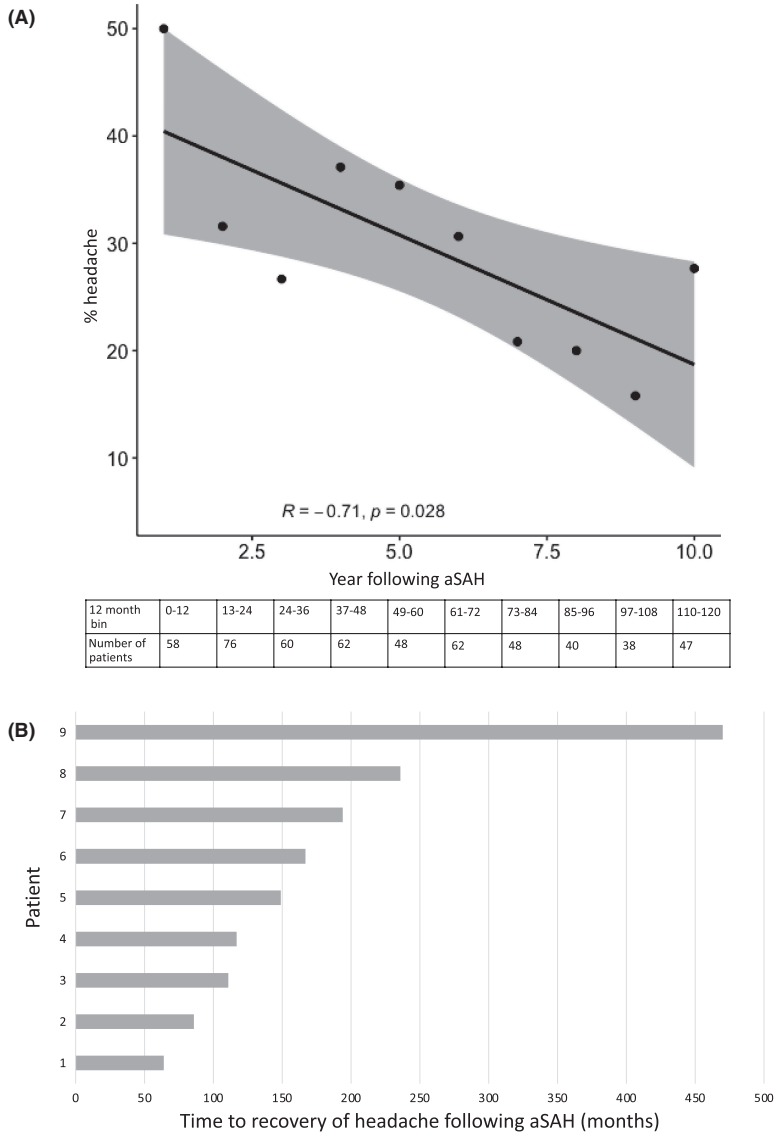
Headache phenotype

A total of 132 aSAH cases and 359 controls had data on headache phenotype from the online questionnaire (Figure 1). Migraine-type headache was more frequent in aSAH cases versus controls, among those with a headache at any time (Table 1B). When limiting the same analysis to those who reported headache in the last 6 months, migraine-type headache was also more frequent after aSAH (Table 1C). There was also a consistent higher frequency of aura and/or prodrome in aSAH cases compared to controls in those with any type of headache (Table 1C), as well as those with a migraine-type headache (Table 1D), in the last 6 months.

DISCUSSION

Headache interfering with usual activity and impairing QoL is more common following aSAH compared to controls, at a median of 7.5 years, and is more likely to be chronic in nature (lasting more than 3 months). The only previous study of headache late after aSAH showed that up to 40.9% of individuals report headache, negatively impacting QoL, at 32.6 months.[4] Our study builds on this by increasing both the sample size and duration of follow up. Headache becomes less prevalent with time, affecting 50% in the first year post-aSAH, but reducing to 28% 10 years later. This reduction likely represents resolution of headache over time and decreasing frequency of headache with older age following aSAH.[11] In the subset of aSAH patients with repeated headache outcome measures, 9/21 (43%) showed complete recovery of headache at a median follow-up of 149 months. Taken together these results are consistent with previous studies which show improvement in headache over time.[2] These findings will help with setting patient expectations and emphasize the importance of understanding and managing headache symptoms in the long term as they can take many years to resolve.

Migraine-type headache was more common following aSAH compared to controls. Aura and/or prodrome were also more common regardless of whether the reported headache was migraine-type or not. CSD occurs in migraine aura, may play a role in the initiation of headache,[7] and has been demonstrated in the brain following aSAH.[8] As aura is a dominant feature in headache following aSAH it suggests CSD may play a role in the pathophysiology of persistent headache following aSAH. This has potential therapeutic implications as standard pharmacological management such as acetaminophen ± opioids for post-aSAH headache does not significantly



12 month bin	0-12	13-24	24-36	37-48	49-60	61-72	73-84	85-96	97-108	110-120
Number of patients	58	76	60	62	48	62	48	40	38	47

FIGURE 2 (A) Change in frequency of headache over time, divided into 12 month bins up to 10 years following aneurysmal subarachnoid hemorrhage (aSAH). (B) Time to recovery of persistent post-aSAH headache, for the 9 out of 21 individuals who were assessed at least twice in the UK Biobank allowing recovery to be recorded.

improve the headache.[12] Additional therapies such as magnesium have been shown to reduce headache pain scores although the change was not clinically significant.[13,14] Migraine preventative treatments, including those which have been shown to influence CSD in migraine, may therefore offer superior analgesia for persistent headache following aSAH.

Limitations

In the UK Biobank information on a number of factors which may be relevant to headache following aSAH is absent, including WFNS grade, presence of preexisting headache/migraine, and radiological severity of aSAH. In addition, this study does not use validated headache questionnaires which may limit the translation of these results to external datasets. Future studies should include the missing variables and utilize a validated headache questionnaire when analyzing persistent headache after aSAH.

The UK Biobank and this study are biased towards individuals with good outcome as participants are required to attend detailed assessment center visits following aSAH, favoring better performing individuals with fewer neurological sequelae. In addition, in this study only a subset of individuals attended assessment centers twice after aSAH or completed an online questionnaire, which again is likely to select for individuals with better outcome, introducing selection bias into the headache time course and phenotype analysis. The smaller sample size in the phenotype analysis also limits the power of this study to compare phenotype between cases and controls. Selection bias is a feature of the UK Biobank but is not necessarily a major limitation of this study. Anecdotally, patients with the best clinical outcome following aSAH are more likely to be bothered by symptoms such as headache in comparison to poorer outcome individuals who are often preoccupied with more severe neurological deficits. Therefore, the information generated by this study is most likely to be applied to individuals with better outcome, as included in this study. Future prospective studies should include larger sample sizes and multiple serial time points to provide greater insight into persistent headache after aSAH.

CONCLUSIONS

Persistent headache occurs in the long term following aSAH. Although it may last for years, it gradually improves over time. In some cases, the post-aSAH headache has migrainous features and while underpowered, this study has provided the data needed for larger prospective studies to confirm these findings and to warrant clinical trials of migraine treatment for selected patients with a post-aSAH headache with migrainous features.

AUTHOR CONTRIBUTIONS

Study concept and design: Ben Gaastra, Harry Carmichael, Ian Galea, Diederik Bulters. *Analysis and interpretation of data*: Ben Gaastra, Harry Carmichael, Ian Galea, Diederik Bulters. *Drafting of the manuscript*: Ben Gaastra, Harry Carmichael. *Revising it for intellectual content*: Ben Gaastra, Harry Carmichael, Ian Galea, Diederik Bulters. *Final approval of the completed manuscript*: Ben Gaastra, Harry Carmichael, Ian Galea, Diederik Bulters.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the UK Biobank (https://www.ukbiobank.ac.uk) by application.

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REFERENCES

1. Headache Classification Committee of the International Headache Society (IHS). The International Classification of Headache Disorders, 3rd edition. *Cephalalgia*. 2018;38(1):1-211. doi:10.1177/0333102417738202
2. Hong CK, Joo JY, Kim YB, et al. The course of headache in patients with moderate-to-severe headache due to aneurysmal subarachnoid hemorrhage: a retrospective cross-sectional study. *Headache*. 2015;55(7):992-999. doi:10.1111/head.12612
3. Gerner ST, Reichl J, Custal C, et al. Long-term complications and influence on outcome in patients surviving spontaneous subarachnoid hemorrhage. *Cerebrovasc Dis*. 2020;49(3):307-315. doi:10.1159/000508577
4. Huckhagel T, Klinger R, Schmidt NO, Regelsberger J, Westphal M, Czorlich P. The burden of headache following aneurysmal subarachnoid hemorrhage: a prospective single-center cross-sectional analysis. *Acta Neurochir (Wien)*. 2020;162(4):893-903. doi:10.1007/s00701-020-04235-7
5. Nader ND, Ignatowski TA, Kurek CJ, Knight PR, Spengler RN. Clonidine suppresses plasma and cerebrospinal fluid concentrations of TNF-alpha during the perioperative period. *Anesth Analg*. 2001;93(2):363-369. doi:10.1097/00000539-200108000-00026
6. Calabrese LH, Dodick DW, Schwedt TJ, Singhal AB. Narrative review: reversible cerebral vasoconstriction syndromes. *Ann Intern Med*. 2007;146(1):34-44. doi:10.7326/0003-4819-146-1-200701020-00007
7. Zhang X, Levy D, Noseda R, Kainz V, Jakubowski M, Burstein R. Activation of meningeal nociceptors by cortical spreading depression: implications for migraine with aura. *J Neurosci*. 2010;30(26):8807-8814. doi:10.1523/JNEUROSCI.0511-10.2010
8. Dreier JP, Major S, Manning A, et al. Cortical spreading ischaemia is a novel process involved in ischaemic damage in patients with aneurysmal subarachnoid haemorrhage. *Brain*. 2009;132(Pt 7):1866-1881. doi:10.1093/brain/awp102

9. Sudlow C, Gallacher J, Allen N, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*. 2015;12(3):e1001779. doi:10.1371/journal.pmed.1001779
10. Yousef K, Crago E, Fisher A, Mahmoud K, Lagattuta T, Hravnak M. Grading scales in subarachnoid hemorrhage: which scale to control for when studying outcomes. *Crit Care Med*. 2019;47(1):2. doi:10.1097/01.ccm.0000550795.32974.8f
11. Glisic EK, Gardiner L, Josti L, et al. Inadequacy of headache management after subarachnoid hemorrhage. *Am J Crit Care*. 2016;25(2):136-143. doi:10.4037/ajcc2016486
12. Morad AH, Tamargo RJ, Gottschalk A. The longitudinal course of pain and analgesic therapy following aneurysmal subarachnoid hemorrhage: a cohort study. *Headache*. 2016;56(10):1617-1625. doi:10.1111/head.12908
13. Hile GB, Cook AM. Treatment of headache in aneurysmal subarachnoid hemorrhage: multimodal approach. *Interdiscip Neurosurg*. 2020;22:100857. doi:10.1016/j.inat.2020.100857
14. Dorhout Mees SM, Bertens D, van der Worp HB, Rinkel GJ, van den Bergh WM. Magnesium and headache after aneurysmal subarachnoid haemorrhage. *J Neurol Neurosurg Psychiatry*. 2010;81(5):490-493. doi:10.1136/jnnp.2009.181404

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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A.2.4 Publication 4: Long-term fatigue following aneurysmal subarachnoid haemorrhage and the impact on employment

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This study is based on data from the UK Biobank. The study was originally conceived as a research project for Harry Carmichael as part of his medical degree, which I co-supervised. I subsequently further developed the concept, curated the data and performed all the analyses included in this manuscript. I authored the manuscript with input from the co-authors after completion of the first draft.

Specific contribution: Primary role in study design, data curation and analysis. Authored first draft of manuscript.

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ORIGINAL ARTICLE

Long-term fatigue following aneurysmal subarachnoid haemorrhage and the impact on employment

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Abstract

Background and purpose: Fatigue is common following aneurysmal subarachnoid haemorrhage (aSAH) but little is known about its frequency, prognosis and impact on employment. The aim of this study was to assess the frequency of fatigue, whether it changes over time and the relationship to employment in the long term.

Methods: This was a retrospective observational study of aSAH cases and matched controls from the UK Biobank. The presence of fatigue was compared between cases and controls using the chi-squared test. The change in frequency over time was assessed using Spearman's rank correlation coefficient. The effect of fatigue on employment was assessed using mediation analysis.

Results: Fatigue is more common following aSAH compared to matched controls (aSAH 18.7%; controls 13.7%; $\chi^2 = 13.0$, $p < 0.001$) at a mean follow-up of 123 months. Fatigue gradually improves over time with significant fatigue decreasing by 50% from ~20% in the first year to ~10% after a decade ($p = 0.04$). Fatigue significantly mediated 24.0% of the effect of aSAH status on employment.

Conclusions: Fatigue is common following aSAH and persists in the long term. It gradually improves over time but has a major impact on aSAH survivors, significantly contributing to unemployment following haemorrhage. Further work is required to develop treatments and management strategies for fatigue with a view to improving this symptom and consequently employment following aSAH.

KEYWORDS
employment, fatigue, outcome, subarachnoid haemorrhage

INTRODUCTION

Aneurysmal subarachnoid haemorrhage (aSAH) is a devastating form of stroke associated with significant morbidity and mortality. It affects younger people than other stroke types, resulting in a disproportionately high socio-economic impact due to loss of productive employment and the long-term healthcare burden [1]. Survivors of aSAH can suffer a wide range of neurological deficits ranging from physical disability to less obvious, yet life changing, sequelae including cognitive [2], psychological [3] and auditory deficits [4, 5]. These disabilities contribute to unemployment following aSAH, with up to 50% of previously employed individuals not returning to work at 1 year following haemorrhage [6].

Fatigue is another common consequence of aSAH with one analysis reporting a weighted mean fatigue frequency of 73.6% in the first year, falling to 50.7% thereafter, based on five published studies [7]. Of the studies included in that analysis, where the subarachnoid haemorrhage was confirmed to be aneurysmal, the maximum follow-up time period was 4 years. Very little is known about the long-term prognosis of fatigue following aSAH.

Fatigue has significant implications for patients and has been associated with reduced quality of life and impaired return to work following aSAH [3, 8, 9]. A number of factors have been reported to predict fatigue following aSAH including smoking, impaired consciousness, hydrocephalus, anxiety and depression [8–11].

The aim of this study was (i) to assess the frequency and phenotype of fatigue in the long term following aSAH; (ii) to identify whether the frequency of fatigue changes over time; and (iii) to assess whether fatigue mediated any of the effect of aSAH on employment status.

METHODS

This was a retrospective case–control study using data from the UK Biobank, a major biomedical database [12]. This study includes information on 502,497 participants with informed consent, aged 40–69 at the time of recruitment between 2006 and 2010 (application ID 49305). The study is reported in accordance with the STROBE statement for case-controlled studies [13] and has both national REC (16/NW/0274) and institutional approval (ERGO 49253).

Fatigue

Fatigue was assessed in the UK Biobank at assessment centre visits using the question 'Over the past 2 weeks, how often have you felt tired or had little energy?' (data field 2080). Individuals were categorized as suffering significant fatigue if they reported tiredness or little energy for more than half the time. A subset of individuals answered questions about fatigue phenotype (Table 1), scored using a 7-point scale with a score of 1 indicating strong disagreement and 7 strong agreement. Where applicable correction for multiple testing was performed using the Benjamini–Hochberg procedure with a false discovery rate of 5%.

Aneurysmal subarachnoid haemorrhage population

Aneurysmal subarachnoid haemorrhage cases were identified from the UK Biobank using International Classification of Diseases (ICD) 9 (data field 41271), ICD-10 (data field 41270), self-reported medical conditions (data field 20002) and primary care data (data field 42040). Individuals were excluded if the subarachnoid haemorrhage was non-aneurysmal in nature or if there was a trauma code documented within 30 days of diagnosis (see Table S1 for inclusion and exclusion codes). aSAH cases were included in this study if they had data on fatigue subsequent to the diagnosis of aSAH.

Control population

A single matched control population was identified from the UK Biobank using propensity score matching with a nearest neighbour method and a case:control ratio of 1:4. Individuals were matched according to age at follow-up, sex, smoking status and presence of anxiety or depression which have been shown to influence fatigue following aSAH [8, 10, 11]. Smoking status was dichotomized into current smoker or not (data field 20116). Anxiety and depression were dichotomized on whether the individual had seen a doctor for nerves, anxiety, tension or depression (data field 2090). Individuals with missing data on fatigue or covariates were excluded from the control pool available for matching.

Primary analysis

The chi-square test was used to compare frequency of fatigue between cases and controls. The *t* test was used to compare fatigue phenotype domains. Spearman's rank correlation coefficient was used to assess the relationship between frequency of fatigue and time.

Severity of clinical presentation and complications of aSAH, such as hydrocephalus, have been shown to be predictive of fatigue [9, 10]. Logistic regression was used to explore whether these features were associated with significant fatigue in this dataset. The dependent variable was significant fatigue with the variable of interest as the independent variable in addition to age, sex, smoking status and presence of anxiety/depression. The presence of hydrocephalus was defined using the Office of Population Censuses and Surveys Classification of Interventions and Procedures (version 4) codes (data field 41272). A201 (drainage of ventricle of brain) and A124 (creation of ventriculo-peritoneal shunt) at the time of or within 1 year of diagnosis were used. The World Federation of Neurological Surgeons

TABLE 1 Questions included from the UK Biobank on fatigue phenotype

Data field	Question
120119	Motivation is lower when fatigued
120120	Exercise brings on fatigue
120121	Easily fatigued
120122	Fatigue interferes with physical functioning
120123	Fatigue causes frequent problems
120124	Fatigue prevents sustained physical functioning
120125	Fatigue interferes with carrying out certain duties and responsibilities
120126	Fatigue is amongst the three most disabling symptoms
120127	Fatigue interferes with work, family or social life

(WFNS) grade is a measure of the severity of clinical presentation and the strongest known predictor of outcome following aSAH [14]. WFNS grade is not available in the UK Biobank but length of stay, which is strongly correlated with WFNS [15], was used as a surrogate.

Mediation analysis

To explore whether significant fatigue mediated any component of the effect of aSAH on employment status, causal mediation analysis using a natural effects model was performed utilizing the package medflex [16]. This method has been shown to be superior when analysing a binary mediator and outcome [17]. A non-parametric bootstrap procedure with 1000 samples was used to derive standard errors and *p* values. This was performed in the aSAH and matched control cohorts, additionally controlling for the Townsend deprivation score [18] (data field 189) and education status, dichotomized into people holding a college or university degree at the time of initial assessment in the UK Biobank or not (data field 6138). Employment status was dichotomized into good and poor, with poor employment defined as 'unemployed' or 'unable to work because of sickness or disability' (data field 6142). The proportion of the effect of aSAH status on employment mediated by fatigue was calculated using the method described by VanderWeele [19].

To provide context and assess the relative importance of fatigue to employment a further mediation analysis was performed exploring what proportion of the effect of aSAH status on employment was mediated by persistent headache, another common sequela of aSAH [20]. Headache was defined as present or absent using data field 6159 ('In the last month have you experienced headache that interfered with usual activity?') and the same causal mediation analysis was performed.

All analyses were performed in R (version 3.6.2, R Foundation for Statistical Computing).

RESULTS

A total of 869 aSAH cases were identified from the UK Biobank. 829 were eligible for inclusion with data available on fatigue. 479,617 individuals were eligible for inclusion in the control cohort and 3316 controls were matched with a mean standard difference <0.004 (see Table 2 for demographics of individuals included in the study and Figure 1 for the flowchart of aSAH cases included).

Primary analysis

Significant fatigue was more frequent in cases compared to controls (aSAH 18.7%; controls 13.7%; $\chi^2 = 13.0$, $p < 0.001$) at a mean follow-up of 123 months. Length of stay and hydrocephalus were not significant predictors of fatigue following aSAH in this cohort ($p = 0.940$ and $p = 0.150$, respectively). After correction for multiple testing four fatigue phenotypes were more significant in the aSAH cohort compared to controls: 'fatigue interferes with work, family or social life', 'fatigue is amongst the three most disabling symptoms', 'fatigue causes frequent problems' and 'easily fatigued' (Table 3). This suggests that fatigue has an impact in almost all domains of life and significantly impairs a patient's quality of life.

The frequency of significant fatigue decreased by half from 19.6% in the first year following aSAH to 11.1% in the eleventh year with a significant relationship between frequency of fatigue and time ($R_S = -0.62$, $p = 0.04$, Figure 2).

Mediation analysis

Unemployment or inability to work due to sickness/disability was significantly more frequent in the aSAH population (aSAH 18.7%; controls 5.9%; $\chi^2 = 138.9$, $p < 0.001$). Mediation analysis identified that the estimated natural indirect effect of aSAH status on employment

TABLE 2 Demographics of aSAH and matched controls included in study

	aSAH cohort	Control cohort
Total sample size, *n*	829	3316
Subset completing phenotype questionnaire	121 (14.6%)	619 (18.7%)
Age at time of follow-up		
Mean (±SD) years	58 (±7.1)	58 (±7.1)
Sex		
Male	336 (40.5%)	1348 (40.7%)
Female	493 (59.5%)	1968 (59.3%)
Depression or anxiety		
Present	326 (39.3%)	1308 (39.4%)
Absent	503 (60.7%)	2008 (60.1%)
Smoking status		
Current smoker	138 (16.6%)	556 (16.8%)
Not current smoker	691 (83.4%)	2760 (83.2%)
Education status		
College or university degree	223 (26.9%)	1032 (31.1%)
No college or university degree	605 (73.0%)	2262 (68.2%)
Missing	1 (0.0%)	22 (0.0%)
Townsend deprivation score		
Mean (±SD) months	−1.0 (±3.2)	−1.3 (±3.2)
Time to follow-up		
Mean (±SD) months	123 (±116)	–
Length of stay		
Median (IQR) days	7 (11)	–
Missing	304 (36.7%)	–
Hydrocephalus	40 (4.8%)	–

Abbreviations: aSAH, aneurysmal subarachnoid haemorrhage; IQR, interquartile range; SD, standard deviation.

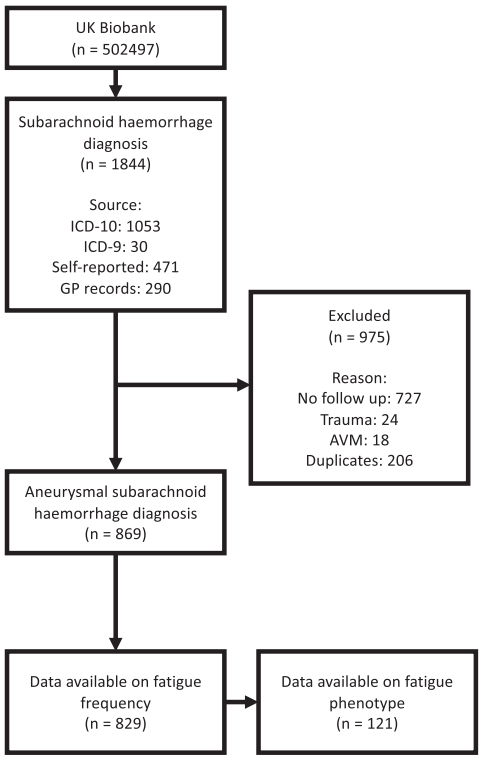

FIGURE 1 aSAH sample inclusion flowchart for UK Biobank

that was mediated by fatigue was significant, with an odds ratio of 1.21 (95% confidence interval [CI] 1.07–1.36, $p = 0.001$). The odds ratio for the estimated natural direct effect of aSAH status on employment was 2.97 (95% CI 2.51–3.49, $p < 0.001$). The proportion of the effect of aSAH on employment mediated by fatigue was 24.0%.

By comparison the estimated natural indirect effect of aSAH status on employment that was mediated by headache was significant, with an odds ratio of 1.06 (95% CI 1.02–1.11, $p = 0.004$). The proportion of the effect of aSAH on employment mediated by headache was 8.3%.

DISCUSSION

In this large sample size, it is demonstrated that fatigue is more common following aSAH compared to matched controls and persists in the long term, with a mean follow-up of over 10 years. Significant fatigue, defined as present for greater than 50% of the time, gradually improves over time in about half of patients, but has important implications. aSAH survivors report that it is one of the most disabling symptoms impacting quality of work, social and family life. In keeping with this, it is demonstrated that fatigue makes a large contribution to unemployment and inability to work due to sickness/disability following aSAH. This information will be helpful to counsel patients regarding the duration and prognosis of fatigue following aSAH and emphasizes the importance of management strategies to improve this disabling symptom and consequently promote a return to employment.

Kutlubaev et al. [7] reported a weighted mean frequency of fatigue of 73.6% in the first year following aSAH using five studies. This is much greater than the 19.6% reported in this study; however, a number of studies used by Kutlubaev et al. defined fatigue as present or absent based on a single binary question inflating the frequency of fatigue by including any self-reported fatigue. In the present study fatigue is defined as significant if present for greater than 50% of the time and the frequency is in keeping with other studies that focus on the presence of significant fatigue [21, 22]. In the UK Biobank dataset, if fatigue is defined as the occurrence of any fatigue, it is present in 80.4% in the first year in keeping with the frequency reported by Kutlubaev et al. [7].

In this study it is shown that the frequency of fatigue significantly improves over time, decreasing by about 50% from around 20% in the first year to 10% after a decade. A recent study of 356 patients also reported that the prevalence of fatigue gradually decreased from 1 to 7 years post-aSAH, although the decrease was not statistically significant [10]. In this study a larger sample size is included and a longer follow-up explaining the greater significance of our results. It is also shown that length of stay, a surrogate of severity of clinical presentation, and hydrocephalus are not predictors of fatigue following aSAH. These results differ from the same recent study [10]. This may be because the UK Biobank favours good outcome individuals due to the requirement to engage in detailed follow-up assessments. Both severity of clinical presentation and hydrocephalus are predictive of poor outcome [23] and are consequently underrepresented in this cohort, limiting our ability to study their association with fatigue. However, it may also be a real observation. It would be easy to rationalize that, once treated, hydrocephalus does not increase fatigue, supported by a further study which showed an association between acute but not chronic treatment of hydrocephalus [9]. Also, although it would be easy to assume more severe haemorrhages result in more severe fatigue, it is possible that patients with worse outcomes have lower activity levels and are more focused on their functional deficits and relatively underreport fatigue. This fits with our anecdotal observations that often some of the best performing patients are most limited by fatigue.

Unemployment is common following aSAH with up to 50% reporting impaired return to work [24]. A number of factors have been implicated in return to work following aSAH including independence at discharge, consciousness at admission [25] and cognitive deficits following aSAH [2]. Fatigue has also been implicated [8, 9] and this study emphasizes the importance of fatigue to employment by demonstrating that it mediates a significant proportion of the effect of aSAH on employment. The long follow-up time (mean over 10 years) in this cohort further emphasizes the

TABLE 3 Comparison of fatigue phenotype questions between aneurysmal subarachnoid haemorrhage (aSAH) and control cohorts using the *t* test

Data field *Question*	Mean score in aSAH cohort	Mean score in control cohort	*p* value
120119 *Motivation is lower when fatigued*	5.07	4.94	0.50
120120 *Exercise brings on fatigue*	3.35	3.07	0.20
120121 *Easily fatigued*	3.88	3.36	0.014[a]
120122 *Fatigue interferes with physical functioning*	3.89	3.70	0.35
120123 *Fatigue causes frequent problems*	3.23	2.75	0.020[a]
120124 *Fatigue prevents sustained physical functioning*	3.36	2.96	0.059
120125 *Fatigue interferes with carrying out certain duties and responsibilities*	3.43	3.04	0.056
120126 *Fatigue is amongst the three most disabling symptoms*	3.42	2.81	0.0067[a]
120127 *Fatigue interferes with work, family or social life*	3.37	2.82	0.0089[a]

Note: Benjamini–Hochberg method with false discovery rate of 5% employed to correct for multiple testing.

[a]Signifies significant *p* values.

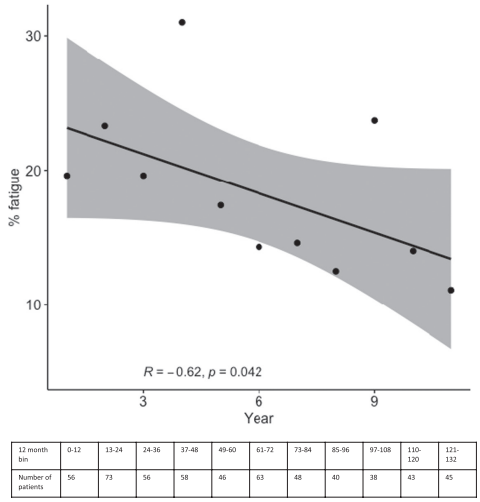

FIGURE 2 Change in frequency of fatigue over time, divided into 12-month bins. Data beyond 11 years were not included due to the sparsity of data in each annual bin

importance of fatigue as it has impact even at such a late stage after aSAH. To emphasize the importance of fatigue on employment the contribution of fatigue was compared to that of another common sequela of aSAH, persistent headache, demonstrating that fatigue is a much more dominant factor (24.0% vs. 8.3%). A previous study further supports the relative importance of fatigue with cognition also contributing a much smaller effect on employment (24.0% vs. 6.6% [2]).

Both fatigue and unemployment impair quality of life following aSAH [8, 26] emphasizing the importance of managing the symptom of fatigue following aSAH, especially as it persists in the long term and impacts employment. At present there are no pharmacological therapies to improve fatigue following stroke [27], but there are non-pharmacological strategies which can improve the symptoms of fatigue [28]. Uptake of these strategies following aSAH in addition to ongoing pharmacological trials (e.g., NCT 03209830) may help to improve fatigue with subsequent benefits for survivors' employment and quality of life.

Limitations

As UK Biobank participants are required to attend multiple very detailed assessment centre visits this study is biased towards individuals with a better outcome and more motivation. In comparison to poor outcome individuals who are preoccupied by functional deficits, aSAH cases included in this study are more likely to be aware of symptoms such as fatigue. Consequently, caution should be taken when applying these results to poorer outcome individuals.

In this study, a single question ('Over the past 2 weeks, how often have you felt tired or had little energy?') was used to assess

frequency of fatigue. Future prospective studies should use more detailed assessments of fatigue, including validated tools such as the Chalder fatigue scale [29] or the fatigue severity scale [30], to provide greater insight into the nature of fatigue following aSAH. In addition, a number of factors have been shown to influence fatigue following aSAH including the presence of anxiety and depression [11]. In this study this is controlled for by matching cases and controls for the presence of anxiety/depression but more comprehensive fatigue assessment tools may be able to further elucidate the role of these factors in post-aSAH fatigue. This study was also unable to assess change in fatigue on an individual level due to lack of serial measurement of fatigue and future studies should also include repeated measures of fatigue to give further detailed information on change in fatigue over time.

Finally, in this analysis, data were only available on employment status following aSAH and consequently it was not possible to study change in employment status before and after aSAH. This finding needs to be confirmed using employment data from individuals before and after aSAH.

CONCLUSION

Fatigue is more common following aSAH compared to matched controls and persists in the long term. Fatigue gradually improves over time with significant fatigue decreasing by about 50% from around 20% in the first year to 10% after a decade. Fatigue negatively impacts quality of life and employment following aSAH. Further work is required to develop treatments and management strategies for fatigue following aSAH with a view to improving quality of life and employment.

AUTHOR CONTRIBUTIONS

IG and DB conceived the study. All authors contributed to the study design. The first draft of the manuscript was written by BG and HC; all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the UK Biobank (https://www.ukbiobank.ac.uk) by application.

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REFERENCES

1. Taylor TN, Davis PH, Torner JC, Holmes J, Meyer JW, Jacobson MF. Lifetime cost of stroke in the United States. *Stroke*. 1996;27(9):1459-1466.
2. Gaastra B, Ewbank F, Tapper W, Bulters D, Galea I. Long-term cognitive outcome following aneurysmal subarachnoid haemorrhage. *J Stroke Cerebrovasc Dis*. 2022;31(1):106184.
3. Visser-Meily JM, Rhebergen ML, Rinkel GJ, van Zandvoort MJ, Post MW. Long-term health-related quality of life after aneurysmal subarachnoid hemorrhage: relationship with psychological symptoms and personality characteristics. *Stroke*. 2009;40(4):1526-1529.
4. Gaastra B, Ashokumar M, Bulters D, Campbell N, Galea I. Auditory outcome following aneurysmal subarachnoid haemorrhage. *J Neurol Sci*. 2021;434:120125.
5. Campbell N, Verschuur C, Mitchell S, et al. Hearing impairment after subarachnoid hemorrhage. *Ann Clin Transl Neurol*. 2019;6(3):420-430.
6. Quinn AC, Bhargava D, Al-Tamimi YZ, Clark MJ, Ross SA, Tennant A. Self-perceived health status following aneurysmal subarachnoid haemorrhage: a cohort study. *BMJ Open*. 2014;4(4):e003932.
7. Kutlubaev MA, Barugh AJ, Mead GE. Fatigue after subarachnoid haemorrhage: a systematic review. *J Psychosom Res*. 2012;72(4):305-310.
8. Western E, Nordenmark TH, Sorteberg W, Karic T, Sorteberg A. Fatigue after aneurysmal subarachnoid hemorrhage: clinical characteristics and associated factors in patients with good outcome. *Front Behav Neurosci*. 2021;15:633616.
9. Buunk AM, Groen RJM, Wijbenga RA, et al. Mental versus physical fatigue after subarachnoid hemorrhage: differential associations with outcome. *Eur J Neurol*. 2018;25(11):1313-e113.
10. Western E, Sorteberg A, Brunborg C, Nordenmark TH. Prevalence and predictors of fatigue after aneurysmal subarachnoid hemorrhage. *Acta Neurochir*. 2020;162(12):3107-3116.
11. Passier PE, Post MW, van Zandvoort MJ, Rinkel GJ, Lindeman E, Visser-Meily JM. Predicting fatigue 1 year after aneurysmal subarachnoid hemorrhage. *J Neurol*. 2011;258(6):1091-1097.
12. Sudlow C, Gallacher J, Allen N, et al. UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*. 2015;12(3):e1001779.
13. von Elm E, Altman DG, Egger M, et al. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol*. 2008;61(4):344-349.
14. Jaja BNR, Saposnik G, Lingsma HF, et al. Development and validation of outcome prediction models for aneurysmal subarachnoid haemorrhage: the SAHIT multinational cohort study. *BMJ*. 2018;360:j5745.
15. Yousef K, Crago E, Fisher A, Mahmoud K, Lagattuta T, Hravnak M. 3: Grading scales in subarachnoid hemorrhage: which scale to control for when studying outcomes. *Crit Care Med*. 2019;47(1):2.
16. Steen J, Loeys T, Moerkerke B, Vansteelandt S. Medflex: an R package for flexible mediation analysis using natural effect models. *J Stat Softw*. 2017;76(11):1-46.

17. Rijnhart JJM, Valente MJ, Smyth HL, MacKinnon DP. Statistical mediation analysis for models with a binary mediator and a binary outcome: the differences between causal and traditional mediation analysis. *Prev Sci*. 2021;1-11. doi:10.1007/s11121-021-01308-6. Epub ahead of print.
18. Mackenbach JP. Health and deprivation. Inequality and the north: by P. Townsend, P. Phillimore and A. Beattie (eds.) Croom Helm Ltd, London, 1987 221 pp., ISBN 0-7099-4352-0, £8.95. *Health Policy*. 1988;10(2):207-206.
19. VanderWeele TJ. Mediation analysis: a practitioner's guide. *Annu Rev Public Health*. 2016;37:17-32.
20. Huckhagel T, Klinger R, Schmidt NO, Regelsberger J, Westphal M, Czorlich P. The burden of headache following aneurysmal subarachnoid hemorrhage: a prospective single-center cross-sectional analysis. *Acta Neurochir*. 2020;162(4):893-903.
21. Noble AJ, Baisch S, Mendelow AD, Allen L, Kane P, Schenk T. Posttraumatic stress disorder explains reduced quality of life in subarachnoid hemorrhage patients in both the short and long term. *Neurosurgery*. 2008;63(6):1095-1104. discussion 04–5.
22. Rödholm M, Starmark JE, Svensson E, Von Essen C. Asthenoemotional disorder after aneurysmal SAH: reliability, symptomatology and relation to outcome. *Acta Neurol Scand*. 2001;103(6):379-385.
23. Galea JP, Dulhanty L, Patel HC, UK and Ireland Subarachnoid Hemorrhage Database Collaborators. Predictors of outcome in aneurysmal subarachnoid hemorrhage patients: observations from a multicenter data set. *Stroke*. 2017;48(11):2958-2963.
24. Nussbaum ES, Mikoff N, Paranjape GS. Cognitive deficits among patients surviving aneurysmal subarachnoid hemorrhage. A contemporary systematic review. *Br J Neurosurg*. 2021;35(4):384-401.
25. Westerlind E, Persson HC, Sunnerhagen KS. Working capacity after a subarachnoid haemorrhage: a six-year follow-up. *J Rehabil Med*. 2017;49(9):738-743.
26. Passier PE, Visser-Meily JM, Rinkel GJ, Lindeman E, Post MW. Life satisfaction and return to work after aneurysmal subarachnoid hemorrhage. *J Stroke Cerebrovasc Dis*. 2011;20(4):324-329.
27. Wu S, Kutlubaev MA, Chun HY, et al. Interventions for post-stroke fatigue. *Cochrane Database Syst Rev*. 2015;2015(7):CD007030.
28. Su Y, Yuki M, Otsuki M. Non-pharmacological interventions for post-stroke fatigue: systematic review and network meta-analysis. *J Clin Med*. 2020;9(3):621.
29. Chalder T, Berelowitz G, Pawlikowska T, et al. Development of a fatigue scale. *J Psychosom Res*. 1993;37(2):147-153.
30. Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. *Arch Neurol*. 1989;46(10):1121-1123.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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A.3 Chapter 3

A.3.1 Publication 5: CRP (C-reactive protein) in outcome prediction after subarachnoid haemorrhage and the role of machine learning

Citation: Gaastra B, Barron P, Newitt L, et al. CRP (C-Reactive Protein) in Outcome Prediction After Subarachnoid Hemorrhage and the Role of Machine Learning. *Stroke*. Oct 2021;52(10):3276-3285. doi:10.1161/STROKEAHA.120.030950

This study is based on data from two sources: (1) the Simvastatin in Aneurysmal Subarachnoid Haemorrhage (STASH) trial, and (2) data collected by Peter Barron, Laura Newitt and Simran Chhugani from the Wessex Neurological Centre as part of medical degree research projects. I curated and analysed the data with statistical input from Diederik Bulters and Ben Macarthur. I authored the manuscript with input from the co-authors after completion of the first draft.

Specific contribution: Primary role in study design, data curation and analysis. Authored first draft of manuscript.

Stroke

ORIGINAL CONTRIBUTION

CRP (C-Reactive Protein) in Outcome Prediction After Subarachnoid Hemorrhage and the Role of Machine Learning

Ben Gaastra, MRCS; Peter Barron; Laura Newitt, BMBS; Simran Chhugani; Carole Turner, MSc; Peter Kirkpatrick, FRCS (SN); Ben MacArthur, PhD; Ian Galea, PhD; Diederik Bulters, FRCS (SN)

BACKGROUND AND PURPOSE: Outcome prediction after aneurysmal subarachnoid hemorrhage (aSAH) is challenging. CRP (C-reactive protein) has been reported to be associated with outcome, but it is unclear if this is independent of other predictors and applies to aSAH of all grades. Therefore, the role of CRP in aSAH outcome prediction models is unknown. The purpose of this study is to assess if CRP is an independent predictor of outcome after aSAH, develop new prognostic models incorporating CRP, and test whether these can be improved by application of machine learning.

METHODS: This was an individual patient-level analysis of data from patients within 72 hours of aSAH from 2 prior studies. A panel of statistical learning methods including logistic regression, random forest, and support vector machines were used to assess the relationship between CRP and modified Rankin Scale. Models were compared with the full Subarachnoid Hemmorhage International Trialists' (SAHIT) prediction tool of outcome after aSAH and internally validated using cross-validation.

RESULTS: One thousand and seventeen patients were included for analysis. CRP on the first day after ictus was an independent predictor of outcome. The full SAHIT model achieved an area under the receiver operator characteristics curve (AUC) of 0.831. Addition of CRP to the predictors of the full SAHIT model improved model performance (AUC, 0.846, P=0.01). This improvement was not enhanced when learning was performed using a random forest (AUC, 0.807), but was with a support vector machine (AUC of 0.960, P<0.001).

CONCLUSIONS: CRP is an independent predictor of outcome after aSAH. Its inclusion in prognostic models improves performance, although the magnitude of improvement is probably insufficient to be relevant clinically on an individual patient level, and of more relevance in research. Greater improvements in model performance are seen with support vector machines but these models have the highest classification error rate on internal validation and require external validation and calibration.

GRAPHIC ABSTRACT: An online graphic abstract is available for this article.

Key Words: C-reactive protein ■ machine learning ■ prognosis ■ subarachnoid hemorrhage ■ support vector machine

Aneurysmal subarachnoid hemorrhage (aSAH) is a devastating form of stroke. Up to 50% of patients are estimated to die,[1] and of the survivors, 50% have cognitive impairment and 40% are unable to return to their previous work.[2] Predicting which patients will have poor outcome after aSAH would be valuable to prognosticate and guide treatment. Better models would also improve our ability to control for covariates and reduce sample sizes for research studies.

Several outcome prediction tools following aSAH have been developed.[3] The best available is the Subarachnoid Hemmorhage International Trialists' (SAHIT) prediction

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For Sources of Funding and Disclosures, see page xxx.

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Nonstandard Abbreviations and Acronyms

aSAH	aneurysmal subarachnoid hemorrhage
AUC	area under the receiver operator characteristics curve
CRP	C-reactive protein
mRS	modified Rankin Scale
STASH	Simvastatin in Aneurysmal Subarachnoid Haemorrhage
SVM	support vector machine
WFNS	World Federation of Neurological Surgeons

tool, developed from over 10 000 patients and validated in a separate cohort of over 3000 patients.[4] Three predictive models were generated: a core, neuroimaging, and full model. The models performed well, with good discrimination (area under the receiver operator characteristics curve [AUC], 0.76–0.81). The authors report R^2 statistic values of 22% to 31%, meaning that up to 78% of variance in outcome after aSAH is not explained by the predictors included in the SAHIT models. To improve on this, it will be necessary to either develop better statistical methods, or include additional predictors, not incorporated in SAHIT.

CRP (C-reactive protein) is an acute phase reactant made in the liver and released into the blood in response to inflammation. It is routinely available making it an ideal addition to predictive models. Both serum and cerebrospinal fluid CRP levels rise following aSAH and peak around day 3 to 4 post ictus.[5–7] Elevated levels were associated with poor outcome following aSAH in 2 small series of <100 patients.[5,6] A further study of 178 patients highlighted the problem that although CRP levels on admission were associated with outcome, with their sample size they were not independently predictive.[8] Another study of 803 patients reported CRP was only an independent predictor in good grade (World Federation of Neurological Surgeons [WFNS] grade 1 or 2) but not poor grade (WFNS 3–5) patients with aSAH.[9] In summary, although there is evidence CRP is associated with outcome after aSAH, it is unclear if this is independent from other known predictors or across all aSAH grades and, therefore, if it should be added to prediction models such as SAHIT.

The SAHIT predictive tool, along with the majority of other outcome prediction tools for aSAH, was developed using the classical statistical learning method of logistic regression,[3,4] which is a generalized linear model. More modern machine learning methods offer advantages over classical statistics as they are better able to analyze complex high dimensional data, account for nonlinear associations between predictors, and thereby generate models that generalize more successfully to unseen data.[10,11] Although machine learning techniques have shown promise for the prediction of cerebral vasospasm and delayed cerebral ischemia,[12,13] only simple decision trees have been applied to outcome prediction. These have been shown to have comparable performance to logistic regression methods.[14–16] More powerful machine learning methods, including ensemble methods, such as random forests, and nonlinear methods, such as support vector machines (SVMs), have not been explored in outcome prediction after aSAH, and use of these methods may improve predictive models.[17]

The aims of this study are therefore to (1) assess if CRP is an independent predictor of outcome after aSAH; (2) develop improved predictive models of outcome after aSAH by including CRP in machine learning models.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request. Predictive models are reported in keeping with the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) statement (Checklist in the Data Supplement).

Patient Data

Patients with aSAH who had at least one recorded CRP and WFNS on day 0, 1, or 2 after ictus were eligible for inclusion. Deidentified individual patient-level data were identified from two sources:

1. The Wessex Neurological Centre (University Hospital Southampton, United Kingdom) neurovascular database. Following National Research Ethics Service and Health Research Authority approval (17/LO/0964), prospectively collected data from patients with aSAH aged over 18 years old admitted to the Wessex Neurological Centre between August 2009 and September 2017 was selected. Additional clinical data for serial CRP and WFNS values were collected from the electronic and paper notes retrospectively. Outcomes were collected prospectively by a specialist nurse trained in assessment of the modified Rankin Scale (mRS) and confirmed retrospectively in the paper notes.
2. The randomized multicentre controlled trial STASH (Simvastatin in Aneurysmal Subarachnoid Haemorrhage)[18]

Variables/Predictors

The following variables/predictors were available:

1. Age
2. CRP (mg/L) on day 0, 1, and 2
3. WFNS grade on day 0, 1, and 2
4. Treatment status (clip, coil, or no treatment)
5. Fisher grade on admission
6. Past medical history of hypertension
7. Aneurysm size (≤12 mm, 13–24 mm, or >24 mm)
8. Aneurysm location (anterior cerebral artery, middle cerebral artery, internal carotid artery, posterior circulation)

All data were treated as categorical apart from age and CRP which were considered as continuous variables. Aneurysm size categories were defined as per the SAHIT predictive tool.[4]

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The variables/predictors available are the same as the complete SAHIT predictive tool with the addition of CRP.

Outcome

Outcome was defined as best mRS score 3 to 6 months post ictus. All patients from the STASH trial had outcome at 6 months, patients from the Wessex Neurological Centre had outcome at 3 to 6 months.

Outcome was dichotomized into good (mRS score, 0–3) and poor outcome (mRS score, 4–6). This dichotomization was chosen to match the SAHIT predictive tool.[4]

Missing Data

Missing CRP and WFNS values were imputed using multiple imputation with a predictive mean matching method[19] to generate 5 imputations. Imputation was only employed if data were missing at random. Other missing data were coded with a dummy variable to allow for inclusion of patients with missing data in the predictive models.

CRP and WFNS Inclusion

Binary logistic regression and AUC analysis were used to compare the predictive values of CRP on day 0, 1, and 2. Reported AUC values were pooled across the 5 imputed datasets using Rubin Rules.[20] To identify best predictive day the DeLong test[21] was used to assess for difference in AUC, as this cannot be implemented for pooled AUCs it is reported for the first imputed dataset. If no difference was identified, the earlier time point was used to minimize confounding of CRP rise with secondary illness such as aspiration pneumonia. The best-performing day was used for the rest of the analysis. The process was repeated to identify the best predictive performing day of WFNS recording.

Model Development

A variety of statistical learning methods were used to develop the outcome prediction models.

Binary logistic regression was used at first incorporating the same predictors as in the SAHIT predictive tool (age, WFNS, past medical history of hypertension, Fisher grade, size and location of aneurysm, treatment status). The model was further developed by the addition of CRP as a predictor.

The supervised classification machine learning methods random forest and SVMs were employed and trained on all predictors available. Both these methods were optimized to improve predictive performance. In the random forest, the number of variables considered at each split of the tree and the number of trees was manually optimized. Both linear and nonlinear SVMs (using a radial basis function kernel) were trained and all model parameters were optimized with 10-fold cross-validation. Nominal categorical predictors were converted to dummy variables to allow inclusion in the SVM model.

Model Performance

Models were compared to the full SAHIT predictive tool. Individual model performance was assessed using the AUC. Reported AUC values were pooled across the 5 imputed datasets using Rubin's Rules and reported with 95% CIs.[20] The DeLong test was used to compare AUCs[21] and is reported for the first imputed dataset. The net reclassification improvement and integrated discrimination improvement were used to assess the improvement in model performance after addition of predictors.

To identify the proportion of variance explained by the logistic regression models, a variance-function-based R^2 statistic was used.[22] To control for collinearity, dominance analysis was used to analyze the importance of individual predictors to a multivariable regression model.[23] Variable importance from random forests were reported by Gini index and mean decrease in accuracy and for the SVM by ranking the coefficients of the support vectors.

Model Validation

Logistic regression models and SVMs were trained using 10-fold cross-validation to avoid overfitting. Random forests were optimized using out-of-bag error, a natural measure that approaches the leave one out cross-validation error as the number of trees gets large. Throughout training a data split into train and test data sets in proportions two thirds and one third, respectively, was used. Average error is reported across the 5 imputed datasets.

Model Calibration

Internal model calibration was assessed using a calibration test based on a likelihood ratio statistic method described by Nattino et al[24] designed to assess prediction tools based on dichotomous outcomes. When required the estimates produced by the classifiers were calibrated using isotonic regression and Platt scaling.[25]

Sensitivity Analyses

Analysis was repeated using mRS dichotomized into 0 to 2 as good outcome and 3 to 6 as poor outcome. This is the same dichotomization used by Turner et al. in the study reporting CRP as an independent predictor of outcome in good grade but not poor grade patients with aSAH.[9]

Further sensitivity analyses were also completed using study site, alternative aneurysm size categories, follow-up time as a covariate, and imputation rather than dummy variables for missing Fisher grade, hypertension, and aneurysm size.

Statistical Analysis

All analyses were conducted using R software (version 3.6.2, R Foundation for Statistical Computing).

RESULTS

Patient Inclusion, Missing Data, and Demographics

A total of 1017 patients were included in the study: 552 from the Wessex Neurological Centre neurovascular database and 465 from the STASH trial (Figure 1 details patient inclusion flow chart).

All patients had both WFNS and CRP data on at least one of day 0, 1, or 2. CRP data was missing on day 0, 1,

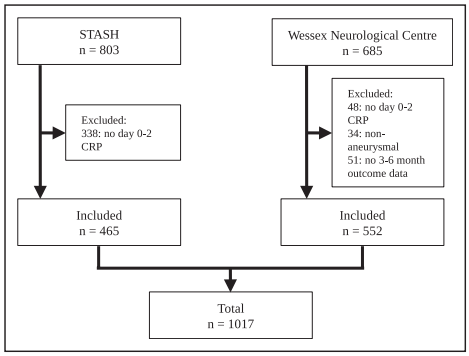

Figure 1. Flow chart for patients included in study.
CRP indicates C-reactive protein; and STASH, Simvastatin in Aneurysmal Subarachnoid Hemorrhage.

and 2 in 760 (75%), 450 (44%) and 373 (37%) patients respectively. WFNS data was missing on day 0, 1, and 2 in 499 (49%), 332 (33%) and 281 (28%) patients, respectively. Any missing CRP and WFNS data for day 0, 1, and 2 were imputed.

Table 1 details demographic data on patients included in the study and details other missing data. WFNS is presented on day 1 (see below).

CRP and WFNS

Figure I in the Data Supplement shows histograms of day 0, 1, and 2 CRP values. Univariate logistic regression showed that CRP on all 3 days was significantly associated with outcome after aSAH (P<0.002). The pooled AUCs for day 0, 1, and 2 CRP were 0.557 (0.472–0.638), 0.685 (0.630–0.734), and 0.709 (0.673–0.757), respectively. The AUCs for day 1 and 2 were both significantly improved compared to day 0 CRP (P<0.001). There was no significant difference between the AUCs for day 1 and 2 CRP (P=0.192). Day 1 CRP was, therefore, used for the remainder of the analysis. The R^2 for day 1 CRP was 8%. Given the distribution of CRP seen in Figure I in the Data Supplement, log CRP was also assessed as a predictor of outcome, but it did not improve predictive performance (P=0.998) and was, therefore, not used in the analysis.

Univariate logistic regression showed that WFNS on day 0, 1, and 2 was significantly associated with outcome after aSAH (P<0.001). The pooled AUCs for day 0, 1, and 2 WFNS were 0.690 (0.638–0.736), 0.763 (0.723–0.799), and 0.757 (0.710–0.799), respectively. The AUCs for day 1 and 2 were both significantly improved compared to day 0 WFNS (P<0.003). There was no significant difference between the AUCs for day 1 and 2 WFNS (P=0.48). Day 1 WFNS was, therefore, used for the remainder of the analysis. The R^2 for day 1 WFNS was 15%,

Table 1. Demographic Data From Patients Included in Study

	Wessex Neurological Centre	STASH	Total
Total (n)	552	465	1017
Age, y	56 (21–79)	50 (20–69)	54 (20–79)
Sex	M: 160	M: 157	M: 318
	F: 392	F: 308	F: 700
WFNS (day 1)			
I	236	185	421
II	83	122	205
III	36	36	72
IV	93	70	163
V	104	52	156
Fisher			
1	8	5	13
2	31	62	93
3	127	162	289
4	191	235	426
Missing	195	1	196
Treatment status			
Clip	131	168	299
Coil	405	287	692
No treatment	16	10	26
Location			
Middle cerebral artery	101	109	210
Anterior cerebral artery	240	134	374
Posterior circulation	77	187	264
Internal cerebral artery	134	35	169
Hypertension			
Yes	141	0	141
No	411	0	411
Missing	0	465	465
Size			
≤12 mm	413	0	413
13–24 mm	19	0	19
>24 mm	0	0	0
Missing	120	465	585
Outcome			
Good	451	389	840
Poor	101	76	177

WFNS reported for the first imputed dataset. F indicates female; M, male; STASH, Simvastatin in Aneurysmal Subarachnoid Haemorrhage; and WFNS, World Federation of Neurological Surgeons.

similar to that reported in other studies.[4] Day 1 and 2 WFNS is likely to be postresuscitation, compared with day 0, which is keeping with the finding that postresuscitation WFNS is more predictive of outcome than preresuscitation.[26]

If both CRP and WFNS are used as predictors in a multivariate logistic regression model, they both remain significantly associated with outcome (CRP: P<0.001;

WFNS: $P<0.001$), suggesting that the prognostic effect of CRP is independent of WFNS.

Predictive Models of Outcome

Logistic Regression Model

Inputting the same predictors as the full SAHIT predictive tool, hereby referred to as the SAHIT model, in this dataset using a logistic regression model generates a pooled AUC of 0.831 (0.797–0.860; R^2=24%), results comparable to those reported by Jaja et al.[4]

When CRP is added as a predictor to the full SAHIT model, it remains an independent predictor of outcome ($P<0.001$). The pooled AUC for this model is 0.846 (0.814–0.873; R^2=27%). The AUC is significantly improved compared to the SAHIT model alone in this dataset (P=0.01). The net reclassification improvement and integrated discrimination improvement were calculated for the addition of CRP to the SAHIT model and demonstrated the addition of CRP significantly improved model performance (P=0.03 and $P<0.001$, respectively).

The general dominance of each individual predictor in the multivariable regression model is reported in Figure 2. WFNS, Fisher grade, treatment status, and CRP were highlighted as the strongest predictors in the model.

Random Forest

A random forest was trained using 500 trees on the SAHIT predictors and CRP and manually optimized to consider 2 predictors at each split of the tree. Out-of-bag error was 17% with a pooled model AUC of 0.807 (0.766–0.843). The AUC is significantly worse when compared to the regression model trained on the same data ($P<0.001$). A random forest was also trained on the SAHIT predictors and similarly optimized, achieving a pooled AUC of 0.782 (0.739–0.820) and performing significantly worse than the regression model trained on the SAHIT predictors ($P<0.001$).

CRP, age, and WFNS were highlighted as the three most important variables to the predictive model as assessed by the mean decrease in Gini index; treatment status, WFNS, and CRP were highlighted as the three most important variables to the predictive model as assessed by the mean decrease in accuracy (Figure 2).

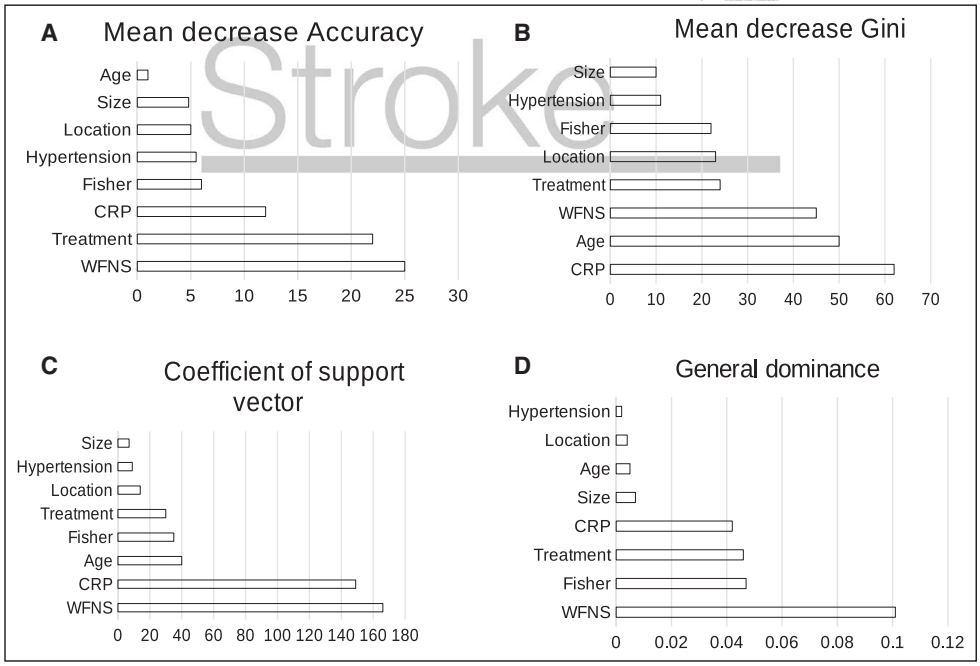

Figure 2. Relative importance of predictors for the first imputed dataset.
A, Mean decrease in accuracy (random forest). **B**, Mean decrease in Gini index (random forest). **C**, Coefficients of support vector machines. **D**, General dominance of predictors reported as R^2 statistic (logistic regression). CRP indicates C-reactive protein; and WFNS, World Federation of Neurological Surgeons.

Support Vector Machine

An SVM was trained on the SAHIT predictors and CRP and optimized using 10-fold cross-validation. The best-performing model was achieved using a radial basis function kernel with model parameters: cost (related to model regularization) of 4 and a γ (related to the radius of influence of the support vectors) of 2. This model achieved 1% misclassification with a pooled AUC of 0.960 (0.932–0.977), performing significantly better than both the regression model ($P<0.001$) and the random forest ($P<0.001$) trained on the same data.

WFNS, CRP, and age were also highlighted as the three most important predictive variables in the SVM (Figure 2).

When an SVM is trained using the same predictors as the SAHIT model and optimized using 10-fold cross-validation the best-performing model was achieved using a radial basis function kernel with a cost of 4 and a γ of 2. The model achieved 5% misclassification with an AUC of 0.895 (0.850–0.927). This AUC was improved compared to the full SAHIT model ($P=0.004$).

Table 2 and Table I (in the Data Supplement) summarize the performance of the predictive models.

Model Validation

All logistic regression models were internally validated using 10-fold cross-validation. For all models, the average classification error rate on the test data was 12% for the model using the same predictors as SAHIT and 11% for the model using CRP in addition. The SVM was also internally validated using 10-fold cross-validation. The average classification error rate in the test data was 20% for the model using the same predictors as SAHIT and 19% for the model using CRP in addition. As described above, the random forest had an out-of-bag error rate of 17%.

Model Calibration

Internal model calibration was assessed for the logistic regression and SVM models. The random forest was not assessed as it did not improve the performance of the logistic regression.

Using the calibration test described by Nattino et al,[24] the logistic regression models trained on the SAHIT predictors both with and without CRP did not demonstrate evidence of miscalibration ($P=0.237$ and $P=0.413$, respectively). Both the SVM trained on the SAHIT predictors alone and with CRP demonstrated evidence of miscalibration ($P<0.001$, Figure 3). To calibrate both these models, isotonic regression was applied to the estimates produced by the SVMs. The calibrated models were reassessed with the calibrated SVM trained on the SAHIT predictors alone showing no evidence of miscalibration ($P=0.859$) and the SVM trained with the addition of CRP demonstrating improved calibration (Figure 3) but with ongoing evidence of miscalibration ($P<0.001$). Platt scaling failed to improve on isotonic regression (Figure 3).

The calibrated SVM without CRP achieved an AUC of 0.857 (0.824–0.890) and with CRP of 0.920 (0.894–0.947).

Table 2. Performance of Predictive Models Comparing SAHIT Predictors and SAHIT+CRP

	AUC for SAHIT predictors	AUC for SAHIT predictors+CRP
Logistic regression	0.831 (0.797–0.860)	0.846 (0.814–0.873)
Random forest	0.782 (0.739–0.820)	0.807 (0.766–0.843)
Support vector machine	0.895 (0.850–0.927)	0.960 (0.932–0.977)

AUCs reported with 95% CI. AUC indicates area under the receiver operator characteristics curve; CRP, C-reactive protein; and SAHIT, Subarachnoid Haemorrhage International Trialists'.

Sensitivity Analysis

None of the sensitivity analyses significantly altered the results of the models (please see in the Data Supplement).

DISCUSSION

Several models have been developed to predict outcome after aSAH.[3] The SAHIT predictive tool is based on the largest population and has been rigorously validated.[4] It is, therefore, the most widely accepted. However, it only explains up to 31% of the variation in outcome, meaning other predictors not in the model may play a significant role in outcome prediction. In this study, we aimed to assess whether CRP is an additional independent predictor of outcome after aSAH and whether incorporating it into a range of outcome prediction models improved performance.

We have shown in a cohort of 1017 patients that day 1 CRP is an independent predictor of outcome controlling for all other known key predictors.

Figure 4 displays the effect on probability of poor outcome for each predictor included in the multivariable logistic regression models. In terms of relative importance, this study shows that CRP is a key predictor of outcome after aSAH with an $R^2=8\%$. In the regression analysis controlling for collinearity, it is the fourth most important predictor as assessed by general dominance after WFNS, Fisher grade, and treatment status (see Figure 2). It should be noted that in this nomenclature, all the effect of treatment status was due to poor performance of patients not treated and that CRP had a much greater influence on outcome than clipping or coiling, which had almost identical outcomes (Figure 4). Both the SVM and Random Forest models identified WFNS, CRP, and age as most important. This emphasizes the importance of including CRP in future prediction models.

However, logistic regression models including CRP only achieved an R^2 of 27%. This suggests that despite the inclusion of CRP, there still remain other unknown but significant predictors of outcome after aSAH. Further

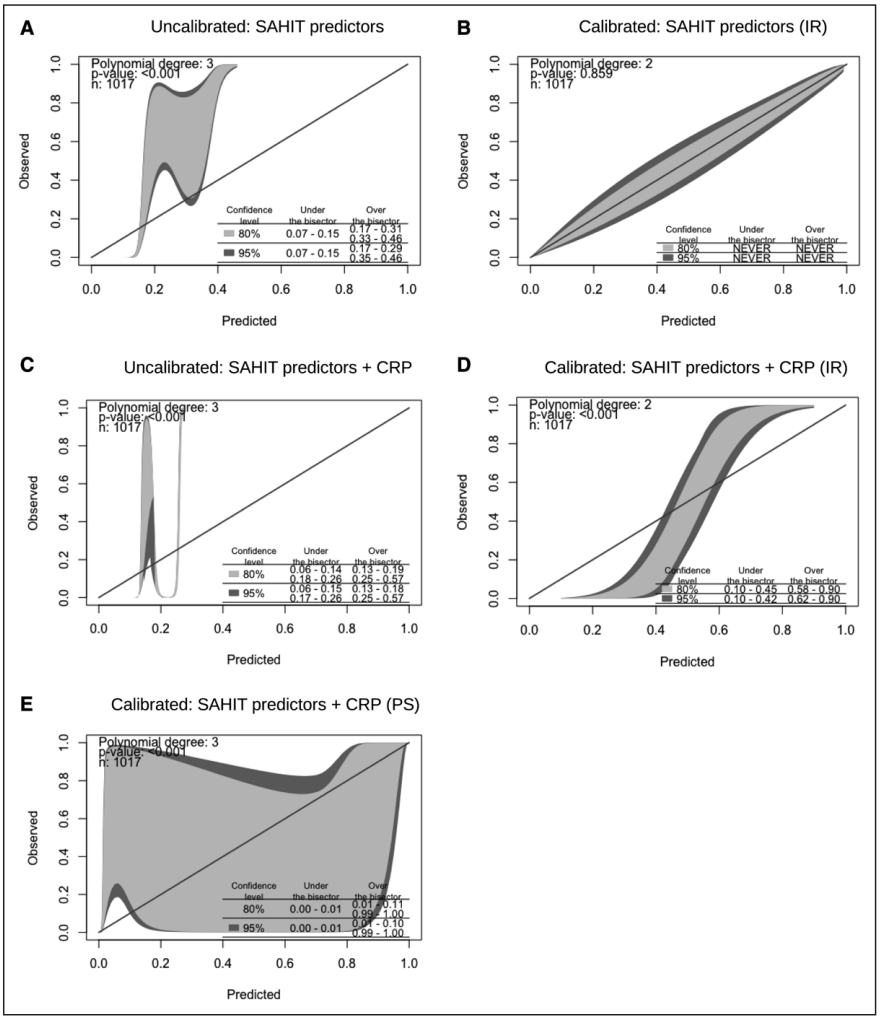

Figure 3. Calibration plots for support vector machine models.
Calibration belt plots for support vector machines trained on SAHIT predictors before and after calibration (A and B) and trained on SAHIT predictors+CRP (C-reactive protein) before and after calibration (**C**, **D**, and **E**). **B** and **D**, Calibrated with isotonic regression (IR) and **E** with Platt scaling (PS). SAHIT indicates Subarachnoid Haemorrhage International Trialists'.

studies need to be designed to look for these unknown predictors if we are to improve prognostication. These studies require unbiased analysis of a wide range of predictors including the consideration of predictors beyond the standard demographic, clinical, and radiological variables, such as proteomics and genetic data.

We have gone on to consider how CRP can best be incorporated into prediction models and demonstrate that incorporation of day 1 CRP to traditional multivariate logistic regression models significantly improves predictive performance above that of the full SAHIT predictive tool.

Random forests did not improve upon logistic regression. This may be inherent to our study design. Random forests are well suited to categorical classification problems with multiple classes and may perform better using Rankin class rather than dichotomization into good and poor outcome.

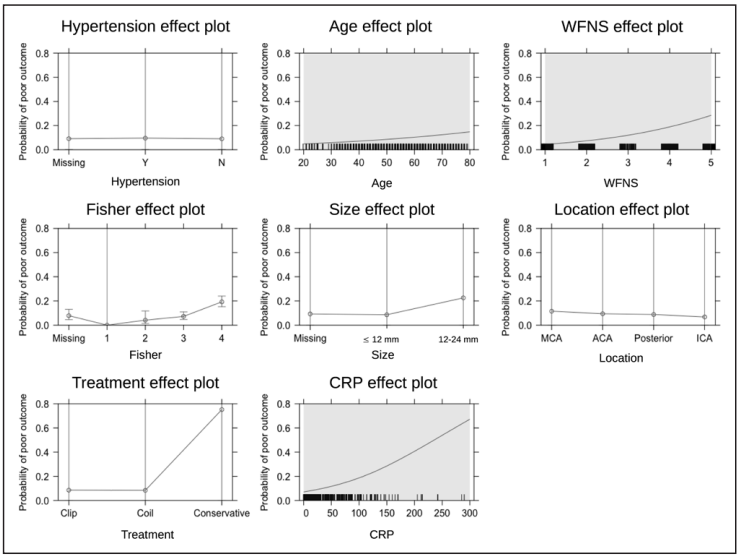

Figure 4. Effect plots for predictors included in multivariable logistic regression model for the first imputed dataset.
CRP indicates C-reactive protein; and WFNS, World Federation of Neurological Surgeons.

The SVM was superior to logistic regression with a marked improvement in discrimination (AUC, 0.960). However, it should be noted that the SVM models had the greatest error on internal validation suggesting overfitting. External validation is necessary to validate the improvements achieved. However, given machine learning methods are of greatest performance benefit in large datasets with high dimensional data and future research is likely to involve larger datasets with more predictors, this observation is relevant and these methods are likely to become increasingly important.[10,11]

None of the logistic regression models showed evidence of miscalibration, but the SVM models did. This could be improved with isotonic regression and Platt scaling. Following calibration, the SVM trained on the SAHIT predictors alone was well-calibrated, but despite clear improvements, the SVM using CRP in addition still showed some evidence of miscalibration (Figure 3). Calibration reduced the AUC of both models but both still markedly outperformed the logistic regression models trained on the same predictors. The fact the model cannot be fully calibrated suggests evidence of overfitting and future studies should ensure SVM models are calibrated on external datasets to minimize this.

Generalisability

In the development of these models, we considered if simplified models, not requiring intensive processing, could be derived. Details of these are available in the Data Supplement. They were unable to capture the improvements seen from utilizing CRP and SVMs. Therefore, use of any of the models in this article would require use of an online calculator.

In the logistic regression models, the addition of CRP to the SAHIT predictors improved outcome prediction (pooled AUC 0.846 compared with 0.831), although statistically significant this is unlikely to influence clinical management on an individual patient basis. Implementation of an online calculator is a significant barrier to adoption and it is notable that even SAHIT itself is mainly used as a research tool, and only routinely used clinically in a minority of institutions. Therefore, given the improvements from addition of the SAHIT predictors to WFNS are larger than that afforded by CRP, this seems unlikely to be widely adopted outside of research.

In order to generate predictive tools, which influence clinical management, they will need to show much greater benefit over WFNS alone. If the magnitude of improvement seen with SVMs can be externally validated, an online calculator with the addition of SVMs as well as CRP may be able to achieve this.

This analysis was limited to patients within 72 hours of ictus and cannot be applied to patients outside of this window. We have shown that CRP (and WFNS) is less predictive on day 0 than either day 1 or 2. It might be expected that CRP becomes confounded by other intervening treatments and complications over time. We have not observed any differences in CRP between coiled and clipped patients on day 1 in this study suggesting this is too early for treatment to be significant. We have,

however, seen such differences at later time points and that the predictive value of CRP drops after day 2 (data not presented). This may explain why previous studies which included patients whose CRP was obtained within 120 hours of ictus have demonstrated that CRP is an independent predictor of outcome in good grade, but not poor grade, patients with aSAH.[9] This distinction is important, as it is poor grade patients where prognostication is of particular importance when guiding potentially invasive and costly treatment strategies, and it would not be possible to use CRP in prognostic models if these only applied to a subgroup of patients.

Limitations

In this study, all patients had CRP and WFNS available on at least one of day 0, 1, or 2. Day 1 CRP and WFNS were used in the analysis and were missing in 450 (44%) and 332 (33%) patients, respectively. Data was missing at random for both day 1 CRP and WFNS with respect to outcome (CRP: P=0.134 [Kruskal-Wallis test]; WFNS: P=0.665 [χ^2 test]). All patients with missing data had day 1 CRP/WFNS imputed using the CRP/WFNS data available from the day before or after (or both). Multiple imputation has been used with pooled AUCs reported. Consequently, although there are missing CRP and WFNS data, robust methodology has been used to account for this. It was not possible to report a DeLong test result to compare pooled AUCs from different predictive models and we, therefore, have reported the result for the first imputed dataset.

A limitation of this study is that the predictive models have not been validated in an external dataset. The models have, however, been internally validated using cross-validation and out-of-bag error. In previous outcome prediction models after aSAH using smaller datasets, a validation set approach dividing data into train and test data sets has commonly been presented. For smaller data sets, this method is superior to cross-validation to avoid overfitting of data; however, as sample sizes increase the utility of cross-validation improves.[27] A sensitivity analysis using a validation set approach with data divided into train and test datasets with proportions 70% and 30% showed error rates consistent with those identified by cross-validation.

Like many studies of outcome in aSAH, this study is limited by its outcome measure, mRS, which was not developed for aSAH and may not be sufficiently sensitive to identify the nuances of aSAH. An SAH-specific outcome score has been developed but is awaiting external validation.[28] The use of scores like this may improve prognostication in this unique condition. This study also uses dichotomized outcome data and future efforts using the full ordinal nature of the mRS may also improve models.

Outcome was recorded at 3 to 6 months in this study in keeping with SAHIT.[4] This range means that these predictive models do not predict outcome at a specific time point. To achieve this, larger datasets with specific outcome times would be required. However, the sensitivity analysis including time of follow-up as a covariate showed no change in the significance of the results, and model performance was not affected.

Some covariates, such as hypertension and aneurysm size, were not available in the STASH data set, which may have influenced the prediction models. This is a limitation of using retrospective data, but may be addressed in external validation and future prospective studies.

Finally, several radiological scales have been shown to have greater predictive value for outcome than the Fisher scale including the modified Fisher Score, Claassen score, Barrow Neurological Institute score, and Hijdra score. Unfortunately, these were not available in this dataset. This has historically been the case for many datasets including SAHIT. However, future studies should use newer scores with greater prognostic value.

Conclusions

This study demonstrates that day 1 CRP is an independent predictor of outcome after aSAH. Its incorporation into predictive models of outcome after aSAH improves model performance over that seen using the SAHIT predictors alone. The machine learning method SVM generates further significant improvement in prognostic modeling, but requires validation and calibration in an external cohort.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Materials

Expanded Methods, Results, and Discussion
Online Tables I–V
Online Figures I–III
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REFERENCES

1. Stegmayr B, Eriksson M, Asplund K. Declining mortality from subarachnoid hemorrhage: changes in incidence and case fatality from 1985 through 2000. *Stroke*. 2004;35:2059–2063. doi: 10.1161/01.STR.0000138451.07853.b6

ORIGINAL CONTRIBUTION

2. Al-Khindi T, Macdonald RL, Schweizer TA. Cognitive and functional outcome after aneurysmal subarachnoid hemorrhage. *Stroke*. 2010;41:e519–e536. doi: 10.1161/STROKEAHA.110.581975
3. Jaja BN, Cusimano MD, Etminan N, Hanggi D, Hasan D, Ilodigwe D, Lantigua H, Le Roux P, Lo B, Louffat-Olivares A, et al. Clinical prediction models for aneurysmal subarachnoid hemorrhage: a systematic review. *Neurocrit Care*. 2013;18:143–153. doi: 10.1007/s12028-012-9792-z
4. Jaja BNR, Saposnik G, Lingsma HF, Macdonald E, Thorpe KE, Mamdani M, Steyerberg EW, Molyneux A, Manoel ALO, Schatlo B, et al; SAHIT collaboration. Development and validation of outcome prediction models for aneurysmal subarachnoid haemorrhage: the SAHIT multinational cohort study. *BMJ*. 2018;360:j5745. doi: 10.1136/bmj.j5745
5. Fountas KN, Tasiou A, Kapsalaki EZ, Paterakis KN, Grigorian AA, Lee GP, Robinson JS Jr. Serum and cerebrospinal fluid C-reactive protein levels as predictors of vasospasm in aneurysmal subarachnoid hemorrhage. Clinical article. *Neurosurg Focus*. 2009;26:E22. doi: 10.3171/2009.2.FOCUS08311
6. Romero FR, Cataneo DC, Cataneo AJ. C-reactive protein and vasospasm after aneurysmal subarachnoid hemorrhage. *Acta Cir Bras*. 2014;29:340–345. doi: 10.1590/s0102-86502014000500009
7. Höllig A, Stoffel-Wagner B, Clusmann H, Veldeman M, Schubert GA, Coburn M. Time courses of inflammatory markers after aneurysmal subarachnoid hemorrhage and their possible relevance for future studies. *Front Neurol*. 2017;8:694. doi: 10.3389/fneur.2017.00694
8. Juvela S, Kuhmonen J, Siironen J. C-reactive protein as predictor for poor outcome after aneurysmal subarachnoid haemorrhage. *Acta Neurochir (Wien)*. 2012;154:397–404. doi: 10.1007/s00701-011-1243-7
9. Turner CL, Budohoski K, Smith C, Hutchinson PJ, Kirkpatrick PJ, Murray GD; STASH collaborators. Elevated baseline C-reactive protein as a predictor of outcome after aneurysmal subarachnoid hemorrhage: data from the simvastatin in aneurysmal subarachnoid hemorrhage (STASH) trial. *Neurosurgery*. 2015;77:786–792. doi: 10.1227/NEU.0000000000000963
10. Bzdok D, Krzywinski M, Altman N. Points of significance: machine learning: a primer. *Nat Methods*. 2017;14:1119–1120. doi: 10.1038/nmeth.4526
11. Bzdok D, Altman N, Krzywinski M. Statistics versus machine learning. *Nat Methods*. 2018;15:233–234. doi: 10.1038/nmeth.4642
12. Dumont TM. Prospective assessment of a symptomatic cerebral vasospasm predictive neural network model. *World Neurosurg*. 2016;94:126–130. doi: 10.1016/j.wneu.2016.06.110
13. Ramos LA, van der Steen WE, Sales Barros R, Majoie CBLM, van den Berg R, Verbaan D, Vandertop WP, Zijlstra IJAJ, Zwinderman AH, Strijkers GJ, et al. Machine learning improves prediction of delayed cerebral ischemia in patients with subarachnoid hemorrhage. *J Neurointerv Surg*. 2019;11:497–502. doi: 10.1136/neurintsurg-2018-014258
14. Hostettler IC, Muroi C, Richter JK, Schmid J, Neidert MC, Seule M, Boss O, Pangalu A, Germans MR, Keller E. Decision tree analysis in subarachnoid hemorrhage: prediction of outcome parameters during the course of aneurysmal subarachnoid hemorrhage using decision tree analysis. *J Neurosurg*. 2018;129:1499–1510. doi: 10.3171/2017.7.JNS17677
15. Liu J, Xiong Y, Zhong M, Yang Y, Guo X, Tan X, Zhao B. Predicting long-term outcomes after poor-grade aneurysmal subarachnoid hemorrhage using decision tree modeling. *Neurosurgery*. 2020;87:523–539. doi: 10.1093/neuros/nyaa052
16. de Toledo P, Rios PM, Ledezma A, Sanchis A, Alen JF, Lagares A. Predicting the outcome of patients with subarachnoid hemorrhage using machine learning techniques. *IEEE Trans Inf Technol Biomed*. 2009;13:794–801. doi: 10.1109/TITB.2009.2020434
17. Shi Z, Hu B, Schoepf UJ, Savage RH, Dargis DM, Pan CW, Li XL, Ni QQ, Lu GM, Zhang LJ. Artificial intelligence in the management of intracranial aneurysms: current status and future perspectives. *AJNR Am J Neuroradiol*. 2020;41:373–379. doi: 10.3174/ajnr.A6468
18. Kirkpatrick PJ, Turner CL, Smith C, Hutchinson PJ, Murray GD; STASH Collaborators. Simvastatin in aneurysmal subarachnoid haemorrhage (STASH): a multicentre randomised phase 3 trial. *Lancet Neurol*. 2014;13:666–675. doi: 10.1016/S1474-4422(14)70084-5
19. van Buuren S, Groothuis-Oudshoorn K. Mice: multivariate imputation by chanied equations in R. *J Stat Softw*. 2011;45:1–67. doi: 10.18637/jss.v045.i03
20. Rubin DB. *Multiple Imputation for Nonresponse in Surveys*. New York: John Wiley and Sons; 2004.
21. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*. 1988;44:837–845.
22. Zhang D. A coefficient of determination for generalized linear models. *Am Stat*. 2017;71:310–316.
23. Kraha A, Turner H, Nimon K, Zientek LR, Henson RK. Tools to support interpreting multiple regression in the face of multicollinearity. *Front Psychol*. 2012;3:44. doi: 10.3389/fpsyg.2012.00044
24. Nattino G, Finazzi S, Bertolini G. A new calibration test and a reappraisal of the calibration belt for the assessment of prediction models based on dichotomous outcomes. *Stat Med*. 2014;33:2390–2407. doi: 10.1002/sim.6100
25. Huang Y, Li W, Macheret F, Gabriel RA, Ohno-Machado L. A tutorial on calibration measurements and calibration models for clinical prediction models. *J Am Med Inform Assoc*. 2020;27:621–633. doi: 10.1093/jamia/ocz228
26. van Donkelaar CE, Bakker NA, Veeger NJ, Uyttenboogaart M, Metzemaekers JD, Eshghi O, Mazuri A, Foumani M, Luijckx GJ, Groen RJ, et al. Prediction of outcome after subarachnoid hemorrhage: timing of clinical assessment. *J Neurosurg*. 2017;126:52–59. doi: 10.3171/2016.1.JNS152136
27. Vabalas A, Gowen E, Poliakoff E, Casson AJ. Machine learning algorithm validation with a limited sample size. *PLoS One*. 2019;14:e0224365. doi: 10.1371/journal.pone.0224365
28. Pace A, Mitchell S, Casselden E, Zolnourian A, Glazier J, Foulkes L, Bulters D, Galea I. A subarachnoid haemorrhage-specific outcome tool. *Brain*. 2018;141:1111–1121. doi: 10.1093/brain/awy003
29. Zhang Z, Zhang H, Khanal MK. Development of scoring system for risk stratification in clinical medicine: a step-by-step tutorial. *Ann Transl Med*. 2017;5:436. doi: 10.21037/atm.2017.08.22

A.4 Chapter 4

A.4.1 Publication 6: Haemoglobin scavenging in intracranial bleeding: biology and clinical implications

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I am joint first author. This review was authored by members of the HATCH consortium. All co-authors summarised the literature relating to a component of the review topic. This information was then assimilated into the final manuscript which was written in three sections. I authored one of the sections with the remaining two written by Ian Galea and Diederik Bulters. All co-authors contributed to subsequent drafts of the manuscript.

Specific contribution: Primary role in manuscript authorship alongside Ian Galea and Diederik Bulters.

REVIEWS

Haemoglobin scavenging in intracranial bleeding: biology and clinical implications

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Abstract | Haemoglobin is released into the CNS during the breakdown of red blood cells after intracranial bleeding. Extracellular free haemoglobin is directly neurotoxic. Haemoglobin scavenging mechanisms clear haemoglobin and reduce toxicity; these mechanisms include erythrophagocytosis, haptoglobin binding of haemoglobin, haemopexin binding of haem and haem oxygenase breakdown of haem. However, the capacity of these mechanisms is limited in the CNS, and they easily become overwhelmed. Targeting of haemoglobin toxicity and scavenging is, therefore, a rational therapeutic strategy. In this Review, we summarize the neurotoxic mechanisms of extracellular haemoglobin and the peculiarities of haemoglobin scavenging pathways in the brain. Evidence for a role of haemoglobin toxicity in neurological disorders is discussed, with a focus on subarachnoid haemorrhage and intracerebral haemorrhage, and emerging treatment strategies based on the molecular pathways involved are considered. By focusing on a fundamental biological commonality between diverse neurological conditions, we aim to encourage the application of knowledge of haemoglobin toxicity and scavenging across various conditions. We also hope that the principles highlighted will stimulate research to explore the potential of the pathways discussed. Finally, we present a consensus opinion on the research priorities that will help to bring about clinical benefits.

Porphyrin ring
A complex aromatic chemical structure consisting of four modified pyrrole rings with the capacity to coordinate a central metal ion; protoporphyrin IX is the porphyrin in haemoglobin.

Degradation of red blood cells (RBCs) after haemorrhage and intravascular or extravascular haemolysis results in extracellular release of haemoglobin. Within the CNS, cell-free haemoglobin and its breakdown products are neurotoxic and cause secondary brain injury[1,2]. Two scenarios can lead to haemoglobin coming into direct contact with brain parenchyma: vascular rupture (as occurs during various forms of intracranial haemorrhage) and low-grade leakage of haemoglobin into the brain. Haemoglobin-induced neurotoxicity is thought to have an important role in the pathophysiology of several neurological conditions. These conditions were traditionally thought to be those characterized by acute macroscopic bleeding, such as intracranial haemorrhage, but evidence suggests that extracellular haemoglobin also plays an important role in several neurological conditions, including superficial siderosis[3], Alzheimer disease[4] and progressive multiple sclerosis[5].

In this Review, we consider the emerging importance of extracellular haemoglobin in the brain and its clinical implications. First, we present the cellular and molecular mechanisms of haemoglobin neurotoxicity and clearance, focusing on the special environment of the CNS. Subsequently, we focus on the clinical implications of extracellular haemoglobin within the CNS, reviewing the evidence that links the pathophysiology of cell-free haemoglobin to the clinical presentations and outcomes of several neurological conditions. Finally, we discuss current and emerging treatment strategies designed to augment the clearance of haemoglobin and/or alleviate its toxicity. We also present the outcomes of a meeting of an expert panel in which a consensus opinion on the key research questions that need to be answered was formulated. We hope that the principles highlighted will encourage research into the effects of extracellular haemoglobin in the brain and exploration of the potential for clinical benefits.

Neurotoxicity of haemoglobin

Haemoglobin consists of four globin chains tightly associated with a haem group, which itself consists of a porphyrin ring that coordinates an iron atom in the Fe^{2+}, Fe^{3+} or Fe^{4+} oxidative state. When outside RBCs, haemoglobin

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Key points

- Extracellular haemoglobin induces cell death, mainly via oxidation and inflammation.
- Mechanisms in the brain that can mitigate haemoglobin toxicity and enable its clearance are easily overwhelmed by extensive haemolysis after intracranial haemorrhage.
- Variation in genes related to haemoglobin binding and metabolism influence outcomes after subarachnoid haemorrhage or intracerebral haemorrhage.
- Treatments that mitigate haemoglobin toxicity and increase clearance might have clinical benefits for patients with intracranial bleeding.
- Research priorities include prospective genetic association studies and an improved basic scientific understanding of the mechanism of haemoglobin toxicity and its clearance from the brain.

is oxidized to methaemoglobin and dissociates into dimers. Subsequent modification of globin forms hemichromes, and these degrade into haem, which releases iron. The toxicity of haemoglobin is undisputed — neurotoxicity is observed in vitro[1] and after intracerebral injection of haemoglobin in vivo[2]. Neurons seem to be more vulnerable to this toxicity than glia[1]. The toxicity of haemoglobin is multifactorial but mainly seems to be mediated by four factors: oxidation, inflammation, nitric oxide scavenging and oedema.

Oxidation

Haem mediates oxidation: in its ferrous (Fe^{2+}) and ferric (Fe^{3+}) states, haem can react with hydrogen peroxide (which is released by neutrophils, for example) or endogenous lipid hydroperoxides (which are formed by lipoxygenases) to form a highly reactive ferryl (Fe^{4+}) form. Such reactions of ferric haem also form a globin-based radical[6]. Ferryl haem and globin-based radicals react directly with lipids and proteins to form free radicals, which self-propagate in the presence of molecular oxygen.

Free radicals lead to destructive modification of membranes, lipids, proteins and nucleic acids and their associated machinery, thereby critically altering cellular and organ function[7]. Molecular signatures of this process, including covalently modified proteins[8] and oxidized lipids[9], have been detected in the cerebrospinal fluid (CSF) after subarachnoid haemorrhage (SAH). Free haem is thought to be more toxic than haemoglobin, as its lipophilicity enables its intercalation into membranes[10].

Haem releases Fe, which drives cell death via Fe-dependent lipid oxidation — known as ferroptosis[11] — which can be suppressed by the lipid reactive oxygen species inhibitor ferrostatin 1, iron chelators and lipophilic antioxidants.

Inflammation

The pro-inflammatory effects of haemoglobin breakdown products are mediated in several ways. Methaemoglobin[12] and haem[13] are ligands of Toll-like receptor 4 (TLR4), which is expressed by microglia and macrophages; activation of TLR4 causes these cells to secrete tumour necrosis factor (TNF), triggering nuclear factor-κB (NF-κB) activation, inflammation and necroptosis[14]. Haem can also activate the NLRP3 (NOD-, LRR- and pyrin domain-containing 3) inflammasome, leading to IL-1β synthesis[15], and can induce IL-1α production from mixed glia in culture, which in turn causes neuronal death[16].

Nitric oxide depletion

Nitric oxide is depleted by haemoglobin in a process that is rapid and irreversible. In this process, reaction of nitric oxide with oxyferrous haemoglobin forms methaemoglobin and nitrate[17]. Nitric oxide is also consumed by oxygen radicals after SAH[18]; for example, nitric oxide can react with superoxide to form peroxynitrite. Endothelial nitric oxide synthase that is exposed to peroxynitrite does not function normally and produces superoxide instead of nitric oxide[19,20]; this superoxide causes further nitric oxide consumption.

Nitric oxide is a vasodilator produced by endothelial cells, neurons and microglia. It regulates cerebral vascular tone[21] and is a potent platelet inhibitor. Several adverse effects of reduced nitric oxide as a result of haemoglobin-mediated depletion have been reported. Evidence suggests that depletion of nitric oxide contributes to microthrombosis[22] in cerebral vessels in SAH in humans and animal models, and is associated with poor clinical outcomes[23–25]. Reduced bioavailability of nitric oxide lowers the threshold for cortical spreading depolarization[26], an electrical phenomenon observed in the presence of high potassium and haemoglobin levels[27], which leads to spreading ischaemia and neuronal death. Nitric oxide bioavailability at the vasculature is also important in vasospasm; observations of haemoglobin-induced vasospasm in the acute phase of preclinical SAH models[28–30] gave rise to the long-held belief that an absolute cerebral nitric oxide deficiency was the cause, but evidence that nitric oxide metabolism is phasic after SAH has changed this view[31–37]. Nitric oxide depletion has not been studied in intracerebral haemorrhage, despite evidence that a reduction in perihaematomal blood flow is delayed[38] (as would be expected secondary to nitric oxide depletion) and unresponsive to osmotic therapy[39] (suggesting that the reduced blood flow is unrelated to high intracranial pressure).

Oedema

Several lines of evidence show that cerebral oedema is induced by haemoglobin and its breakdown products. Intraparenchymal injection of haemoglobin breakdown products increased the water and sodium

Hemichromes
Haemoglobin molecules that can no longer react with oxygen, characterized by the binding of the distal histidine to the central Fe atom; hemichromes can occur spontaneously and reversibly, but denaturation of haemoglobin alters its quaternary structure, facilitating the process and forming irreversible hemichromes.

Globin-based radical
Reactions between Fe^{3+} and peroxides extract an electron from the protein globin chain, leaving it in the radical state (with an unpaired electron), making it highly reactive; this free radical is commonly on the phenoxyl group of a tyrosine residue, and can migrate.

Necroptosis
A form of regulated cell death, morphologically akin to necrosis, triggered by TNF and regulated by caspase 8; it is dependent on receptor-interacting protein kinases 1 and 3.

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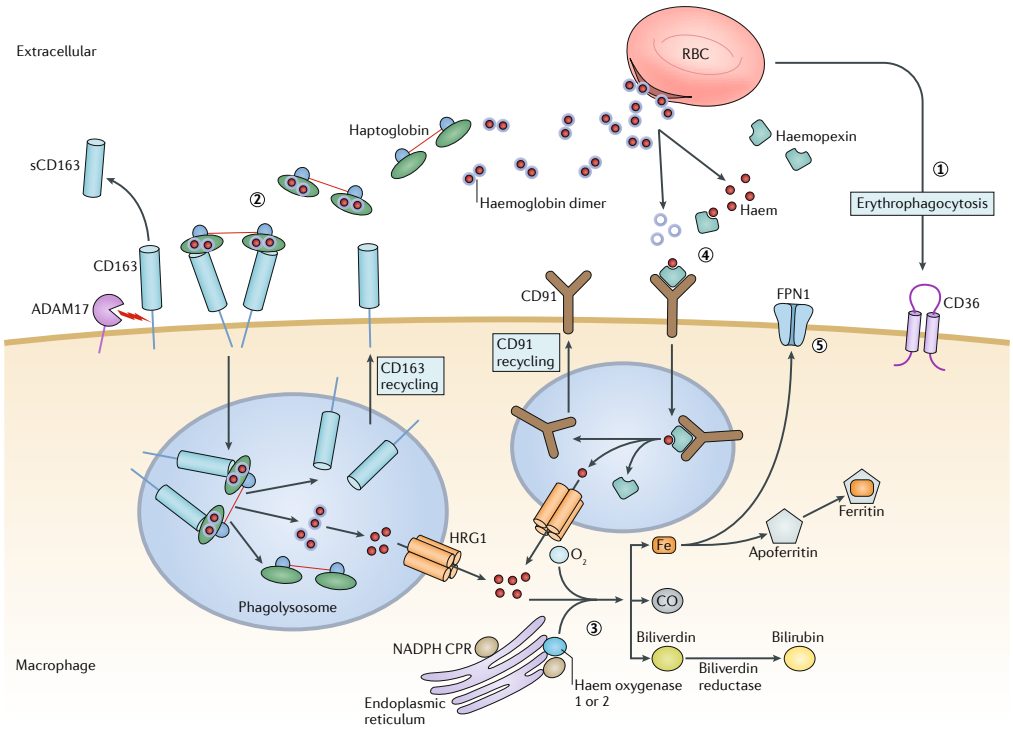

Fig. 1 | **Haemoglobin scavenging pathways in humans.** Multiple receptor mediated-pathways can prevent the toxicity of haemoglobin and its breakdown products haem and iron. Red blood cells (RBCs) can be directly phagocytosed following detection by the CD36 receptor on macrophages (step 1). Free haemoglobin dimers can be captured by haptoglobin, and the haptoglobin–haemoglobin complex can be taken up by macrophages via CD163 (step 2). CD163 can be shed from the cell surface after subarachnoid haemorrhage owing to the action of the enzyme disintegrin and metalloproteinase domain-containing protein 17 (ADAM17), and this process might reduce the efficiency of haemoglobin scavenging. In the phagolysosome, haemoglobin is then broken down to release haem, which is in turn broken down by haem oxygenases to generate iron (step 3). Similarly, free extracellular haem can be captured by haemopexin and transported into macrophages via CD91 (step 4), where it is also broken down to produce free iron (step 3). The resulting iron is transported from macrophages via the ferroportin 1 (FPN1; also known as SLC40A1) channel (step 5). HRG1, haem-responsive gene 1 protein homologue (also known as SLC48A1); NADPH–CPR, NADPH–cytochrome P450 reductase; sCD163, soluble CD163.

content in the brains of rats[40]. After haemoglobin injection into the brains of rats, matrix metalloproteinase 9 (MMP-9) was upregulated, resulting in blood–brain barrier (BBB) disruption[41]; acute MMP-9 inhibition attenuated the consequent cerebral oedema[42]. For these reasons, delayed perihaematomal oedema in intracerebral haemorrhage is thought to be at least partly caused by haemoglobin and its breakdown products[43]. Cerebral oedema is more marked in intracerebral haemorrhage than in SAH[44], possibly because haemoglobin is in a parenchymal location after intracerebral haemorrhage and is therefore closer to the BBB. In keeping with this speculation, delayed global cerebral oedema is less common after SAH[44] and is seen in association with diffuse ischaemia[45], suggesting a different pathophysiology.

Haemoglobin clearance

Several endogenous mechanisms have the potential to clear the haemoglobin, haem and iron that are released into the brain following a haemorrhage and RBC lysis (FIG. 1). These mechanisms are discussed below.

Erythrophagocytosis

Erythrophagocytosis is one mechanism by which abnormal RBCs are cleared. Abnormal RBCs exteriorize phosphatidylserine, and evidence suggests that macrophages recognize phosphatidylserine via CD36, leading to erythrophagocytosis[46,47]. Iron and haem are subsequently exported from the macrophages to the extracellular milieu via, for example, ferroportin[48] and feline leukaemia virus subgroup C receptor 1 (FLVCR1)[49],

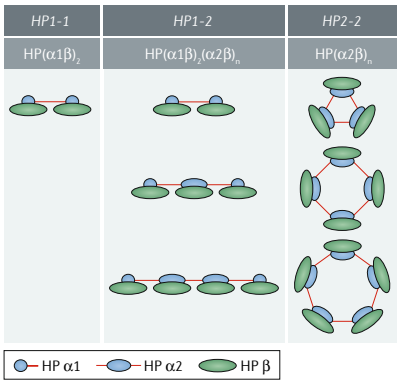

Fig. 2 | **Isoforms and possible structures of haptoglobin according to genotype.** Haptoglobin is encoded by the *HP1* and *HP2* alleles. *HP1* encodes an α1-chain and a β-chain that are linked by a disulfide bond. The α1-chain has another free cysteine residue (red) that enables dimerization to form the $HP(\alpha1\beta)_2$ dimer (left). This dimer is the only form of haptoglobin present in homozygotes for *HP1*. *HP2* encodes a duplicated α-chain called α2 and a β-chain. The α2-chain has two free cysteines that enable formation of cyclic polymers of increasing size, HP(α2β)n, where $n \geq 3$ (right). These cyclic polymers are found in homozygotes for *HP2*. In heterozygotes that express *HP1* and *HP2*, linear polymers form $HP(\alpha1\beta)_2(\alpha2\beta)n$, where $n \geq 0$ (REF.[218]).

respectively. Estimates suggest that 40% of the iron originating from haemoglobin can be released within 24 h of RBC uptake by macrophages. Although erythrophagocytosis might seem to be an effective clearance mechanism, macrophages that ingest more than two RBCs undergo cell death, leading to release of deleterious haem and iron into the extracellular matrix[50]. Consequently, the contribution of erythrophagocytosis to the prevention of haem-derived toxicity is probably small.

Haptoglobin and CD163

Extravasated RBCs that avoid erythrophagocytosis undergo spontaneous lysis as a result of, for example, free radical damage or complement-mediated attack[51], leading to release of haemoglobin. In this situation, the haemoglobin dimer is immediately and irreversibly bound by haptoglobin, an acute-phase glycoprotein, in one of the strongest noncovalent interactions known to occur naturally[52].

Three types of haptoglobin polymers exist (FIG. 2), generated by combined expression of two alleles: *HP1* and *HP2*. The three polymer types are referred to as HP1-1, HP1-2 and HP2-2. The functional differences between the different haptoglobin types are unclear and controversial (BOX 1). Functional differences might also differ between the CNS and the periphery as a result of CNS specialization (BOX 2). Resolving the controversies around the functional differences is important because haptoglobin could have potential as a therapeutic agent, and functional differences have implications for the selection of haptoglobin types to be tested in clinical trials.

Haptoglobin is mainly synthesized by the liver and reticuloendothelial system and is not normally synthesized within the brain. Instead, haptoglobin diffuses into the CSF from the blood, meaning that higher polymeric forms of the protein are not present in the CSF in the healthy state[53] and levels of haptoglobin in the brain are much lower than levels in the blood in the healthy state[54]. Within each millilitre of intracranial blood, the molar amount of cell-free haemoglobin produced by haemolysis is approximately 250-fold greater than the amount that the haptoglobin present in the same volume is able to bind to.

Current evidence points to two main functions of the haptoglobin–haemoglobin complex: protection against redox activity[55–58] and haemoglobin clearance via the CD163 membrane receptor[59]. Crystal structure analyses of the haptoglobin–haemoglobin complex have revealed reactive iron and pro-oxidative tyrosine residues close to the haptoglobin–haemoglobin interface[60], structural features that explain the ability of haptoglobin to prevent oxidative reactions and delay the release of haem. The binding of haptoglobin by haemoglobin exposes a neoepitope on the haptoglobin β-chain[61] that enables CD163 to recognize the complex and initiate endocytosis. The affinity of CD163 for the haptoglobin–haemoglobin complex is tenfold higher than for uncomplexed haemoglobin[62].

Some evidence suggests that astrocytes[63] and oligodendrocytes[64] express haptoglobin in pathological states. Given that oligodendrocytes and astrocytes are so abundant, local production of haptoglobin by these cells is an endogenous mechanism with potential to protect the brain against extravascular haemoglobin toxicity. However, this mechanism is clearly not enough, as most haemoglobin in the CSF after SAH is not bound to haptoglobin[54]. Furthermore, given that haptoglobin protects haemoglobin from auto-oxidation and that haemoglobin auto-oxidation interferes with haptoglobin binding, the scarcity of haptoglobin might render haemoglobin unscavengeable by CD163 and haptoglobin with time[65]; the extent to which this occurs requires further study.

One study has suggested that the lack of haptoglobin in the brain is compounded by saturation of the CD163 uptake system, as haptoglobin–haemoglobin complexes were detected in the CSF after SAH[54]. Intrathecal shedding of membrane-bound CD163 was also observed (FIG. 1), which resulted in high levels of soluble CD163 (sCD163) in the CSF after SAH[54] and is likely to exacerbate saturation of the uptake system. However, sCD163 might be important in haptoglobin-independent pathways that detoxify free haemoglobin under conditions of severe haemolysis, such as haemorrhage. sCD163 and immunoglobulin G (IgG) can interact with free haemoglobin, and the sCD163–haemoglobin–IgG complex undergoes endocytosis into monocytes via the crystallizable fragment (Fc)-γ receptor[66].

Haemopexin and CD91

Unscavenged haemoglobin releases haem, which is sequestered with very high affinity by haemopexin[67]. Haemopexin is expressed by neurons and glia[68]; one study has shown that ~90% of all haemopexin in the

Acute-phase glycoprotein
A protein that is secreted in large amounts by the liver during acute systemic inflammation.

Box 1 | **Functional differences between haptoglobin types**

Many studies have investigated the functional differences between the HP1-1, HP1-2 and HP2-2 types of haptoglobin, which are expressed by homozygotes for the *HP1* allele and heterozygotes and homozygotes for the *HP2* allele, respectively; these studies have been reviewed elsewhere[103]. Expression levels differ reproducibly by type: haptoglobin expression is greater with the genotype *HP1-1* than expression with the genotype *HP1-2*, which is in turn greater than that with the genotype *HP2-2* (REF.[203]). Some studies[204–206] have shown that HP1-1 is associated with better reduction of the redox potential of haemoglobin than is HP2-2 (REFS[204–206]), but other studies have not replicated this observation[175,207,208]. In complex with haemoglobin, HP2-2 seems to have a higher binding affinity for CD163 than does HP1-1 (REFS[59,209]). With respect to CD163-mediated cellular uptake of haptoglobin–haemoglobin complexes, different studies have indicated that HP1-1 is more effective, that HP2-2 is more effective and that they are equally effective[175,209,210], suggesting that there is little or no difference in this aspect of haptoglobin function. Secretion of anti-inflammatory IL-10 in response to CD163 binding of the haptoglobin–haemoglobin complex differs according to type: secretion is several-fold higher with HP1-1 than with HP2-2 (REF.[211]).

brain is produced intrathecally in the healthy state[69]. This production in the brain is in sharp contrast to that of haptoglobin, suggesting that the brain is geared towards clearing haem more effectively than haemoglobin. However, CSF levels of haemopexin are tenfold lower than levels in the circulation, suggesting that the capacity for haem binding in the brain is relatively low[69]. The haem–haemopexin complex binds to CD91 (also known as LRP-1), which is expressed in neurons and glia[70] and is subsequently cleared by endocytosis[71] (FIG. 1). In a study of 30 patients with SAH, free haem was still detectable in the CSF after SAH[69], indicating that the haemopexin–CD91 system cannot cope with the demands imposed on it after SAH.

After endocytosis by any cell type, haem is degraded by haem oxygenase, resulting in production of equimolar amounts of iron, carbon monoxide and biliverdin (FIG. 1). Biliverdin is then converted to bilirubin by biliverdin reductase[72]. Two active isoforms of haem oxygenase exist: haem oxygenase 1, the expression of which is induced by haem and other forms of oxidative stress, mostly within glia, macrophages and endothelial cells[73], and haem oxygenase 2, which is constitutively expressed in most cell types, including neurons[74]. These enzymes are catalytically active only inside cells, as they require the microsomal NADPH–cytochrome P450 reductase to cleave haem[72]. Excess iron produced during this reaction is either transported out of cells (for example, via ferroportin[75]) or rapidly stored within ferritin — a heteropolymeric cage that can store up to 4,500 ferric ions as ferrihydrite aggregates in its core[76] — in a safe but readily available form[77]. The reactive nature of noncomplexed iron means it has the potential to cause intracellular toxicity while it is shuttled inside cells. When the size of this so-called cellular labile iron pool[78] increases, it can, for instance, participate in the Fenton reaction[79] and activate the NLRP3 inflammasome[80]. Under conditions of iron overload, breakdown of ferritin within the lysosomes results in intracellular deposition of haemosiderin, which is insoluble but might not be redox inert[76], so could be detrimental.

Haemoglobin uptake by macrophages converts them to a so-called Mhem phenotype, with antioxidative properties and reduced inflammatory cytokine expression[81,82] but still an undesirable overall profile that results in increased vascular permeability and microvascular inflammation[83]. Moreover, phagocytosis of iron-laden cell carcasses and debris results in iron-laden macrophages and microglia, which are pro-inflammatory[84,85]. Hence, although haemoglobin scavenging reduces the inflammatory effects of haemoglobin, some of these effects still occur.

Fenton reaction
A reaction in which Fe^{2+} causes disproportionation of a peroxide bond (in hydrogen peroxide or organic peroxides) to produce highly reactive hydroxyl (·OH) and hydroperoxyl (·OOH) radicals.

Microparticles
Micrometre-sized membrane-bound vesicles released by a variety of cell types; microparticles retain the molecules associated with, or embedded in, the original membranes, and these molecules retain their function, thus acting in a paracrine fashion.

Haemoglobin in neurological disorders

Extravascular haemoglobin and its breakdown products have been implicated in the pathophysiology of several neurological conditions, with evidence ranging from very strong in some to very weak in others. In the following sections, we review the evidence implicating haemoglobin in several conditions, focusing on conditions that produce macroscopic extravascular blood, in which the evidence is most robust. Animal studies are cited where evidence in humans is lacking or where experimental intervention has proven causality suggested by human observational data.

Subarachnoid haemorrhage

Neurological injury after SAH occurs in two stages: early brain injury (within 72 h) and delayed brain injury, including delayed cerebral ischaemia, which presents days to weeks after haemorrhage. Early brain injury is associated with a transient increase in intracranial pressure and a decrease in cerebral blood flow, resulting in initiation of a toxic cascade that includes global cerebral ischaemia and cerebral oedema[44,86,87]. The presence of haemoglobin and its breakdown products in the brain along with the toxic cascade initiated during early brain injury is thought to have a key role in the development of delayed brain injury[88].

Reported CSF concentrations of haemoglobin after SAH vary from 3 μM to 250 μM (REFS[36,89–93]). Some of this variation is likely to be due to the time points at which haemoglobin levels were measured and the specific species of haemoglobin being measured; the average of these reported values is ~20 μM. Haem can also be detected in CSF after SAH; in one study, the concentration was ~100 μg/ml (REF.[69]). Ferritin levels in the CSF also increase markedly as early as 1 day after SAH: the upper limit of the reference range is 12 ng/ml, and the mean concentration on day 11 after SAH was 1,750 ng/ml (REFS[94,95]). In addition, CSF iron levels increase from a mean of 2.3 μg/dl in controls to 27.9 μg/dl on day 5 after SAH, with no apparent further increase[95].

Haptoglobin levels in human CSF rise rapidly after SAH, as expected after an injection of blood into the subarachnoid space, before declining, presumably owing to clearance of haptoglobin–haemoglobin complexes[96,97]. A subsequent rise in haptoglobin levels has been observed, accompanied by a parallel rise in haemoglobin, suggesting saturation of the CD163-mediated scavenging pathway[54,96]; the precise contribution of intrathecal haptoglobin synthesis to the haptoglobin rise remains to be determined. Haptoglobin has been detected in association with microparticles in the CSF after SAH, and levels are higher in patients with vasospasm than in patients without[97]. The precise source

Box 2 | **CNS specialization and haemoglobin scavenging**

The anatomical specialization of the brain reduces the first-line defence against the toxicity of extracellular haemoglobin via haptoglobin and CD163 scavenging for several reasons, discussed below.

The blood–brain barrier

The blood–brain barrier, although not an absolute barrier[212], imposes a size restriction on molecules that can diffuse from the blood into the brain parenchyma[213]. Haptoglobin is mainly produced by the liver and granulocytes[214] and is not normally synthesized within the intrathecal compartment. For these reasons, the presence of polymeric forms of haptoglobin (FIG. 2) depends on blood–brain barrier permeability[53].

Solute drainage

The brain's drainage system for interstitial fluid and solutes is size-selective, and clearance of molecules with a molecular mass >200 kDa is limited[215–217]. The size of the simplest dimeric form of haptoglobin in complex with haemoglobin is below this threshold (162 kDa), but the highest-order polymer of haptoglobin[218] saturated with haemoglobin is 1,760 kDa. Drainage of ovalbumin–immunoglobulin G immune complexes with molecular masses of 500–2,000 kDa (REF.[219]) is severely impeded[220]; therefore, exit of haemoglobin in complex with larger haptoglobin species from the brain would be restricted.

Immunological privilege

In the uninflamed state, the brain contains few CD163-positive macrophages and no granulocytes[221]. During inflammation, myelomonocytic entry into the brain occurs but is delayed and low in magnitude[222]. These limitations could limit the number of CD163 binding sites available to scavenge haptoglobin–haemoglobin complexes[54] and limit haptoglobin upregulation in the brain compared with elsewhere.

Cellular traffic

The reticuloendothelial system is responsible for haemoglobin clearance. Although a route for cellular traffic out of the brain via lymphatic drainage of the cerebrospinal fluid exists[223], no high-throughput monocyte or macrophage exit system from the brain has been found.

of this microparticle-derived haptoglobin is yet to be studied.

Haptoglobin genotype has been associated with outcomes after SAH. Specifically, the *HP2* allele has been associated with an increased risk of cerebral vasospasm in several observational studies, although these studies were assessed as being at moderate risk of bias (Supplementary Table 1)[54,98–100]. In a prospective cohort study that included 133 patients, the *HP2* allele was associated with increased cerebral salt wasting after SAH (OR 4.94, 95% CI 1.78–17.43, $P = 0.01$)[101]. In one study that included 193 patients with SAH, the *HP2* allele was also linked to worse functional outcomes[102], although other smaller studies have not demonstrated this link[99–101]. A meta-analysis published in 2017 indicated that the *HP2* allele confers a small effect on short-term outcomes but not on long-term outcomes[103], although the studies included were small, varied in design and did not include correction for prognostically relevant covariates. For these reasons, the conclusion should be viewed with caution until the results of an ongoing analysis of data from these studies at the individual patient level are known[104], or until a larger, well-designed, prospective cohort study is conducted. In a mouse model of SAH, animals that were homozygous for a murine equivalent of the human *HP2* allele experienced more marked cerebral vasospasm than wild-type mice that were homozygous for *HP1*[105]. However, the effect of haptoglobin differs between humans and mice: haptoglobin increases the affinity of haemoglobin for CD163 in humans but not in mice[59,62], so the mechanism underlying the observed difference in vasospasm between the transgenic and wild-type mice is unclear. In humans, haptoglobin facilitates haemoglobin uptake into cells, so differences in iron retention might occur if one haptoglobin isoform is better than the other at scavenging haemoglobin. Investigation of the CD91–haem–haemopexin scavenging pathway after SAH in humans has produced surprising results. In one small prospective cohort study with 30 participants, CSF levels of haemopexin were elevated to a mean of 133.8 μg/ml in one-third of patients with SAH; the upper limit among healthy controls was 32.6 μg/ml. Among the patients with elevated haemopexin levels, the rate of delayed cerebral ischaemia was higher and functional outcomes were poorer than among patients with normal haemopexin levels[69]. Given that haemopexin is presumed to have a neuroprotective effect, these results are unexpected, and further understanding of this system and its role in SAH is required.

A role for haem oxygenase 1 after SAH has been indicated by various studies. In one study, haem oxygenase 1 mRNA expression was increased in cells in human CSF, and this increase correlated with haematoma volume[106]. In small studies, elevated CSF levels of haem oxygenase after SAH in humans have been associated with an increased incidence of vasospasm[92] and unfavourable functional outcomes[93]; however, as haem oxygenase 1 is an intracellular enzyme, these studies are complicated by the fact that detection of the enzyme might purely reflect tissue damage. By contrast, a similarly sized study of CSF from humans after SAH indicated a reduced incidence of vasospasm among patients with elevated levels of ferritin and bilirubin, which are products of haem oxygenase 1 activity[95]. In animal models of SAH, haem oxygenase 1 overexpression in brain tissue protected against vasospasm[107] and suppression of haem oxygenase activity increased cerebral vasospasm and neuronal apoptosis[106,108]. The conflicting results in humans clearly demonstrate that the effects of haem oxygenase 1 activity in humans after SAH need further study.

Intracerebral haemorrhage

Intracerebral haemorrhage leads to primary and secondary neurological injury. The primary injury results from a rapid rise in brain volume and intracranial pressure due to haematoma formation. Secondary injury develops as a result of the physical effects of the initial haemorrhage, an inflammatory response to the haematoma and the release of blood breakdown products[109]. Perihaematomal oedema is a key component of the secondary injury; this phenomenon follows a triphasic pattern that comprises early ionic oedema followed by early vasogenic oedema and delayed vasogenic oedema. Delayed vasogenic oedema is thought to be driven by blood breakdown products[43]. Evidence from studies in humans and animals indicates that haemoglobin scavenging pathways are active after intracerebral haemorrhage and are linked to outcomes.

The haptoglobin–CD163 scavenging system has been shown to be active after intracerebral haemorrhage. In a prospective cohort study that had a low risk of bias

(Supplementary Table 1) and included 94 patients with intracerebral haemorrhage, the *HP2* allele was associated with a reduced chance of a favourable outcome (OR 0.13, $P = 0.018$) after intracerebral haemorrhage but was not associated with mortality after intracerebral haemorrhage[110]. Evidence from animal models demonstrates that haptoglobin is protective after intracerebral haemorrhage, as induction of hypohaptoglobinaemia and genetic knockout of haptoglobin in rodent models results in worse neurological deficits after intracerebral haemorrhage than in untreated and wild-type animals[64,111].

In human brain tissue harvested from around intracerebral haemorrhage haematomas[112] and in animal models of intracerebral haemorrhage[113], increased levels of CD163-positive microglia and macrophages have been detected. In a study of 54 patients with intracerebral haemorrhage, serum sCD163 levels above the average level in the group were associated with increased haematoma absorption and improved neurological recovery[114]. In an animal study, knockout of CD163 in a mouse model of intracerebral haemorrhage led to early beneficial effects but harmful late effects, indicating biphasic functions of CD163 in the mouse that are yet to be fully eludicated[115]. In murine neuronal cultures exposed to haemoglobin and after intracerebral injection of autologous blood in piglets, neuronal expression of CD163 was associated with neuronal toxicity, which was proposed to be related to neuronal uptake of toxic haemoglobin[116,117]. Neuronal CD163 expression has yet to be confirmed in humans. Nevertheless, the evidence together indicates that the haptoglobin–CD163 pathway has an important protective role in intracerebral haemorrhage. However, if manipulation of this pathway is considered as a therapeutic strategy, care must be taken to ensure that the potentially protective role of CD163-positive microglia and macrophages is not counteracted by toxicity caused by iron overload in the brain.

Evidence from animal studies indicates that the haemopexin–CD91 scavenging pathway provides neuroprotection after intracerebral haemorrhage. In a study of a mouse model of intracerebral haemorrhage, haemopexin and CD91 levels within brain tissue were elevated, and administration of recombinant CD91 reduced various pathological indices and neurological deficits[118]. In haemoglobin injection and collagenase injection mouse models of intracerebral haemorrhage, haemopexin knockout led to greater neuronal damage and worse outcomes than in mice that expressed haemopexin[119,120]. Further supporting evidence comes from a study in which intracerebroventricular injection of a haemopexin-encoding recombinant adeno-associated viral vector to induce overexpression of haemopexin in a mouse model of intracerebral haemorrhage resulted in improved functional outcomes relative to outcomes in mice that received a control vector[121].

The role of haem oxygenases in haemoglobin scavenging after intracerebral haemorrhage seems to vary according to the isoform. Upregulation of the inducible haem oxygenase 1 in the brain, notably in the microglia and astrocytes, has been demonstrated in animal models of intracerebral haemorrhage[122–129]. In a mouse model, selective overexpression of haem oxygenase 1 in astrocytes reduced mortality and improved short-term neurological outcomes up to 7 days after injury[130,131]. Pharmacological induction of haem oxygenase 1 with cobalt protoporphyrin IX in a mouse model of intracerebral haemorrhage was associated with worse neurological function in the short term (days 1–3) but improved neurological function at 28 days[132] when compared with vehicle-treated mice. Conversely, haem oxygenase 1 knockout in a mouse model of intracerebral haemorrhage improved neurological function at 24 h after the insult in comparison with outcomes in wild-type mice, although this benefit was no longer apparent at 72 h (REF.[73]). This evidence suggests that haem oxygenase 1 has a protective effect after intracerebral haemorrhage, but the enzyme might have harmful effects in the short term.

Investigation of the effects of haem oxygenase 2 in intracerebral haemorrhage has been performed in mouse knockout models, but the findings are conflicting. In the collagenase injection model of intracerebral haemorrhage, haem oxygenase 2 knockout has been associated with increased neuronal damage[133,134]. By contrast, in the blood injection model, haem oxygenase 2 knockout was protective, with a weak and variable effect on neurological outcome[135,136]. Studies in stroma-free haemoglobin injection models of intracerebral haemorrhage have yielded similarly conflicting results: haem oxygenase 2 knockout has been associated with increased[119] and decreased[136] neuronal damage. The metabolism of haem over time might provide an explanation for the different outcomes in such models.

Traumatic brain injury

Traumatic brain injury (TBI) can be complicated by the presence of extravascular blood within one or more of the extradural, subdural, subarachnoid, intraventricular or intraparenchymal compartments. Blood breakdown products are thought to lead to secondary brain injury[137]; therefore, haem and haemoglobin scavenging might have a key role in minimizing this injury after TBI. The presence of intracranial blood is also a risk factor for the development of post-traumatic seizures[138], and the free haemoglobin and reactive oxygen species that it produces are thought to play an important role[2,139]. Furthermore, as for aneurysmal SAH, traumatic SAH can lead to cerebral vasospasm and delayed neurological deficits, although the clinical manifestation of vasospasm after traumatic SAH is less severe and the pathogenesis might be different[140].

The role of haptoglobin after TBI is not well defined. In humans, haptoglobin levels are elevated in the serum[141] and the CSF[142] after TBI, and the main source of elevated haptoglobin in TBI is thought to be the liver[141]. Homozygous expression of the *HP1* allele has been associated with worse neuropsychological outcomes (assessed with the Verbal Intelligence Quotient and Finger Tapping Test) at 1 month and 12 months after TBI in humans relative to outcomes in people with other *HP* genotypes, but the association was not observed at 6 months or 10 years after injury[143]. In an adult mouse model of TBI, haptoglobin-knockout mice exhibited fewer neurological deficits than did wild-type mice, but lesion volumes did not differ[144]. In the same model in

older adult mice, haptoglobin-knockout mice had larger lesion volumes but neurological deficits did not differ from those of wild-type mice[144]. This evidence indicates that age-dependent effects of haptoglobin might influence outcomes after TBI[144]. Immunoreactive CD163-positive cells are elevated in perilesional areas in a rat TBI model, suggesting that haemoglobin scavenging mechanisms are upregulated after TBI[145].

Whether haptoglobin influences post-traumatic seizures is also unclear. One study has been conducted to investigate the association of haptoglobin genotype with post-traumatic seizures[143]. This study included only 50 patients but demonstrated no significant association between haptoglobin genotype and the development of post-traumatic seizures after TBI. Similarly, little is known about the role of haptoglobin in vasospasm after traumatic SAH. Determining whether haptoglobin does affect these outcomes is an area of interest for future research.

Animal studies suggest that haem oxygenases are involved in the response to TBI. Haem oxygenase 1 levels are elevated after TBI in animal models[146–148]. In an adult mouse model, knockout of haem oxygenase 2 was associated with significantly greater neuronal loss after TBI than in wild-type mice[149]. Haem oxygenase 2 knockout in an immature rat model was associated with larger lesions at 1 week after injury, but not at 2 weeks, than in wild-type mice, and a tendency towards worse neurological recovery[150].

Superficial siderosis

Superficial siderosis is a neurodegenerative condition characterized by chronic low-grade subarachnoid bleeding from a wide range of sources, whether microscopic or macroscopic[151]. A human pathological study has revealed an intense reaction of microglia, astrocytes and Bergmann glia, and the presence of axonal debris[3]. Experimental studies in rabbits have shown that the glial reaction is related to the RBC fraction of blood, indicating that haemoglobin is the likely culprit[152]. In addition, pronounced upregulation of haem oxygenase 1 and ferritin as well as iron deposition are seen in superficial siderosis in humans[3] and animals[152,153]. Intracisternal administration of the haem oxygenase inhibitor tin protoporphyrin in rabbits decreased iron accumulation but not the microglial response[153]. Whether *HP* genotype and the efficiency of haemoglobin scavenging affect clinical outcomes in superficial siderosis is unclear but is the subject of an ongoing study[154].

Other conditions

The conditions discussed above are associated with macroscopic release of blood into the CNS. A more gradual build-up of extracorpuscular haemoglobin has been explored as part of the aetiology of several other neurological conditions. For instance, raised serum levels of free haemoglobin polypeptides have been demonstrated in patients with multiple sclerosis and have been shown to correlate with neurodegeneration[5]. Elevated haemoglobin levels have been identified in brain tissue from humans with Alzheimer disease[4], and these levels correlated with amyloid burden[155]. This observation raises the possibility that circulating free haemoglobin enters the CNS through a leaky BBB to modify disease course. Some evidence has also linked free haemoglobin to idiopathic generalized epilepsy and Parkinson disease, but the evidence (Supplementary Box 1) is either sparse or conflicting in all these conditions, and further research is required.

Haemoglobin as a therapeutic target

Several treatment strategies are intended to clear haemoglobin or mitigate its toxic and other deleterious consequences, and many of these strategies have been studied (Supplementary Table 2). In this section, we discuss these strategies, focusing on their application in SAH and intracerebral haemorrhage, the conditions in which most evidence has shown haemoglobin to mediate toxicity.

Physical augmentation of clearance

An extravasated blood clot acts as a static source of large amounts of extracellular haemoglobin, so its surgical removal is a rational therapeutic approach to prevent the harmful consequences of cell-free haemoglobin. Reduction of the clot burden in SAH via direct surgical removal has shown promise in a primate model of SAH, in which removal was associated with reduced vasospasm[156]. However, in this model, the clot was focally placed in the Sylvian fissure; reproducing complete evacuation in real SAH, in which blood is distributed more widely, is challenging owing to the anatomy of the subarachnoid space. Indeed, the rate of clot resolution in a study of 413 patients with SAH does not seem to differ between conventional surgery and coiling[157].

Intrathecal thrombolytics have also been used to clear the blood clot. A meta-analysis of the five available randomized controlled trials (RCTs) showed that administration of thrombolytics (tissue plasminogen activator or urokinase) rather than a placebo or no treatment was associated with a significant reduction in angiographic vasospasm, delayed cerebral ischaemia, poor outcome and chronic hydrocephalus[158]. However, the analysed data were from generally small exploratory studies in which limited blinding measures were used; therefore, the risk of bias was considerable. Furthermore, in one of the studies, an intrathecal vasodilator was administered with the thrombolytic, which might have accounted for some of the improvement in outcome[159]. In an RCT that included 60 patients with SAH, intraventricular thrombolysis with concomitant low-frequency head-motion therapy after SAH did not lead to clinical improvements[160]. However, in a small RCT that included 12 patients with SAH, intrathecal thrombolysis (compared with placebo) worsened inflammation, an effect that could attenuate the benefits of rapid clot clearance[161]. A new trial that will include 440 patients has been registered and is designed to conclusively address the efficacy of intrathecal thrombolysis[162]. Recruitment of patients with SAH to a phase I trial of an alternative approach to clot removal has begun in the United States; insertion of a closed-loop lumbar intrathecal filtration device will be used to remove RBCs in the CSF[163].

Coiling
The endovascular placement of platinum coils in the aneurysmal sac to decrease blood flow into the sac and initiate thrombus formation; this process seals the aneurysm from blood flow in the artery and prevents re-bleeding.

An alternative approach to physical clearance of haemoglobin is CSF diversion via a ventriculostomy or lumbar drain to increase CSF drainage and reduce CSF levels of haemoglobin. Retrospective studies showed that use of a lumbar drain after SAH lowered the incidence of delayed cerebral ischaemia and improved outcomes[164,165]. In another study of patients with SAH, the incidence of vasospasm and patient outcomes did not differ whether lumbar drainage or ventricular drainage was used[166]. Only one prospective study of CSF diversion via lumbar drainage in nonhydrocephalic patients with SAH has been conducted[167]. This high-quality RCT, which was at low risk of bias (Supplementary Table 2), included 210 patients and showed that lumbar drainage (versus no drainage) reduced the incidence of delayed cerebral ischaemia from 35% to 21% but had no effect on long-term outcomes[168].

The above findings suggest that the effects of CSF diversion are limited, at least in nonhydrocephalic patients, as the diverted CSF would have otherwise drained through endogenous routes. Furthermore, the use of CSF drainage devices will not have an appreciable effect on the local tissue concentrations of haemoglobin and its metabolites adjacent to the solid clot, as the clot continues to release haemoglobin. Nevertheless, studies conducted to date might have lacked the power necessary to detect a difference in clinical outcome, and larger studies might reveal a long-term benefit of CSF drainage. In one large prospective RCT that is underway, known as EARLYDRAIN, the effects of lumbar drainage within 72 h of the ictus will be studied; recruitment of 300 patients is complete, and the results are forthcoming[168].

Mechanical evacuation of the clot in intracerebral haemorrhage is equally as controversial as in SAH. Clot volume can be similar to that after SAH, but intracerebral haemorrhage affects a much smaller surface area than SAH; in the former, the clot is focal within the brain parenchyma, whereas in the latter, the blood clot coats the surface of the cortex. Two large, randomized clinical trials that included a total of 1,634 patients with intracerebral haemorrhage showed no benefit of early surgical evacuation compared with initial medical treatment[169,170]. Although the primary outcome was clear, its generalizability is more complex. Patients who were assigned to initially receive medical treatment were allowed to cross over to surgery in the following hours or days if their clinical condition changed, which might have hampered attempts to demonstrate benefits of surgical clot evacuation because these patients remained in the initial conservative treatment group in the primary intention-to-treat analysis. Furthermore, at the time these trials were designed, most neurosurgeons were of the opinion that at least some patients with intracerebral haemorrhage would benefit from clot removal. As a result, randomization for all patients was felt to be ethically unacceptable, so the studies only included patients for whom the benefit of clot removal was considered by neurosurgeons to be uncertain; if a neurosurgeon felt confident that a patient would benefit from clot evacuation, they were not entered into the trial. There is no practical way to resolve whether these excluded patients would benefit from surgery or not. As a result, despite a lack of objective evidence, many clinicians continue to feel that a subgroup of patients with superficial, moderate-sized clots might benefit from clot reduction. However, whether any benefit of mechanical clot removal arises from a reduction of intracranial pressure or a reduction in toxicity mediated by blood products is unclear. A phase II trial of thrombolysis with recombinant tissue-type plasminogen activator administered through a catheter into the haematoma cavity showed that the reduction in blood volume reduces oedema[171]. A phase III trial for which recruitment of 500 patients has been completed will hopefully clarify whether a reduction in clot burden improves outcomes[172].

Augmentation of haemoglobin scavenging

Haptoglobin. Given that endogenous haptoglobin is consumed and depleted after haemorrhage or haemolysis, haptoglobin supplementation is an appealing treatment strategy[54]. A preliminary study of intrathecal administration (topical at the time of surgery or postoperatively via a cisternal drain) of haptoglobin as a treatment for vasospasm was conducted in 27 patients with SAH in 1979 in Japan, and improvements in vasospasm in many patients suggested some therapeutic benefits[173]. However, the study design is best described as an open-label, uncontrolled study, which precludes any strong conclusions being drawn. A personal communication from a member of the medical team at that time suggests that the treatment was abandoned after one patient developed seizures (M. Miyaoka, personal communication). Furthermore, the haptoglobin used in this study was derived from pooled blood donations, and given the low proportion of individuals who are homozygous for the *HP1* allele (which evidence suggests is even lower in Japan, where the blood was sourced[174]), the patients in the study probably received large amounts of HP2-2. The haptoglobin phenotype of patients was not determined; therefore, any patients included who expressed HP1-1 might not have benefited or might even have been harmed as a result of immunological rejection. Thus, the role of haptoglobin administration for vasospasm prophylaxis and improvement of outcome is not known.

One haptoglobin product, which is ~90% HP2-2 (REF.[175]), is approved in Japan for clinical use as a therapeutic plasma protein for intravenous infusion in patients with haemoglobinaemia associated with intravascular haemolysis in response to a thermal burn, blood transfusion and cardiopulmonary bypass. In a study published in 2017, intraoperative administration of haptoglobin was associated with a lower risk of acute kidney injury in patients undergoing cardiovascular surgery[176]. However, there are currently no neurological indications for treatment with haptoglobin.

The possibility of haptoglobin supplementation as a treatment for patients with SAH is an avenue for further research, but many issues remain to be resolved. More certainty is needed about which haptoglobin type — HP1-1 or HP2-2 — is most beneficial. The mechanism by which endogenous haptoglobin improves outcome is unknown. No clinical haptoglobin preparation is yet

Ventriculostomy
A neurosurgical procedure to drain a cerebral ventricle, typically by inserting an external ventricular drain into the ventricle and allowing the CSF to drain into an external bag.

available, and the optimal concentration and mode of delivery to the site of the clot need to be worked out. No point-of-care test to determine haptoglobin phenotype is available, and the immunogenicity of allogeneic haptoglobin infusion, which might affect its efficacy, has not been systematically studied, although from a safety perspective, only 3 of 4,600 clinically overt adverse events related to blood product transfusion in Japan over an 8-year period were associated with anti-haptoglobin antibodies[177]. The effects of increased protein load in the CNS as a result of haptoglobin infusion are unknown, and the risk of increased iron load due to improved uptake also needs to be determined.

Haemopexin. Unlike haptoglobin, haemopexin is constitutively expressed in the healthy brain, yet its levels in the CSF are still low compared with its levels in the blood. Preclinical data (see Intracerebral haemorrhage above) indicate that intrathecal administration of haemopexin or selective agonists of haemopexin expression are possible therapeutic strategies to neutralize haem toxicity after brain haemorrhage, although no obvious clinical candidates currently exist.

Transcriptional upregulation of antioxidative responses. Genes that encode proteins that are essential for haemoglobin clearance and redox protection are under the transcriptional control of signalling pathways that sense oxidative stress and inflammation (FIG. 3). One of the main transcriptional regulators of the host response to haemoglobin is nuclear factor erythroid 2-related factor 2 (NRF2; encoded by the *NFE2L2* gene). In the brain, NRF2 upregulates haptoglobin expression in oligodendrocytes[64] and CD36-mediated phagocytosis of RBCs[47]. In animal models of SAH, administration of the NRF2 activator sulforaphane, compared with administration of vehicle alone, reduced cerebral vasospasm, brain oedema, BBB leakage, cortical apoptosis and motor deficits[178–180]. This evidence makes NRF2 a candidate target for treatment of SAH. Multiple NRF2 activators have been studied in SAH; one of the most potent and widely studied is sulforaphane. Practically, sulforaphane has limited stability at room temperature, but when complexed with cyclodextrin, its stability is improved, which makes it suitable for clinical use, and a phase II clinical trial of this complex in 90 patients with SAH is currently underway[181].

Another global transcriptional regulator with anti-inflammatory and antioxidant properties[182] is peroxisome proliferator-activated receptor-γ (PPARγ; encoded by the *PPARG* gene). PPARγ inactivates NF-κB and is a transcriptional activator of the genes that encode NRF2 and other redox-protective proteins, such as catalase[182]. In an animal model of intracerebral haemorrhage, PPARγ activators enhanced haematoma clearance by increasing microglial phagocytic activity and upregulating CD36 (REF.[183]). In a rat model of SAH, the PPARγ agonist rosiglitazone reduced vasospasm and improved outcomes[184]. On the basis of this evidence, a phase II study (SHRINC) of the related drug pioglitazone has been started to assess its effect on the rate of haematoma resolution after intracerebral haemorrhage[185].

Some evidence also suggests that co-activation of NRF2 and PPARγ might have additional therapeutic potential. *PPARG* contains an NRF2 response element[186] and *NFE2L2* contains a PPARγ response element[187], so expression of each can activate expression of the other[182].

Mitigation of haemoglobin redox toxicity. The iron chelator deferiprone, which can cross the BBB, has been studied as a potential therapeutic agent in disorders associated with haemoglobin toxicity. In one small dose escalation study that included 20 patients, an intravenous dose of deferiprone up to 62 mg/kg daily for 3 days after intracerebral haemorrhage seemed to be safe[188]. However, an RCT of deferiprone at the same intravenous dose for 5 days was halted owing to concerns over acute respiratory distress syndrome[189]. A multicentre phase III RCT of a lower dose (32 mg/kg daily for 3 days) in 294 patients is now underway[190]. A previous study that included 42 patients has suggested that intravenous deferiprone at 32 mg/kg daily for 3 days reduces intracerebral haemorrhage oedema compared with no treatment[191]. This study was well designed and at relatively low risk of bias for its size (Supplementary Table 2) but was not sufficiently powered to examine clinical outcomes, which were similar in both arms at 30 days after intracerebral haemorrhage. The effects of deferiprone will also be studied in SAH in an RCT that has been registered[192].

Deferiprone has also been studied in superficial siderosis. A small (10 patients), single-arm, open-label study of 30 mg/kg oral deferiprone daily for 90 days suggested a satisfactory safety profile and decreased haemosiderin deposition assessed with MRI[193]. This study was followed by a larger and longer observational study of 38 patients, 30 of whom completed the follow-up[194]. After 2 years of deferiprone treatment, 19 patients reported no disease progression or an improvement in at least one neurological domain; disease progression would be expected over this time period. In half of the 16 patients for whom a complete MRI assessment was available in this study, a reduction in haemosiderin was observed over the 2 years. Another single-arm, non-randomized study of deferiprone in superficial siderosis is ongoing[195]. Although the observations to date are promising, the nature of such small open-label studies means they are at high risk of bias.

Several other drugs have potential for reducing haemoglobin redox toxicity and have either been investigated or are under active investigation (BOX 3). Perhaps the most robustly investigated therapeutic agent for SAH is tirilazad, a nonglucocorticoid 21-aminosteroid that inhibits lipid peroxidation. However, a Cochrane analysis of five trials of tirilazad, which were assessed as being at low risk of bias, had high follow-up rates and involved a total of 3,821 patients with SAH, identified no clinical benefit[196].

Modulation of inflammation. Inflammation after intracerebral haemorrhage or SAH can be modulated in multiple ways, and many therapeutic avenues are currently under investigation. Most are not specific to haemoglobin scavenging, but one potential treatment is IL-1 receptor antagonist (IL1-RA), which

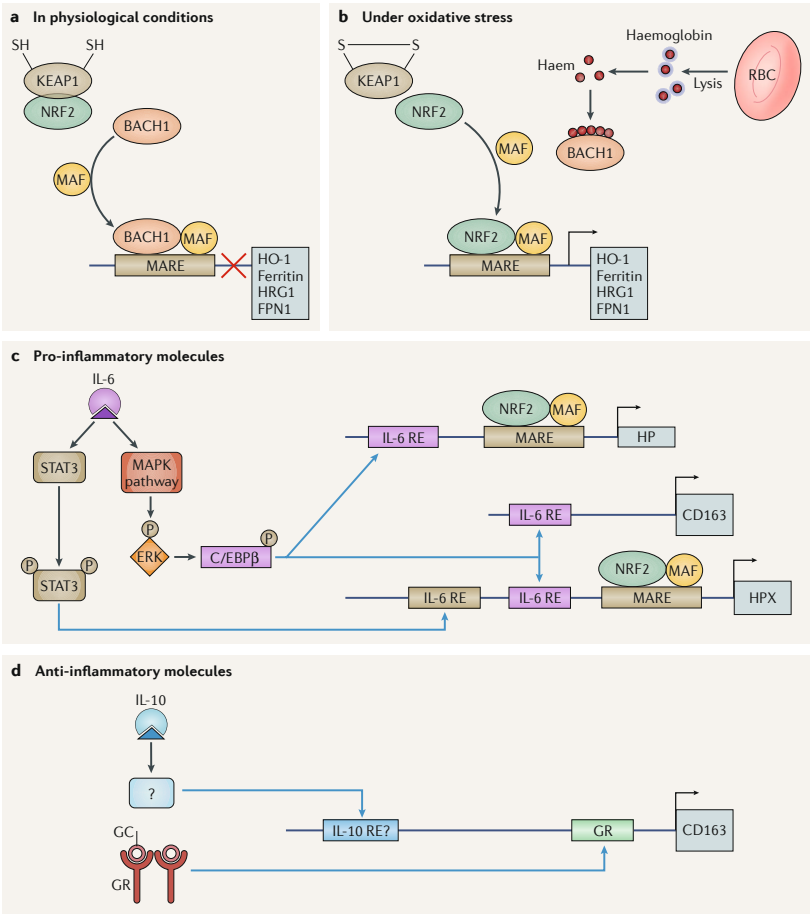

Fig. 3 | **Transcriptional regulation of genes involved in haemoglobin scavenging and redox protection.** One of the main transcriptional regulators of genes involved in haemoglobin scavenging and redox protection is nuclear factor erythroid 2-related factor 2 (NRF2), a redox-sensitive transcription factor that binds to response elements (REs) upstream of a wide range of such genes[237]. **a** | In physiological conditions, NRF2 is targeted for ubiquitylation by Kelch-like ECH-associated protein 1 (KEAP1), and transcriptional regulator protein BACH1 can bind to V-maf musculoaponeurotic fibrosarcoma oncogene homologue (MAF), which enables it to bind to MAF recognition elements (MAREs). BACH1 binds to a subset of NFR2 REs and suppresses transcription of the downstream genes. **b** | Under oxidative stress, KEAP1 is inactivated by oxidation of sulfhydryl (thiol) groups on certain cysteine residues; internal disulfide bridge formation is depicted as an example, but other reactions between KEAP1 and NRF2 inducers can occur, such as alkylation of the cysteine thiol groups. Upon KEAP1 inactivation, NRF2 is released. Red blood cell (RBC) lysis leads to haem binding of BACH1, causing BACH1 to dissociate from MAREs. NRF2 is therefore able to bind to MAF and MAREs, activating transcription of protective genes, such as those that encode haem oxygenase 1 (HO1), ferritin heavy and light chains, haem-responsive gene 1 protein homologue (HRG1; also known as SLC48A1) and ferroportin 1 (FPN1; also known as SLC40A1). **c** | During inflammation, release of IL-6 leads to phosphorylation of signal transducer and activator of transcription 3 (STAT3) and CCAAT/enhancer-binding protein-β (C/EBPβ), which bind to IL-6 REs that activate transcription of downstream genes involved in haemoglobin scavenging, including those that encode haptoglobin (HP), CD163 and haemopexin (HPX). NRF2 is upregulated in response to inflammation and cerebral insults[238] and can bind to MAREs that activate transcription of the same genes. **d** | Anti-inflammatory molecules, such as IL-10 and glucocorticoids (GCs), upregulate transcription of the CD163 gene. Question marks indicate uncertain elements of the pathway. GR, glucocorticoid receptor; GRE, glucocorticoid response element; ERK, extracellular-signal-regulated kinase; MAPK, mitogen-activated protein kinase.

Box 3 | **Drugs with potential for reducing haemoglobin redox toxicity**

Minocycline
Minocycline is an antibiotic but is also an iron chelator and antioxidant. Extensive safety data are available for this drug, and small clinical studies to determine its effects after intracerebral haemorrhage and subarachnoid haemorrhage (SAH) are ongoing[224–226].

Selenium and paracetamol
Trials of selenium[227] and paracetamol[228] to reduce oxidative stress after SAH were registered >10 years ago, but the status of these trials is unclear, and no results have been published to date.

Hydrogen-rich saline
Exogenous hydrogen supplementation with intraperitoneal administration of hydrogen-rich saline in a rat model of SAH reduced oxidative stress and inflammatory markers, whereas physiological saline did not[229]; on this basis, an early clinical trial of orally administered hydrogen-rich water versus regular water after SAH is underway[230].

NXY-059
NXY-059 is a free radical scavenger that has been tested in a commercially sponsored randomized controlled trial involving 603 patients with intracerebral haemorrhage. The results were reported as a safety study and concluded that NXY-059 is safe[231], but no reductions in deaths or poor outcomes were noted and no further progress has been made.

Ferrostatin 1, liproxstatin and zileuton
Ferrostatin 1 was identified in 2012 as a specific inhibitor of ferroptosis[232] and inhibits neuronal death as a result of haemoglobin and free Fe^{2+} (REF.[233]). In a mouse model of intracerebral haemorrhage, ferrostatin 1 treatment was associated with reduced lesion volumes and improvements in neurological function[233]. These findings have been replicated with the ferroptosis inhibitors liproxstatin 1 and zileuton[234]. Their effects are thought to result predominantly from free radical trapping but also lipoxygenase inhibition[235]. Owing to the relative novelty of these agents, no human studies have been conducted. Zileuton is a good candidate for a clinical study, as it is currently used as maintenance therapy for asthma.

Necrostatin 1
Necrostatin 1 (NEC-1) is a necroptosis inhibitor. After intraventricular administration in an animal model of intracerebral haemorrhage, NEC-1 reduced haematoma volume, cell death and oedema, and improved neurobehavioural outcomes[236].

specifically reduces haem-induced neuronal death in organotypic slice cultures[16]. A dose-finding study in 25 patients with SAH demonstrated brain penetration with therapeutic levels of IL1-RA and good safety[197]. A further single-blinded, open-label study that included 136 patients with SAH has demonstrated that IL1-RA reduces inflammatory markers[198], and the drug is currently being trialled in a definitive RCT aiming to recruit 1,000 patients with SAH[199].

Increasing nitric oxide bioavailability. Nitric oxide donors could be beneficial after intracerebral haemorrhage and SAH, as they could mitigate the adverse effects of nitric oxide scavenging by haemoglobin. Sodium nitroprusside and glyceryl trinitrate are the nitric oxide donors that have been studied most, but both have profound vasodilatory effects and induce hypotension, which is not tolerable after SAH because cerebral perfusion could be compromised. Sodium nitrite is currently the only clinical option; a study of this drug in 18 patients with SAH showed that it is safe and produced adequate therapeutic levels[200], but a clinical trial that was subsequently started has been terminated for unknown reasons[201]. After intracerebral haemorrhage, more-aggressive blood pressure management is recommended; glyceryl trinitrate has been trialled in this context, albeit for the purpose of blood pressure management[202]. Regardless, this treatment did not improve patient outcomes.

Future directions

A large body of preclinical work has investigated different approaches to ameliorating the secondary brain injury caused by extracellular haemoglobin. Despite promising early results for several agents, few have undergone the further preclinical evaluation required before translation to human studies. A handful of agents have been tested in small clinical studies, but few of these studies have been sufficiently powered to conclusively assess clinical benefits to patients with intracerebral haemorrhage or SAH. Therefore, the need for further research in this area is pressing.

To address this need, the Haemoglobin After Intracranial Haemorrhage (HATCH) consortium held a meeting to arrive at a consensus opinion regarding the immediate priorities for research in intracranial haemolysis that will deliver clinical benefits. The HATCH consortium was formed by invitation of all groups who had published studies of haptoglobin in patients with intracranial haemorrhage and includes groups from the United States, Japan and Europe. Four main research priorities were identified, discussed below.

Improved understanding of mechanisms

The first priority identified was a need for improved basic scientific understanding of the mechanisms of haemoglobin toxicity and its clearance in the brain. Although the basic structures of many of the pathways involved have been described, little is known about the details of their control, their interrelationships and their functional relevance. Examples of aspects that need to be studied are genetic control of expression of haemoglobin scavengers, the kinetics of haemoglobin and its scavengers within the brain, and the relationship between haemoglobin scavenging and inflammation. Specific clinical-grade therapeutics, such as haptoglobin, are needed for preclinical and phase I and phase II human studies. Research might reveal that multiple levels in the haemoglobin and haem scavenging pathways need to be targeted. In order to study these basic mechanisms and evaluate new therapeutics, current preclinical models need improvement. These models need to recapitulate the clearance pathways that are relevant in humans and exhibit consistent functional outcomes that equate to clinical scenarios. Such models will need to be cross-validated between laboratories.

Prospective studies

Research into basic mechanisms will need to be complemented by large, multicentre, prospective observational cohort studies of intracranial haemorrhage that examine the impact of functionally distinct genetic variants of elements of the haemoglobin clearance pathways on clinical outcomes while controlling for covariates that affect prognosis and for ancestral background. Large studies of genetic predisposition to aneurysm formation and the occurrence of

intracerebral haemorrhage have been conducted, but only small, underpowered genetic association studies have been used to assess the clinical outcomes of SAH and intracerebral haemorrhage.

Disease-specific outcome measures

The success of prospective studies relies on the development of robust disease-specific outcome measures. Outcomes after SAH and intracerebral haemorrhage can be thought of in simple terms as being influenced by a combination of primary brain injury and secondary brain injury mediated through extracellular haemoglobin. Current scales for assessing brain injury, such as the modified Rankin scale and the Glasgow Outcome Scale, might be well suited to capture the initial catastrophic injury that results from shearing and a surge in intracranial pressure after very large bleeds, but sensitive measures of long-term cognitive and psychosocial functioning are needed. Such outcome measures are also essential for the success of any future clinical trials of therapeutics.

Point-of-care genotyping

The success of clinical trials might also depend on appropriate risk stratification. Effective stratification might require the development of rapid point-of-care tests for genetic determinants of outcome, such as haptoglobin genotype. These tests might also be of wider clinical benefit in prognostication after intracranial haemorrhage.

Conclusions

Following intracranial haemorrhage, RBCs lyse and release their contents, initiating a cascade of events that results in secondary injury. Extracellular haemoglobin has been identified as a major early mediator of these events. Multiple endogenous mechanisms can mitigate haemoglobin-related pathology in humans; these mechanisms include erythrophagocytosis, the haptoglobin–CD163 pathway, the haemopexin–CD91 pathway and the intracellular haem oxygenases. These systems differ between humans and animals, and between the CNS and the periphery, and detailed functional implications of these differences require further study. The role of extracellular haemoglobin in secondary brain injury means that it is an attractive therapeutic target to minimize such injury and improve outcomes. Several therapeutic approaches are feasible, and many are under investigation, but robust clinical studies are currently lacking. Therefore, further research is needed, and the HATCH consortium has identified four areas of priority for research: improved understanding of toxic and clearance mechanisms, prospective observational studies in intracranial haemorrhage, the development of disease-specific outcome measures, and the development of point-of-care tests for risk stratification. Advances in these areas would facilitate the translation of knowledge of haemoglobin toxicity and clearance mechanisms into clinical benefits.

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1. Regan, R. F. & Panter, S. S. Neurotoxicity of hemoglobin in cortical cell culture. *Neurosci. Lett.* **153**, 219–222 (1993).
2. Rosen, A. D. & Frumin, N. V. Focal epileptogenesis after intracortical hemoglobin injection. *Exp. Neurol.* **66**, 277–284 (1979).
3. Koeppen, A. H. et al. The pathology of superficial siderosis of the central nervous system. *Acta Neuropathol.* **116**, 371–382 (2008).
This article presents the most detailed pathological study of haemoglobin toxicity accompanying superficial siderosis.
4. Slemmon, J. R., Hughes, C. M., Campbell, G. A. & Flood, D. G. Increased levels of hemoglobin-derived and other peptides in Alzheimer's disease cerebellum. *J. Neurosci.* **14**, 2225–2235 (1994).
5. Lewin, A. et al. Free serum haemoglobin is associated with brain atrophy in secondary progressive multiple sclerosis. *Wellcome Open Res.* **1**, 10 (2016).
This is an intriguing study showing that extracellular haemoglobin might be important in neurological conditions that are not characterized by macroscopic bleeding.
6. Reeder, B. J., Svistunenko, D. A., Cooper, C. E. & Wilson, M. T. The radical and redox chemistry of myoglobin and hemoglobin: from in vitro studies to human pathology. *Antioxid. Redox Signal.* **6**, 954–966 (2004).
7. Marnett, L. J., Riggins, J. N. & West, J. D. Endogenous generation of reactive oxidants and electrophiles and their reactions with DNA and protein. *J. Clin. Invest.* **111**, 583–593 (2003).
8. Reeder, B. J. et al. Toxicity of myoglobin and haemoglobin: oxidative stress in patients with rhabdomyolysis and subarachnoid haemorrhage. *Biochem. Soc. Trans.* **30**, 745–748 (2002).
9. Suzuki, N. et al. Identification of 5-hydroxy eicosatetraenoic acid in cerebrospinal fluid after subarachnoid hemorrhage. *J. Neurochem.* **41**, 1186–1189 (1983).
10. Balla, G., Jacob, H. S., Eaton, J. W., Belcher, J. D. & Vercellotti, G. M. Hemin: a possible physiological mediator of low density lipoprotein oxidation and endothelial injury. *Arterioscler Thromb.* **11**, 1700–1711 (1991).
11. Yang, W. S. & Stockwell, B. R. Ferroptosis: death by lipid peroxidation. *Trends Cell Biol.* **26**, 165–176 (2016).
12. Kwon, M. S. et al. Methemoglobin is an endogenous toll-like receptor 4 ligand-relevance to subarachnoid hemorrhage. *Int. J. Mol. Sci.* **16**, 5028–5046 (2015).
13. Figueiredo, R. T. et al. Characterization of heme as activator of Toll-like receptor 4. *J. Biol. Chem.* **282**, 20221–20229 (2007).
14. Grootjans, S., Vanden Berghe, T. & Vandenabeele, P. Initiation and execution mechanisms of necroptosis: an overview. *Cell Death Differ.* **24**, 1184–1195 (2017).
15. Dutra, F. F. et al. Hemolysis-induced lethality involves inflammasome activation by heme. *Proc. Natl Acad. Sci. USA* 1 **11**, E4110–E4118 (2014).
16. Greenhalgh, A. D. et al. Interleukin-1 receptor antagonist is beneficial after subarachnoid haemorrhage in rat by blocking haem-driven inflammatory pathology. *Dis. Model. Mech.* **5**, 823–833 (2012).
This study shows that haem causes inflammasome activation after SAH.
17. Joshi, M. S. et al. Nitric oxide is consumed, rather than conserved, by reaction with oxyhemoglobin under physiological conditions. *Proc. Natl Acad. Sci. USA* **99**, 10341–10346 (2002).
18. Kajita, Y. et al. Combined effect of L-arginine and superoxide dismutase on the spastic basilar artery after subarachnoid hemorrhage in dogs. *J. Neurosurg.* **80**, 476–483 (1994).
19. Zou, M. H., Shi, C. & Cohen, R. A. Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. *J. Clin. Invest.* **109**, 817–826 (2002).
20. Sabri, M. et al. Uncoupling of endothelial nitric oxide synthase after experimental subarachnoid hemorrhage. *J. Cereb. Blood Flow Metab.* **31**, 190–199 (2011).
21. Faraci, F. M. & Brian, J. E. Jr. Nitric oxide and the cerebral circulation. *Stroke* **25**, 692–703 (1994).
22. Voetsch, B., Jin, R. C. & Loscalzo, J. Nitric oxide insufficiency and atherothrombosis. *Histochem. Cell Biol.* **122**, 353–367 (2004).
23. Suzuki, S. et al. Cerebral microthrombosis in symptomatic cerebral vasospasm—a quantitative histological study in autopsy cases. *Neurol. Med. Chir.* **30**, 309–316 (1990).
24. Friedrich, B., Muller, F., Feiler, S., Scholler, K. & Plesnila, N. Experimental subarachnoid hemorrhage causes early and long-lasting microarterial constriction and microthrombosis: an in-vivo microscopy study. *J. Cereb. Blood Flow Metab.* **32**, 447–455 (2012).
25. Stein, S. C., Browne, K. D., Chen, X. H., Smith, D. H. & Graham, D. I. Thromboembolism and delayed cerebral ischemia after subarachnoid hemorrhage: an autopsy study. *Neurosurgery* **59**, 781–787; discussion 787–788 (2006).
26. Petzold, G. C. et al. Nitric oxide modulates spreading depolarization threshold in the human and rodent cortex. *Stroke* **39**, 1292–1299 (2008).
27. Dreier, J. P. et al. Nitric oxide scavenging by hemoglobin or nitric oxide synthase inhibition by N-nitro-L-arginine induces cortical spreading ischemia when K^+ is increased in the subarachnoid space. *J. Cereb. Blood Flow Metab.* **18**, 978–990 (1998).
28. Sehba, F. A., Schwartz, A. Y., Chereshnev, I. & Bederson, J. B. Acute decrease in cerebral nitric oxide levels after subarachnoid hemorrhage. *J. Cereb. Blood Flow Metab.* **20**, 604–611 (2000).
29. Kanamaru, K., Waga, S., Kojima, T., Fujimoto, K. & Niwa, S. Endothelium-dependent relaxation of canine basilar arteries. Part 2: inhibition by hemoglobin and cerebrospinal fluid from patients with aneurysmal subarachnoid hemorrhage. *Stroke* **18**, 938–943 (1987).
30. Byrne, J. V., Griffith, T. M., Edwards, D. H., Harrison, T. J. & Johnston, K. R. Investigation of the vasoconstrictor action of subarachnoid haemoglobin in the pig cerebral circulation in vivo. *Br. J. Pharmacol.* **97**, 669–674 (1989).
31. Iqbal, S. et al. Inducible nitric oxide synthase (NOS-2) in subarachnoid hemorrhage: regulatory mechanisms and therapeutic implications. *Brain Circ.* **2**, 8–19 (2016).
32. Woszczyk, A., Deinsberger, W. & Boker, D. K. Nitric oxide metabolites in cisternal CSF correlate with cerebral vasospasm in patients with a subarachnoid

haemorrhage. *Acta Neurochir.***145**, 257–263; discussion 263–254(2003).
33. Suzuki, Y. et al. Nitric oxide metabolites in the cisternal cerebral spinal fluid of patients with subarachnoid hemorrhage. *Neurosurgery* **41**, 807–811; discussion 811–802 (1997).
34. Suzuki, M. et al. Increased levels of nitrite/nitrate in the cerebrospinal fluid of patients with subarachnoid hemorrhage. *Neurosurg. Rev.* **22**, 96–98 (1999).
35. Sadamitsu, D. et al. Cerebrospinal fluid and plasma concentrations of nitric oxide metabolites in postoperative patients with subarachnoid hemorrhage. *Crit. Care Med.* **29**, 77–79 (2001).
36. Rejdak, K. et al. Cerebrospinal fluid nitrite/nitrate correlated with oxyhemoglobin and outcome in patients with subarachnoid hemorrhage. *J. Neurol. Sci.* **219**, 71–76 (2004).
37. Ng, W. H., Moochhala, S., Yeo, T. T., Ong, P. L. & Ng, P. Y. Nitric oxide and subarachnoid hemorrhage: elevated level in cerebrospinal fluid and their implications. *Neurosurgery* **49**, 622–626; discussion 626–627 (2001).
38. Ding, H. Y., Han, X., Lv, C. Z. & Dong, Q. Xenon-CT study of regional cerebral blood flow around hematoma in patients with basal ganglia hemorrhage. *Acta Neurochir. Suppl.* **105**, 161–164 (2008).
39. Kalita, J., Misra, U. K., Ranjan, P., Pradhan, P. K. & Das, B. K. Effect of mannitol on regional cerebral blood flow in patients with intracerebral hemorrhage. *J. Neurol. Sci.* **224**, 19–22 (2004).
40. Huang, F. P. et al. Brain edema after experimental intracerebral hemorrhage: role of hemoglobin degradation products. *J. Neurosurg.* **96**, 287–293 (2002).
41. Katsu, M. et al. Hemoglobin-induced oxidative stress contributes to matrix metalloproteinase activation and blood-brain barrier dysfunction in vivo. *J. Cereb. Blood Flow Metab.* **30**, 1939–1950 (2010).
42. Rosenberg, G. A. & Navratil, M. Metalloproteinase inhibition blocks edema in intracerebral hemorrhage in the rat. *Neurology* **48**, 921–926 (1997).
43. Urday, S. et al. Targeting secondary injury in intracerebral haemorrhage—perihaematomal oedema. *Nat. Rev. Neurol.* **11**, 111–122 (2015).
44. Claassen, J. et al. Global cerebral edema after subarachnoid hemorrhage: frequency, predictors, and impact on outcome. *Stroke* **33**, 1225–1232 (2002).
45. Johshita, H., Kassell, N. F., Sasaki, T. & Ogawa, H. Impaired capillary perfusion and brain edema following experimental subarachnoid hemorrhage: a morphometric study. *J. Neurosurg.* **73**, 410–417 (1990).
46. Fadok, V. A., Warner, M. L., Bratton, D. L. & Henson, P. M. CD36 is required for phagocytosis of apoptotic cells by human macrophages that use either a phosphatidylserine receptor or the vitronectin receptor (alpha v beta 3). *J. Immunol.* **161**, 6250–6257 (1998).
47. Zhao, X. et al. Cleaning up after ICH: the role of Nrf2 in modulating microglia function and hematoma clearance. *J. Neurochem.* **133**, 144–152 (2015).
48. Knutson, M. D., Oukka, M., Koss, L. M., Aydemir, F. & Wessling-Resnick, M. Iron release from macrophages after erythrophagocytosis is up-regulated by ferroportin 1 overexpression and down-regulated by hepcidin. *Proc. Natl Acad. Sci. USA* **102**, 1324–1328 (2005).
49. Yang, Z. et al. Kinetics and specificity of feline leukemia virus subgroup C receptor (FLVCR) export function and its dependence on hemopexin. *J. Biol. Chem.* **285**, 28874–28882 (2010).
50. Kondo, H., Saito, K., Grasso, J. P. & Aisen, P. Iron metabolism in the erythrophagocytosing Kupffer cell. *Hepatology* **8**, 32–38 (1988).
51. Bhakdi, S. & Tranum-Jensen, J. On the cause and nature of C9-related heterogeneity of terminal complement complexes generated on target erythrocytes through the action of whole serum. *J. Immunol.* **133**, 1453–1463 (1984).
52. Nagel, R. L. & Gibson, Q. H. The binding of hemoglobin to haptoglobin and its relation to subunit dissociation of hemoglobin. *J. Biol. Chem.* **246**, 69–73 (1971).
53. Chamoun, V. et al. Haptoglobins as markers of blood-CSF barrier dysfunction: the findings in normal CSF. *J. Neurol. Sci.* **182**, 117–121 (2001).
54. Galea, J. et al. The intrathecal CD163-haptoglobin-hemoglobin scavenging system in subarachnoid hemorrhage. *J. Neurochem.* **121**, 785–792 (2012). **The first detailed description of the CD163–haptoglobin–haemoglobin scavenging system in the human brain, in healthy individuals and after SAH**.
55. Cooper, C. E. et al. Haptoglobin binding stabilizes hemoglobin ferryl iron and the globin radical on tyrosine beta145. *Antioxid. Redox Signal.* **18**, 2264–2273 (2013).
56. Buehler, P. W. et al. Haptoglobin preserves the CD163 hemoglobin scavenger pathway by shielding hemoglobin from peroxidative modification. *Blood* **113**, 2578–2586 (2009).
57. Boretti, F. S. et al. Sequestration of extracellular hemoglobin within a haptoglobin complex decreases its hypertensive and oxidative effects in dogs and guinea pigs. *J. Clin. Invest.* **119**, 2271–2280 (2009).
58. Banerjee, S. et al. Haptoglobin alters oxygenation and oxidation of hemoglobin and decreases propagation of peroxide-induced oxidative reactions. *Free Radic. Biol. Med.* **53**, 1317–1326 (2012).
59. Kristiansen, M. et al. Identification of the haemoglobin scavenger receptor. *Nature* **409**, 198–201 (2001). **A crystal-clear description of how CD163, the receptor for haemoglobin–haptoglobin complexes, was discovered**.
60. Andersen, C. B. et al. Structure of the haptoglobin-haemoglobin complex. *Nature* **489**, 456–459 (2012).
61. Nielsen, M. J. et al. A unique loop extension in the serine protease domain of haptoglobin is essential for CD163 recognition of the haptoglobin-hemoglobin complex. *J. Biol. Chem.* **282**, 1072–1079 (2007).
62. Etzerodt, A. et al. Plasma clearance of hemoglobin and haptoglobin in mice and effect of CD163 gene targeting disruption. *Antioxid. Redox Signal.* **18**, 2254–2263 (2013).
63. Lee, M. Y. et al. Upregulation of haptoglobin in reactive astrocytes after transient forebrain ischemia in rats. *J. Cereb. Blood Flow Metab.* **22**, 1176–1180 (2002).
64. Zhao, X. et al. Neuroprotective role of haptoglobin after intracerebral hemorrhage. *J. Neurosci.* **29**, 15819–15827 (2009). **This is the first experimental study to demonstrate the therapeutic value of haptoglobin and NRF2 activation in intracerebral haemorrhage**.
65. Vallelian, F. et al. The reaction of hydrogen peroxide with hemoglobin induces extensive alpha-globin crosslinking and impairs the interaction of hemoglobin with endogenous scavenger pathways. *Free Radic. Biol. Med.* **45**, 1150–1158 (2008).
66. Subramanian, K., Du, R., Tan, N. S., Ho, B. & Ding, J. L. CD163 and IgG codefend against cytotoxic hemoglobin via autocrine and paracrine mechanisms. *J. Immunol.* **190**, 5267–5278 (2013).
67. Paoli, M. et al. Crystal structure of hemopexin reveals a novel high-affinity heme site formed between two beta-propeller domains. *Nat. Struct. Biol.* **6**, 926–931 (1999).
68. Morris, C. M., Candy, J. M., Edwardson, J. A., Bloxham, C. A. & Smith, A. Evidence for the localization of haemopexin immunoreactivity in neurones in the human brain. *Neurosci. Lett.* **149**, 141–144 (1993).
69. Garland, P. et al. Heme-hemopexin scavenging is active in the brain and associates with outcome after subarachnoid hemorrhage. *Stroke* **47**, 872–876 (2016).
70. Moestrup, S. K., Gliemann, J. & Pallesen, G. Distribution of the alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein in human tissues. *Cell Tissue Res.* **269**, 375–382 (1992).
71. Hvidberg, V. et al. Identification of the receptor scavenging hemopexin-heme complexes. *Blood* **106**, 2572–2579 (2005).
72. Maines, M. D. The heme oxygenase system: a regulator of second messenger gases. *Annu. Rev. Pharmacol. Toxicol.* **37**, 517–554 (1997).
73. Wang, J. & Doré, S. Heme oxygenase-1 exacerbates early brain injury after intracerebral haemorrhage. *Brain* **130**, 1643–1652 (2007).
74. Ewing, J. F. & Maines, M. D. In situ hybridization and immunohistochemical localization of heme oxygenase-2 mRNA and protein in normal rat brain: differential distribution of isozyme 1 and 2. *Mol. Cell Neurosci.* **3**, 559–570 (1992).
75. Boserup, M. W., Lichota, J., Haile, D. & Moos, T. Heterogenous distribution of ferroportin-containing neurons in mouse brain. *Biometals* **24**, 357–375 (2011).
76. Harrison, P. M. & Arosio, P. The ferritins: molecular properties, iron storage function and cellular regulation. *Biochim. Biophys. Acta* **1275**, 161–203 (1996).
77. Kidane, T. Z., Sauble, E. & Linder, M. C. Release of iron from ferritin requires lysosomal activity. *Am. J. Physiol. Cell Physiol.* **291**, C445–C455 (2006).
78. Breuer, W., Shvartsman, M. & Cabantchik, Z. I. Intracellular labile iron. *Int. J. Biochem. Cell Biol.* **40**, 350–354 (2008).
79. Spangler, B. et al. A reactivity-based probe of the intracellular labile ferrous iron pool. *Nat. Chem. Biol.* **12**, 680–685 (2016).
80. Nakamura, K. et al. Activation of the NLRP3 inflammasome by cellular labile iron. *Exp. Hematol.* **44**, 116–124 (2016).
81. Boyle, J. J. et al. Activating transcription factor 1 directs Mhem atheroprotective macrophages through coordinated iron handling and foam cell protection. *Circ. Res.* **110**, 20–33 (2012).
82. Chinetti-Gbaguidi, G., Colin, S. & Staels, B. Macrophage subsets in atherosclerosis. *Nat. Rev. Cardiol.* **12**, 10–17 (2015).
83. Guo, L. et al. CD163+ macrophages promote angiogenesis and vascular permeability accompanied by inflammation in atherosclerosis. *J. Clin. Invest.* **128**, 1106–1124 (2018).
84. Sindrilaru, A. et al. An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. *J. Clin. Invest.* **121**, 985–997 (2011).
85. Zhang, X., Surguladze, N., Slagle-Webb, B., Cozzi, A. & Connor, J. R. Cellular iron status influences the functional relationship between microglia and oligodendrocytes. *Glia* **54**, 795–804 (2006).
86. Schubert, G. A., Seiz, M., Hegewald, A. A., Manville, J. & Thome, C. Hypoperfusion in the acute phase of subarachnoid hemorrhage. *Acta Neurochir. Suppl.* **110**, 35–38 (2011).
87. Bederson, J. B., Germano, I. M. & Guarino, L. Cortical blood flow and cerebral perfusion pressure in a new noncraniotomy model of subarachnoid hemorrhage in the rat. *Stroke* **26**, 1086–1091; discussion 1091–1082 (1995).
88. Brathwaite, S. & Macdonald, R. L. Current management of delayed cerebral ischemia: update from results of recent clinical trials. *Transl Stroke Res.* **5**, 207–226 (2014).
89. Dunne, V. G. & Besser, M. Outcome scoring in neurosurgery: the cognitive outcome score, an alternative to the Glasgow outcome scale. *J. Clin. Neurosci.* **12**, 119–123 (2005).
90. Dunne, V. G., Bhattachayya, S., Besser, M., Rae, C. & Griffin, J. L. Metabolites from cerebrospinal fluid in aneurysmal subarachnoid haemorrhage correlate with vasospasm and clinical outcome: a pattern-recognition 1 H NMR study. *NMR Biomed.* **18**, 24–33 (2005).
91. Mast, H., Poche, H. & Marx, P. Hemoglobin quantitation in subarachnoid hemorrhage. *Klin. Wochenschr.* **65**, 513–515 (1987).
92. Pyne-Geithman, G. J. et al. Bilirubin production and oxidation in CSF of patients with cerebral vasospasm after subarachnoid hemorrhage. *J. Cereb. Blood Flow Metab.* **25**, 1070–1077 (2005).
93. Wang, K. C. et al. Prognostic value of intrathecal heme oxygenase-1 concentration in patients with fisher grade III aneurysmal subarachnoid hemorrhage. *J. Neurosurg.* **121**, 1388–1393 (2014).
94. Petzold, A. et al. Cerebrospinal fluid ferritin level, a sensitive diagnostic test in late-presenting subarachnoid hemorrhage. *J. Stroke Cerebrovasc. Dis.* **20**, 489–493 (2011).
95. Suzuki, H., Muramatsu, M., Kojima, T. & Taki, W. Intracranial heme metabolism and cerebral vasospasm after aneurysmal subarachnoid hemorrhage. *Stroke* **34**, 2796–2800 (2003).
96. Durnford, A. et al. Haemoglobin scavenging after subarachnoid haemorrhage. *Acta Neurochir. Suppl.* **120**, 51–54 (2015).
97. Przybycien-Szymanska, M. M., Yang, Y. & Ashley, W. W. Microparticle derived proteins as potential biomarkers for cerebral vasospasm post subarachnoid hemorrhage. A preliminary study. *Clin. Neurol. Neurosurg.* **141**, 48–55 (2016).
98. Borsody, M., Burke, A., Coplin, W., Miller-Lotan, R. & Levy, A. Haptoglobin and the development of cerebral artery vasospasm after subarachnoid hemorrhage. *Neurology* **66**, 634–640 (2006). **This article presents the first clinical study to show an association between haptoglobin genotype and outcome after SAH**.
99. Leclerc, J. L. et al. Haptoglobin phenotype predicts the development of focal and global cerebral vasospasm and may influence outcomes after aneurysmal subarachnoid hemorrhage. *Proc. Natl Acad. Sci. USA* **112**, 1155–1160 (2015).
100. Ohnishi, H. et al. Haptoglobin phenotype predicts cerebral vasospasm and clinical deterioration after

aneurysmal subarachnoid hemorrhage. *J. Stroke Cerebrovasc. Dis.* **22**, 520–526 (2013).
101. Murthy, S. B. et al. Haptoglobin 2–2 genotype is associated with cerebral salt wasting syndrome in aneurysmal subarachnoid hemorrhage. *Neurosurgery* **78**, 71–76 (2016).
102. Kantor, E. et al. Haptoglobin genotype and functional outcome after aneurysmal subarachnoid hemorrhage. *J. Neurosurgery.* **120**, 386–390 (2014).
103. Gaastra, B., Glazier, J., Bulters, D. & Galea, I. Haptoglobin genotype and outcome after subarachnoid haemorrhage: new insights from a meta-analysis. *Oxid. Med. Cell Longev.* **2017**, 6747940 (2017).
A meta-analysis of all published clinical studies linking haptoglobin genotype and outcome after SAH.
104. Gaastra, B. et al. Haptoglobin after subarachnoid haemorrhage: individual patient level data (IPLD) analysis. *NHS* http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42017070830 (2017).
105. Chaichana, K. L., Levy, A. P., Miller-Lotan, R., Shakur, S. & Tamargo, R. J. Haptoglobin 2–2 genotype determines chronic vasospasm after experimental subarachnoid hemorrhage. *Stroke* **38**, 3266–3271 (2007).
106. Schallner, N. et al. Microglia regulate blood clearance in subarachnoid hemorrhage by heme oxygenase-1. *J. Clin. Invest.* **125**, 2609–2625 (2015).
107. Ono, S., Komuro, T. & Macdonald, R. L. Heme oxygenase-1 gene therapy for prevention of vasospasm in rats. *J. Neurosurg.* **96**, 1094–1102 (2002).
108. Suzuki, H. et al. Heme oxygenase-1 gene induction as an intrinsic regulation against delayed cerebral vasospasm in rats. *J. Clin. Invest.* **104**, 59–66 (1999).
109. Keep, R. F., Hua, Y. & Xi, G. Intracerebral haemorrhage: mechanisms of injury and therapeutic targets. *Lancet Neurol.* **11**, 720–731 (2012).
110. Murthy, S. B. et al. Presence of haptoglobin-2 allele is associated with worse functional outcomes after spontaneous intracerebral hemorrhage. *World Neurosurg.* **83**, 583–587 (2015).
111. Zhao, X. et al. Cytoprotective role of haptoglobin in brain after experimental intracerebral hemorrhage. *Acta Neurochir Suppl.* **111**, 107–112 (2011).
112. Liu, B. et al. CD163/Hemoglobin oxygenase-1 pathway regulates inflammation in hematoma surrounding tissues after intracerebral hemorrhage. *J. Stroke Cerebrovasc. Dis.* **24**, 2800–2809 (2015).
113. Cao, S. et al. Hematoma changes during clot resolution after experimental intracerebral hemorrhage. *Stroke* **47**, 1626–1631 (2016).
114. Xie, W. J., Yu, H. Q., Zhang, Y., Liu, Q. & Meng, H. M. CD163 promotes hematoma absorption and improves neurological functions in patients with intracerebral hemorrhage. *Neural Regen Res.* **11**, 1122–1127 (2016).
115. Leclerc, J. L. et al. The absence of the CD163 receptor has distinct temporal influences on intracerebral hemorrhage outcomes. *J Cereb. Blood Flow Metab.* **38**, 262–273 (2017).
116. Liu, R. et al. CD163 expression in neurons after experimental intracerebral hemorrhage. *Stroke* **48**, 1369–1375 (2017).
117. Chen-Roetling, J. & Regan, R. F. Haptoglobin increases the vulnerability of CD163-expressing neurons to hemoglobin. *J. Neurochem.* **139**, 586–595 (2016).
118. Wang, G. et al. Low-density lipoprotein receptor-related protein-1 facilitates heme scavenging after intracerebral hemorrhage in mice. *J. Cereb. Blood Flow Metab.* **37**, 1299–1310 (2017).
119. Ma, B. et al. Deletion of the hemopexin or heme oxygenase-2 gene aggravates brain injury following stroma-free hemoglobin-induced intracerebral hemorrhage. *J. Neuroinflamm.* **13**, 26 (2016).
120. Chen, L., Zhang, X., Chen-Roetling, J. & Regan, R. F. Increased striatal injury and behavioral deficits after intracerebral hemorrhage in hemopexin knockout mice. *J. Neurosurg.* **114**, 1159–1167 (2011).
121. Leclerc, J. L. et al. Increased brain hemopexin levels improve outcomes after intracerebral hemorrhage. *J. Cereb. Blood Flow Metab.* https://doi.org/10.1177/0271678X16679170 (2016).
This is the first experimental study to demonstrate the therapeutic value of haemopexin after intracerebral haemorrhage.
122. Shang, H. et al. Time course of Keap1-Nrf2 pathway expression after experimental intracerebral haemorrhage: correlation with brain oedema and neurological deficit. *Free Radic. Res.* **47**, 368–375 (2013).
123. Wang, G. et al. Time course of heme oxygenase-1 and oxidative stress after experimental intracerebral hemorrhage. *Acta Neurochir.* **153**, 319–325 (2011).
124. Chen, M. & Regan, R. F. Time course of increased heme oxygenase activity and expression after experimental intracerebral hemorrhage: correlation with oxidative injury. *J. Neurochem.* **103**, 2015–2021 (2007).
125. Wu, J. et al. Iron and iron-handling proteins in the brain after intracerebral hemorrhage. *Stroke* **34**, 2964–2969 (2003).
126. Wagner, K. R. et al. Protein oxidation and heme oxygenase-1 induction in porcine white matter following intracerebral infusions of whole blood or plasma. *Dev. Neurosci.* **24**, 154–160 (2002).
127. Wu, J. et al. Oxidative brain injury from extravasated erythrocytes after intracerebral hemorrhage. *Brain Res.* **953**, 45–52 (2002).
128. Nakaso, K. et al. Co-induction of heme oxygenase-1 and peroxiredoxin I in astrocytes and microglia around hemorrhagic region in the rat brain. *Neurosci. Lett.* **293**, 49–52 (2000).
129. Matz, P. G., Weinstein, P. R. & Sharp, F. R. Heme oxygenase-1 and heat shock protein 70 induction in glia and neurons throughout rat brain after experimental intracerebral hemorrhage. *Neurosurgery* **40**, 152–160; discussion 160–152 (1997).
130. Chen-Roetling, J., Song, W., Schipper, H. M., Regan, C. S. & Regan, R. F. Astrocyte overexpression of heme oxygenase-1 improves outcome after intracerebral hemorrhage. *Stroke* **46**, 1093–1098 (2015).
131. Chen-Roetling, J. et al. Astrocyte heme oxygenase-1 reduces mortality and improves outcome after collagenase-induced intracerebral hemorrhage. *Neurobiol. Dis.* **102**, 140–146 (2017).
132. Zhang, Z. et al. Distinct role of heme oxygenase-1 in early- and late-stage intracerebral hemorrhage in 12-month-old mice. *J. Cereb. Blood Flow Metab.* **37**, 25–38 (2017).
133. Wang, J., Zhuang, H. & Dorė, S. Heme oxygenase 2 is neuroprotective against intracerebral hemorrhage. *Neurobiol. Dis.* **22**, 473–476 (2006).
134. Wang, J. & Dorė, S. Heme oxygenase 2 deficiency increases brain swelling and inflammation after intracerebral hemorrhage. *Neuroscience* **155**, 1133–1141 (2008).
135. Chen-Roetling, J., Cai, Y. & Regan, R. F. Neuroprotective effect of heme oxygenase-2 knockout in the blood injection model of intracerebral hemorrhage. *BMC Res. Notes* **7**, 561 (2014).
136. Qu, Y., Chen, J., Benvenisti-Zarom, L., Ma, X. & Regan, R. F. Effect of targeted deletion of the heme oxygenase-2 gene on hemoglobin toxicity in the striatum. *J. Cereb. Blood Flow Metab.* **25**, 1466–1475 (2005).
137. Lok, J. et al. Intracranial hemorrhage: mechanisms of secondary brain injury. *Acta Neurochir. Suppl.* **111**, 63–69 (2011).
138. Verellen, R. M. & Cavazos, J. E. Post-traumatic epilepsy: an overview. *Therapy* **7**, 527–531 (2010).
139. Meyerhoff, J. L., Lee, J. K., Rittase, B. W., Tsang, A. Y. & Yourick, D. L. Lipoic acid pretreatment attenuates ferric chloride-induced seizures in the rat. *Brain Res.* **1016**, 139–144 (2004).
140. Kramer, D. R., Winer, J. L., Pease, B. A., Amar, A. P. & Mack, W. J. Cerebral vasospasm in traumatic brain injury. *Neurol. Res. Int.* **2013**, 415813 (2013).
141. Yang, S. et al. Elevated serum haptoglobin after traumatic brain injury is synthesized mainly in liver. *J. Neurosci. Res.* **91**, 230–239 (2013).
142. Conti, A. et al. Proteome study of human cerebrospinal fluid following traumatic brain injury indicates fibrin(ogen) degradation products as trauma-associated markers. *J. Neurotrauma.* **21**, 854–863 (2004).
143. Anderson, G. D. et al. Haptoglobin phenotype and apolipoprotein E polymorphism: relationship to posttraumatic seizures and neuropsychological functioning after traumatic brain injury. *Epilepsy Behav.* **16**, 501–506 (2009).
144. Glushakov, A. V., Arias, R. A., Tolosano, E. & Dorė, S. Age-dependent effects of haptoglobin deletion in neurobehavioral and anatomical outcomes following traumatic brain injury. *Front. Mol. Biosci.* **3**, 34 (2016).
145. Zhang, Z., Zhang, Z. Y., Wu, Y. & Schluesener, H. J. Lesional accumulation of CD163+ macrophages/microglia in rat traumatic brain injury. *Brain Res.* **1461**, 102–110 (2012).
146. Okubo, S., Xi, G., Keep, R. F., Muraszko, K. M. & Hua, Y. Cerebral hemorrhage, brain edema, and heme oxygenase-1 expression after experimental traumatic brain injury. *Acta Neurochir. Suppl.* **118**, 83–87 (2013).
147. Liu, Y., Zhang, Z., Luo, B. & Schluesener, H. J. Lesional accumulation of heme oxygenase-1+ microglia/macrophages in rat traumatic brain injury. *Neuroreport* **24**, 281–286 (2013).
148. Fukuda, K. et al. Induction of heme oxygenase-1 (HO-1) in glia after traumatic brain injury. *Brain Res.* **736**, 68–75 (1996).
149. Chang, E. F. et al. Heme oxygenase-2 protects against lipid peroxidation-mediated cell loss and impaired motor recovery after traumatic brain injury. *J. Neurosci.* **23**, 3689–3696 (2003).
150. Yoneyama-Sarnecky, T. et al. Heme oxygenase-2 modulates early pathogenesis after traumatic injury to the immature brain. *Dev. Neurosci.* **32**, 81–90 (2010).
151. Levy, M., Turtzo, C. & Llinas, R. H. Superficial siderosis: a case report and review of the literature. *Nat. Clin. Pract. Neurol.* **3**, 54–58; quiz 59 (2007).
152. Koeppen, A. H. & Borke, R. C. Experimental superficial siderosis of the central nervous system I. Morphological observations. *J. Neuropathol. Exp. Neurol.* **50**, 579–594 (1991).
153. Koeppen, A. H. & Dickson, A. C. Tin-protoporphyrin prevents experimental superficial siderosis in rabbits. *J. Neuropathol. Exp. Neurol.* **61**, 689–701 (2002).
154. National Health Service Research Authority. Clearing haemoglobin in superficial siderosis (CHISS). *NHS* https://www.hra.nhs.uk/planning-and-improving-research/application-summaries/research-summaries/clearing-haemoglobin-in-superficial-siderosis-chiss/ (2016).
155. Wu, C. W. et al. Hemoglobin promotes Abeta oligomer formation and localizes in neurons and amyloid deposits. *Neurobiol. Dis.* **17**, 367–377 (2004).
156. Handa, Y. et al. The effect of timing of clot removal on chronic vasospasm in a primate model. *J. Neurosurg.* **67**, 558–564 (1987).
157. Ibrahim, G. M. et al. Method of aneurysm treatment does not affect clot clearance after aneurysmal subarachnoid hemorrhage. *Neurosurgery* **70**, 102–109; discussion 109 (2012).
158. Kramer, A. H. & Fletcher, J. J. Locally-administered intrathecal thrombolytics following aneurysmal subarachnoid hemorrhage: a systematic review and meta-analysis. *Neurocrit. Care.* **14**, 489–499 (2011).
159. Hänggi, D. et al. A multimodal concept in patients after severe aneurysmal subarachnoid hemorrhage: results of a controlled single centre prospective randomized multimodal phase I/II trial on cerebral vasospasm. *Cent. Eur. Neurosurg.* **70**, 61–67 (2009).
160. Etminan, N. et al. Prospective, randomized, open-label phase II trial on concomitant intraventricular fibrinolysis and low-frequency rotation after severe subarachnoid hemorrhage. *Stroke* **44**, 2162–2168 (2013).
161. Kramer, A. H. et al. Intraventricular fibrinolysis with tissue plasminogen activator is associated with transient cerebrospinal fluid inflammation: a randomized controlled trial. *J. Cereb. Blood Flow Metab.* **35**, 1241–1248 (2015).
162. US National Library of Medicine. *ClinicalTrials.gov* https://www.clinicaltrials.gov/ct2/show/NCT03187405 (2017).
163. US National Library of Medicine. *ClinicalTrials.gov*https://www.clinicaltrials.gov/ct2/show/NCT02872636 (2018).
164. Klimo, P., Kestle, J. R., MacDonald, J. D. & Schmidt, R. H. Marked reduction of cerebral vasospasm with lumbar drainage of cerebrospinal fluid after subarachnoid hemorrhage. *J. Neurosurg.* **100**, 215–224 (2004).
165. Kwon, O. Y., Kim, Y. J., Cho, C. S., Lee, S. K. & Cho, M. K. The utility and benefits of external lumbar CSF drainage after endovascular coiling on aneurysmal subarachnoid hemorrhage. *J. Korean Neurosurg. Soc.* **43**, 281–287 (2008).
166. Sun, C. et al. Choice for the removal of bloody cerebrospinal fluid in postcoiling aneurysmal subarachnoid hemorrhage: external ventricular drainage or lumbar drainage? *Turk. Neurosurg.* **24**, 737–744 (2014).
167. Al-Tamimi, Y. Z. et al. Lumbar drainage of cerebrospinal fluid after aneurysmal subarachnoid hemorrhage: a prospective, randomized, controlled trial (LUMAS). *Stroke* **43**, 677–682 (2012).
This article presents the first definitive trial of CSF drainage after SAH.

168. Bardutzky, J. et al. EARLYDRAIN- outcome after early lumbar CSF-drainage in aneurysmal subarachnoid hemorrhage: study protocol for a randomized controlled trial. *Trials* **12**, 203 (2011).
169. Mendelow, A. D. et al. Early surgery versus initial conservative treatment in patients with spontaneous supratentorial intracerebral haematomas in the International Surgical Trial in Intracerebral Haemorrhage (STICH): a randomised trial. *Lancet* **365**, 387–397 (2005).
170. Mendelow, A. D. et al. Early surgery versus initial conservative treatment in patients with spontaneous supratentorial lobar intracerebral haematomas (STICH II): a randomised trial. *Lancet* **382**, 397–408 (2013).
171. Mould, W. A. et al. Minimally invasive surgery plus recombinant tissue-type plasminogen activator for intracerebral hemorrhage evacuation decreases perihematomal edema. *Stroke* **44**, 627–634 (2013).
172. US National Library of Medicine. *ClinicalTrials.gov* https://www.clinicaltrials.gov/ct2/show/NCT01827046 (2017).
173. Nonaka, T., Watanabe, S., Chigasaki, H., Miyaoka, M. & Ishii, S. Etiology and treatment of vasospasm following subarachnoid hemorrhage. *Neurol. Med. Chir.* **19**, 53–60 (1979).
174. Shindo, S. Haptoglobin subtyping with anti-haptoglobin alpha chain antibodies. *Electrophoresis* **11**, 483–488 (1990).
175. Lipiski, M. et al. Human Hp1-1 and Hp2-2 phenotype-specific haptoglobin therapeutics are both effective in vitro and in guinea pigs to attenuate hemoglobin toxicity. *Antioxid. Redox Signal.* **19**, 1619–1633 (2013).
176. Kubota, K., Egi, M. & Mizobuchi, S. Haptoglobin administration in cardiovascular surgery patients: its association with the risk of postoperative acute kidney injury. *Anesth. Analg.* **124**, 1771–1776 (2017).
177. Muta, T. et al. Anti-haptoglobin antibody detection after febrile non-hemolytic transfusion reactions in a non-haptoglobin-deficient patient. *Transfus. Apher. Sci.* **41**, 171–173 (2009).
178. Chen, G., Fang, Q., Zhang, J., Zhou, D. & Wang, Z. Role of the Nrf2-ARE pathway in early brain injury after experimental subarachnoid hemorrhage. *J. Neurosci. Res.* **89**, 515–523 (2011).
This study is the first demonstration of the potential therapeutic role of NRF2 upregulation after SAH.
179. Zhao, X. D., Zhou, Y. T. & Lu, X. J. Sulforaphane enhances the activity of the Nrf2-ARE pathway and attenuates inflammation in OxyHb-induced rat vascular smooth muscle cells. *Inflamm. Res.* **62**, 857–863 (2013).
180. Zhao, X., Wen, L., Dong, M. & Lu, X. Sulforaphane activates the cerebral vascular Nrf2-ARE pathway and suppresses inflammation to attenuate cerebral vasospasm in rat with subarachnoid hemorrhage. *Brain Res.* **1653**, 1–7 (2016).
181. US National Library of Medicine. *ClinicalTrials.gov* https://www.clinicaltrials.gov/ct2/show/NCT02614742 (2018).
182. Zhao, X. R., Gonzales, N. & Aronowski, J. Pleiotropic role of PPARγ in intracerebral hemorrhage: an intricate system involving Nrf2, RXR, and NF-κB. *CNS Neurosci. Ther.* **21**, 357–366 (2015).
183. Zhao, X. et al. Hematoma resolution as a target for intracerebral hemorrhage treatment: role for peroxisome proliferator-activated receptor gamma in microglia/macrophages. *Ann. Neurol.* **61**, 352–362 (2007).
184. Lin, B. F. et al. Rosiglitazone attenuates cerebral vasospasm and provides neuroprotection in an experimental rat model of subarachnoid hemorrhage. *Neurocrit Care.* **21**, 316–331 (2014).
185. Gonzales, N. R. et al. Design of a prospective, dose-escalation study evaluating the Safety of Pioglitazone for Hematoma Resolution in Intracerebral Hemorrhage (SHRINC). *Int. J. Stroke.* **8**, 388–396 (2013).
186. Cho, H. Y. et al. Nrf2-regulated PPAR{gamma} expression is critical to protection against acute lung injury in mice. *Am. J. Respir. Crit. Care Med.* **182**, 170–182 (2010).
187. Park, E. Y., Cho, I. J. & Kim, S. G. Transactivation of the PPAR-responsive enhancer module in chemopreventive glutathione S-transferase gene by the peroxisome proliferator-activated receptor-gamma and retinoid X receptor heterodimer. *Cancer Res.* **64**, 3701–3713 (2004).
188. Selim, M. et al. Safety and tolerability of deferoxamine mesylate in patients with acute intracerebral hemorrhage. *Stroke* **42**, 3067–3074 (2011).
189. Yeatts, S. D., Palesch, Y. Y., Moy, C. S. & Selim, M. High dose deferoxamine in intracerebral hemorrhage (HI-DEF) trial: rationale, design, and methods. *Neurocrit Care.* **19**, 257–266 (2013).
190. US National Library of Medicine. *ClinicalTrials.gov* https://www.clinicaltrials.gov/ct2/show/NCT02175225 (2018).
191. Yu, Y. et al. The clinical effect of deferoxamine mesylate on edema after intracerebral hemorrhage. *PLoS ONE.* **10**, e0122371 (2015).
192. US National Library of Medicine. *ClinicalTrials.gov* https://www.clinicaltrials.gov/ct2/show/NCT02875262 (2017).
193. Levy, M. & Llinas, R. Pilot safety trial of deferiprone in 10 subjects with superficial siderosis. *Stroke* **43**, 120–124 (2012).
194. Kessler, R. A. et al. Two-year observational study of deferiprone in superficial siderosis. *CNS Neurosci. Ther.* **24**, 187–192 (2017).
195. World Health Organization. International Clinical Trials Registry Platform. *WHO* http://apps.who.int/trialsearch/Trial3.aspx?trialid=JPRN-UMIN000021218 (2016).
196. Zhang, S., Wang, L., Liu, M. & Wu, B. Tirilazad for aneurysmal subarachnoid haemorrhage. *Cochrane Database Syst. Rev.* **17**, CD006778 (2010).
197. Galea, J. et al. Intravenous anakinra can achieve experimentally effective concentrations in the central nervous system within a therapeutic time window: results of a dose-ranging study. *J. Cereb. Blood Flow Metab.* **31**, 439–447 (2011).
198. Galea, J. et al. Reduction of inflammation after administration of interleukin-1 receptor antagonist following aneurysmal subarachnoid hemorrhage: results of the Subcutaneous Interleukin-1Ra in SAH (SCIL-SAH) study. *J. Neurosurg.* **128**, 515–523 (2017).
199. US National Library of Medicine. *ClinicalTrials.gov* https://www.clinicaltrials.gov/ct2/show/NCT03249207 (2017).
200. Oldfield, E. H. et al. Safety and pharmacokinetics of sodium nitrite in patients with subarachnoid hemorrhage: a phase IIa study. *J. Neurosurg.* **119**, 634–641 (2013).
201. US National Library of Medicine. *ClinicalTrials.gov* https://www.clinicaltrials.gov/ct2/show/NCT02176837 (2018).
202. Bath, P. M. et al. Efficacy of nitric oxide, with or without continuing antihypertensive treatment, for management of high blood pressure in acute stroke (ENOS): a partial-factorial randomised controlled trial. *Lancet* **385**, 617–628 (2015).
203. Bjornsson, E. et al. A rare splice donor mutation in the haptoglobin gene associates with blood lipid levels and coronary artery disease. *Hum. Mol. Genet.* **26**, 2364–2376 (2017).
204. Melamed-Frank, M. et al. Structure-function analysis of the antioxidant properties of haptoglobin. *Blood* **98**, 3693–3698 (2001).
205. Asleh, R., Guetta, J., Kalet-Litman, S., Miller-Lotan, R. & Levy, A. P. Haptoglobin genotype- and diabetes-dependent differences in iron-mediated oxidative stress in vitro and in vivo. *Circ. Res.* **96**, 435–441 (2005).
206. Bamm, V. V., Tsemakhovich, V. A., Shaklai, M. & Shaklai, N. Haptoglobin phenotypes differ in their ability to inhibit heme transfer from hemoglobin to LDL. *Biochemistry* **43**, 3899–3906 (2004).
207. Pimenova, T. et al. Quantitative mass spectrometry defines an oxidative hotspot in hemoglobin that is specifically protected by haptoglobin. *J. Proteome Res.* **9**, 4061–4070 (2010).
208. Mollan, T. L. et al. Redox properties of human hemoglobin in complex with fractionated dimeric and polymeric human haptoglobin. *Free Radic. Biol. Med.* **69**, 265–277 (2014).
209. Asleh, R. et al. Genetically determined heterogeneity in hemoglobin scavenging and susceptibility to diabetic cardiovascular disease. *Circ. Res.* **92**, 1193–1200 (2003).
210. Na, N., Ouyang, J., Taes, Y. E. & Delanghe, J. R. Serum free hemoglobin concentrations in healthy individuals are related to haptoglobin type. *Clin. Chem.* **51**, 1754–1755 (2005).
211. Guetta, J., Strauss, M., Levy, N. S., Fahoum, L. & Levy, A. P. Haptoglobin genotype modulates the balance of Th1/Th2 cytokines produced by macrophages exposed to free hemoglobin. *Atherosclerosis* **191**, 48–53 (2007).
212. Bechmann, I., Galea, I. & Perry, V. H. What is the blood-brain barrier (not)? *Trends Immunol.* **28**, 5–11 (2007).
213. Thompson, E. J. in *Proteins of the Cerebrospinal Fluid: Analysis & Interpretation in the Diagnosis and Treatment of Neurological Disease* (ed. Thompson, E. J.) 13–30 (Elsevier, 2005).
214. Andersen, C. B. F. et al. Haptoglobin. *Antioxid. Redox Signal.* **26**, 814–831 (2017).
215. Barua, N. U. et al. Intrastriatal convection-enhanced delivery results in widespread perivascular distribution in a pre-clinical model. *Fluids Barriers CNS.* **9**, 2 (2012).
216. Carare, R. O. et al. Solutes, but not cells, drain from the brain parenchyma along basement membranes of capillaries and arteries: significance for cerebral amyloid angiopathy and neuroimmunology. *Neuropathol. Appl. Neurobiol.* **34**, 131–144 (2008).
217. Iliff, J. J. et al. Brain-wide pathway for waste clearance captured by contrast-enhanced MRI. *J. Clin. Invest.* **123**, 1299–1309 (2013).
218. Larsson, M., Cheng, T.-M., Chen, C.-Y. & Mao, S. J. T. in *Acute Phase Proteins* (ed. Janciauskiene, S.) 163–180 (InTech, 2013).
219. Mosconi, E. et al. Breast milk immune complexes are potent inducers of oral tolerance in neonates and prevent asthma development. *Mucosal Immunol.* **3**, 461–474 (2010).
220. Carare, R. O. et al. Immune complex formation impairs the elimination of solutes from the brain: implications for immunotherapy in Alzheimer's disease. *Acta Neuropathol. Commun.* **1**, 48 (2013).
221. Galea, I., Bechmann, I. & Perry, V. H. What is immune privilege (not)? *Trends Immunol.* **28**, 12–18 (2007).
222. Andersson, P. B., Perry, V. H. & Gordon, S. The acute inflammatory response to lipopolysaccharide in CNS parenchyma differs from that in other body tissues. *Neuroscience* **48**, 169–186 (1992).
223. Engelhardt, B. et al. Vascular, glial, and lymphatic immune gateways of the central nervous system. *Acta Neuropathol.* **132**, 317–338 (2016).
224. US National Library of Medicine. *ClinicalTrials.gov* https://www.clinicaltrials.gov/ct2/show/NCT03040128 (2017).
225. US National Library of Medicine. *ClinicalTrials.gov* https://www.clinicaltrials.gov/ct2/show/NCT01805895 (2018).
226. US National Library of Medicine. *ClinicalTrials.gov* http://www.clinicaltrials.gov/ct2/show/NCT02113176 (2014).
227. World Health Organization. International Clinical Trials Registry Platform. *WHO* http://apps.who.int/trialsearch/Trial2.aspx?TrialID=EUCTR2005-004684-31-GB (2012).
228. US National Library of Medicine. *ClinicalTrials.gov* https://www.clinicaltrials.gov/ct2/show/NCT00585559 (2018).
229. Shao, A. et al. Hydrogen-rich saline attenuated subarachnoid hemorrhage-induced early brain injury in rats by suppressing inflammatory response: possible involvement of NF-κB pathway and NLRP3 inflammasome. *Mol. Neurobiol.* **53**, 3462–3476 (2016).
230. World Health Organization. International Clinical Trials Registry Platform. *WHO* http://apps.who.int/trialsearch/Trial2.aspx?TrialID=ChiCTR-IIR-16009220 (2017).
231. Lyden, P. D. et al. Safety and tolerability of NXY-059 for acute intracerebral hemorrhage: the CHANT Trial. *Stroke* **38**, 2262–2269 (2007).
232. Dixon, S. J. et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* **149**, 1060–1072 (2012).
233. Li, Q. et al. Inhibition of neuronal ferroptosis protects hemorrhagic brain. *JCI Insight* **2**, e90777 (2017).
234. Liu, Y. et al. The 5-lipoxygenase inhibitor zileuton confers neuroprotection against glutamate oxidative damage by inhibiting ferroptosis. *Biol. Pharm. Bull.* **38**, 1234–1239 (2015).
235. Zilka, O. et al. On the mechanism of cytoprotection by ferrostatin-1 and liproxstatin-1 and the role of lipid peroxidation in ferroptotic cell death. *ACS Cent. Sci.* **3**, 232–243 (2017).
236. King, M. D., Whitaker-Lea, W. A., Campbell, J. M., Alleyne, C. H. & Dhandapani, K. M. Necrostatin-1 reduces neurovascular injury after intracerebral hemorrhage. *Int. J. Cell Biol.* **2014**, 495817 (2014).
237. Rushmore, T. H., Morton, M. R. & Pickett, C. B. The antioxidant responsive element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. *J. Biol. Chem.* **266**, 11632–11639 (1991).
238. Sandberg, M., Patil, J., D'Angelo, B., Weber, S. G. & Mallard, C. NRF2-regulation in brain health and disease: implication of cerebral inflammation. *Neuropharmacology* **79**, 298–306 (2014).

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Author contributions
I.G. led the Review with D.B. and B.G. Each author wrote a review of a subtopic. These subtopical reviews were then integrated by D.B., B.G. and I.G. into a unified first draft, which was further revised by all authors. Figures were prepared by A.Z., J.G. and I.G. and were reviewed by all authors.

Competing interests
The authors declare no competing interests.

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Review criteria
PubMed was searched for articles published in English prior to June 2017 using the following search terms: "h(a)emoglobin", "meth(a)emoglobin", "h(a)eme", "h(a)emolysis", "haptoglobin", "CD163", "h(a)emopexin", "CD91 OR LRP1", "HO-1 OR HO1 OR HO-2 OR HO2 OR h(a)eme oxygenase", "bilirubin", "iron", "ferritin" and "h(a)emosiderin" in single combinations with "neurological", "brain", "intracerebral", "intracranial", "subarachnoid", "h(a)emorrhage", "treatment", "trial", "toxic(ity)", "outcome(s)", and "gene(tics)". Clinical trial registries (European Clinical Trials Database, ClinicalTrials.gov, the International Standard Registered Clinical/Social Study Number (ISRCTN) registry and the WHO International Clinical Trials Registry Platform) were searched for treatments in subarachnoid haemorrhage, intracerebral haemorrhage and superficial siderosis. The reference lists of published articles were manually searched for further articles. All clinical studies reviewed were assessed for quality by D.B., B.G. and I.G. by tabulating study design, sample size, risk of bias and study quality using the Cochrane Tool (for trials) or the Newcastle–Ottawa Score (for observational studies), Cohen's effect size and *P* value (Supplementary Tables 1,2).

Supplementary information
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This is meta-analysis of published data. Along with James Glazier I collected, curated and analysed the data, in addition, to writing the manuscript. The remaining co-authors contributed to subsequent drafts of the manuscript. A corrigendum was submitted correcting a minor error in the results and in the presentation of the figures.

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Corrigendum

Corrigendum to "Haptoglobin Genotype and Outcome after Subarachnoid Haemorrhage: New Insights from a Meta-Analysis"

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In the article titled "Haptoglobin Genotype and Outcome after Subarachnoid Haemorrhage: New Insights from a Meta-Analysis" [1], there were errors that have been corrected in the revised version shown below.

(i) The Results section has been updated.

(ii) Table 3 has been corrected.

(iii) Figures 2 and 3 have been corrected.

Abstract

Haptoglobin (Hp) is a plasma protein involved in clearing extracellular haemoglobin and regulating inflammation; it exists in two genetic variants (Hp1 and Hp2). In a meta-analysis of six published studies, we confirm that Hp genotype affects short-term outcome (cerebral vasospasm and/or delayed cerebral ischemia) after subarachnoid haemorrhage (SAH) but not long-term outcome (Glasgow Outcome Score and modified Rankin Scale between one and three months). A closer examination of the heterozygous group revealed that the short-term outcome of Hp2-1 individuals clustered with that of Hp1-1 and not with that of Hp2-2, suggesting that the presence of one Hp1 allele was sufficient to confer protection. Since the presence of the Hp dimer is the only common feature between Hp1-1 and Hp2-1 individuals, the absence of this Hp moiety is most likely to underlie vasospasm in Hp2-2 individuals. These results have implications for prognosis after SAH and will inform further research into Hp-based mechanism of action and treatment.

1. Introduction

Haptoglobin (Hp) is an acute phase protein that binds to extracellular haemoglobin (Hb) with very high affinity. The resulting Hp-Hb complex is scavenged via CD163 expressed by cells of myeloid lineage [1]. There are two Hp alleles, Hp1 and Hp2. Hp2 is a longer protein which arose during an intragenic duplication event affecting exons 3 and 4 in the Hp1 gene. Recent data suggests that this happened at some point very early in human evolution, followed by recurring exonic deletions to reestablish modern Hp1 [2]. Individuals can express one of three Hp genotypes: Hp1-1, Hp2-1, and Hp2-2. The alpha chain of Hp has one cysteine residue in Hp1 but two cysteine residues in Hp2. These cysteine residues can form intermolecular disulphide bonds to give rise to Hp molecules of different sizes [3]. Hp1-1 homozygotes only form Hp dimers and Hp2-2 homozygotes only form higher-order Hp polymers, while Hp2-1 heterozygous individuals form both Hp dimer and higher-order Hp polymers. In Hp2-2 homozygotes, polymers are cyclic (i.e., $Hp(\alpha 2\beta)n$ where $n = 3$ and above). In Hp2-1 heterozygotes, linear polymers form since polymer growth is arrested by Hp1 at both ends (i.e., $Hp(\alpha 1\beta)2(\alpha 2\beta)n$ where $n = 0$ and above) [4].

Aneurysmal subarachnoid haemorrhage (aSAH) carries substantial morbidity and mortality. A common and serious complication of aSAH is that of cerebral vasospasm (CV).

Appendix A

Table 1: Reported differences between Hp types, relevant to SAH.

Function	No difference between Hp types	Difference between Hp types
Hp expression		Serum Hp1-1 is higher than Hp2-2, with Hp2-1 intermediate, in many populations tested, including European (Belgian [37–39], Iceland [40]), East Asian (Japanese [41], Koreans [42]), and African (Black Zimbabweans [43], Gabonese [44], Papuans [45]).
Haemoglobin binding: capacity per Hp monomer	(1) Ultrafiltration assay of uncomplexed Hb [46] (2) Mass spectrometry [24]	
Haemoglobin binding: affinity	(1) Surface plasmon resonance [47] (2) Surface plasmon resonance [24] (3) Spectrophotometric signal of Hp-Hp interaction [48]	
Inhibition of Hb-mediated oxidation	(1) Reduction in low-density lipoprotein oxidation [24] (2) Reduction in Hb intrinsic redox potential [48] (3) Reduction in Hb autooxidation [17]	(1) Hp1-1 is better than Hp2-2 at inhibiting protein and lipid oxidation [49]. (2) Hp1-1 is better than Hp2-2 at inhibiting oxidation of linolenic acid and low-density lipoprotein [46]. (3) Hp1-1 is better than Hp2-2 at inhibiting lipid peroxidation [47].
Interaction with CD163: affinity		(1) Hp2-2 is better than Hp1-1, by surface plasmon resonance and binding of radioiodinated Hp-Hb complexes *in vitro* [1]. (2) Hp2-2 is better than Hp1-1, by binding of radioiodinated Hp-Hb complexes *in vitro* [50].
Interaction with CD163: uptake of Hp-Hb complexes	Plasma half-life of Hp-Hb complexes after injection in guinea pigs [24]	(1) Hp2-2 is better than Hp1-1, by measurement of free Hb in humans [51]. (2) Hp1-1 is better than Hp2-2, by uptake of radioiodinated Hp-Hb complexes in human cells *in vitro* [50].
Effects on inflammation		Binding of Hp1-1-Hb complexes to CD163 results in secretion of the anti-inflammatory cytokine IL-10 [19, 20].

Prolonged or pronounced vasoconstriction of major cerebral blood vessels can lead to delayed cerebral ischemia (DCI), which occurs in up to 30% of individuals who survive aSAH, manifesting as new focal neurological signs and/or deterioration in level of consciousness. Together, CV and DCI contribute to short-term outcome, by increasing short-term morbidity, hospital stay, and costs [5]. Longer-term outcome and functional status after aSAH are typically assessed using the modified Rankin Scale (mRS) or the Glasgow Outcome Scale (GOS).

Hp alleles profoundly affect outcome after intracranial haemorrhage, such as aSAH; Hp2 confers a poorer prognosis, with odds ratios of up to 4 being reported [6]. The underlying mechanism remains to be established. There are three potential biological mechanisms to explain this phenomenon: difference in Hp expression, Hp function, and Hp-Hb complex size. There is agreement that serum Hp expression is highest in Hp1-1 individuals, intermediate in Hp2-1, and lowest in Hp2-2 individuals (Table 1). With respect to functional aspects of Hp relevant to SAH, these include affinity of Hp binding to Hb, Hb binding capacity of Hp, protection from Hb's redox toxicity, affinity to CD163, CD163-mediated uptake, and effects on inflammation (Table 1). There is a lack of agreement as to whether Hp1 and Hp2 differ with respect to some of these aspects, and in which direction, as reviewed in Table 1. The third potential mechanism relates to the fact that the dimer produced by Hp1-1 and Hp2-1 individuals is smaller than other higher-order polymers produced by Hp2-2 individuals. This may be important since solute drainage from the brain along the glymphatic pathway has a size selectivity [7]. Drainage of Hp-Hb complexes from the brain to the circulation may be important since CD163 binding sites are reduced and saturated in the brain [8, 9].

The outcome of Hp2-1 individuals, when compared to that of Hp1-1 and Hp2-2, is likely to shed light on the mechanism underlying the prognostic effect of Hp. Differences in function between Hp types are likely to result in a dose-dependent effect between genotypes, while a predominant effect of the Hp dimer is likely to result in similar outcomes in Hp2-1 and Hp1-1 individuals, but different from Hp2-2 individuals. So far, small sample sizes have precluded meaningful comparison of the outcome of the heterozygous versus homozygous genotypes. We hypothesised that Hp2-2 individuals are at greater risk of poor short- and long-term outcomes after aSAH. A meta-analysis of published studies was performed with the following objectives: (1) to confirm the effect of Hp genotype on outcome after aSAH and (2)

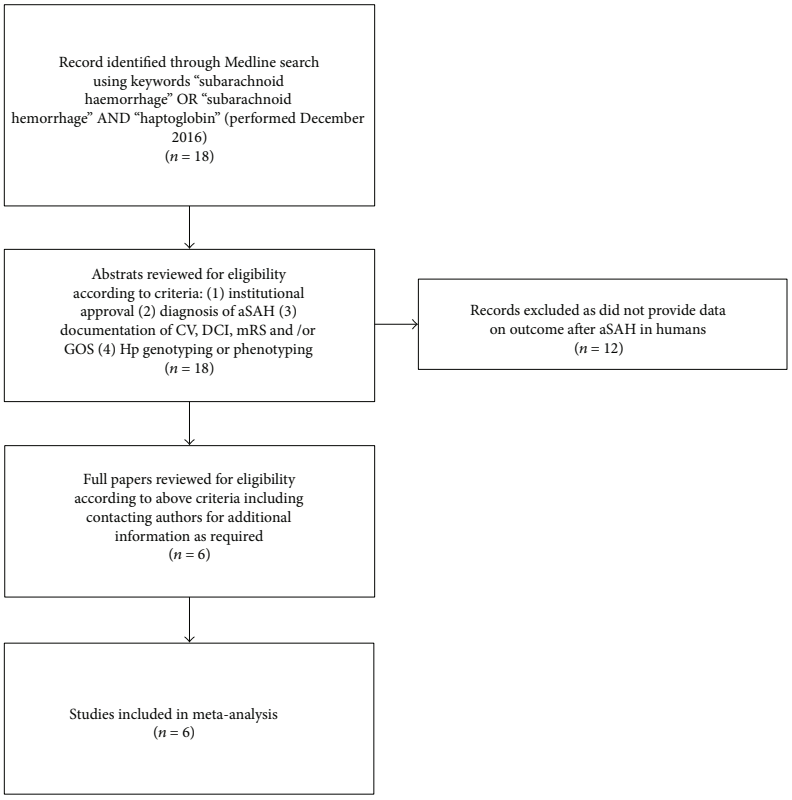

Figure 1: Flow diagram of studies selected for inclusion. If additional information was required, the authors were contacted by email.

to compare the outcome of Hp2-1 individuals with that of Hp1-1 and Hp2-2 individuals and so provide mechanistic insight. In summary, an unfavourable effect of the Hp2-2 genotype on short-term outcome was confirmed. The outcome of Hp2-1 individuals clustered with that of Hp1-1, suggesting that the presence of one Hp1 allele was sufficient to confer protection. Mechanistically, this is in keeping with the hypothesis that the Hp dimer is essential, possibly due to its small size.

2. Materials and Methods

Meta-analysis was conducted in accordance with the PRISMA [10] (Supplementary Table 1 available online at https://doi.org/10.1155/2017/6747940) and Cochrane Collaboration guidelines. Data from six studies were included in the meta-analysis (see Figure 1 for search criteria and Table 2 for summary of studies included; all studies were observational) [6, 8, 11–14]. For each individual study, bias was assessed using the Newcastle-Ottawa Scale for quality assessment of nonrandomised studies [15]. This assessment is based on 3 domains (selection, comparability, and outcome) and allows a study to be scored between 0 and 8. Authors BG, DB, and IG independently scored each study; if there was disagreement between scores, an average was taken. All studies included in this analysis scored 5 and were therefore considered to be at low risk of bias (Supplementary Table 2). Although all studies were assessed to be at low risk of bias, the scoring system highlighted the inclusion of higher Fisher grade patients, limited intrastudy controls, and short duration of follow-up as potential sources of bias. Tests for funnel plot asymmetry were not performed as the meta-analysis only included 6 studies, in keeping with recommendations from the Cochrane Collaboration. Short- and long-term outcomes were derived from the six studies and analysed separately. Short-term outcome was defined as CV and/or DCI during the inpatient period, as determined by any means, including cerebral angiography, transcranial Doppler ultrasonography, and clinical or radiological evidence of DCI. If both CV and DCI values were available, then

Table 2: Summary of studies included in meta-analysis.

Study (year)	Journal	Country	Inclusion/exclusion criteria	Patient number	Short-term outcome*	Long-term outcome*
Leclerc et al. (2015)	Proceedings of the National Academy of Sciences of the United States of America	USA	Inclusion: >18 years, aSAH	Hp1-1: 11 Hp2-1: 39 Hp2-2: 24	Clinical deterioration as a consequence of confirmed delayed cerebral ischemia	—
Murthy et al. (2016)	Neurosurgery	USA	Inclusion: >18 years, aSAH presenting within 24 h of ictus Exclusion: death on arrival, pregnancy, inability to obtain consent	Hp1-1: 29 Hp2-1: 57 Hp2-2: 47	Delayed cerebral ischemia defined as clinical deterioration with radiographic, angiographic, or clinical response to treatment with TCD evidence	GOS at 30 days post discharge
Kantor et al. (2014)	Journal of Neurosurgery	USA	Inclusion: 18–75 years, angiographic diagnosis of aSAH, Fisher grade 2–4, Caucasian Exclusion: preexisting neurological disease or deficit	Hp1-1: 25 Hp2-1: 109 Hp2-2: 59	—	mRS at 3 months
Ohnishi et al. (2013)	Journal of Stroke and Cerebrovascular Diseases	Japan	Inclusion: aSAH treated endovascularly or surgically	Hp1-1: 7 Hp2-1: 39 Hp2-2: 49	Delayed cerebral ischemia defined as development of focal neurology of a drop in GCS of 2 points	mRS at 3 months
Galea et al. (2012)	Journal of Neurochemistry	UK	Inclusion: SAH requiring external ventricular drainage, paired CSF and serum available Exclusion: external ventricular drain infection	Hp1-1: 4 Hp2-1: 21 Hp2-2: 1	Delayed cerebral ischemia defined as development of focal neurology of a drop in GCS of 2 points	—
Borsody et al. (2006)	Neurology	USA	Inclusion: >18 years, known date onset SAH, aSAH suspected, Fisher grade 3-4 Exclusion: diseases which affect Hp or development of VS	Hp1-1: 9 Hp2-1: 12 Hp2-2: 11	Transcranial Doppler (TCD) evidence of "presumed definite" vasospasm or angiogram evidence of vasospasm both by day 14 after SAH	—

*Only outcomes which were available for the meta-analysis are shown.

Table 3: Short and long-term outcome after aSAH.

Comparison groups Group A	Group B	*N* total Group A + B	Odds ratio for poor outcome Group A/B	Z	*p*	Heterogeneity Chi^2	Df	I^2
Short-term outcome								
Hp2-2	Hp1-1	192 (132 + 60)	2.37 (1.12, 5.04)	2.26	0.02*	3.93	3	24%
Hp2-1	Hp1-1	228 (168 + 60)	1.53 (0.74, 3.14)	1.16	0.25	5.92	4	32%
Hp2-2	Hp2-1	300 (132 + 168)	1.90 (1.11, 3.25)	2.34	0.02*	1.79	4	0%
Hp2-2	Hp1-1 & Hp2-1	360 (132 + 228)	2.07 (1.26, 3.41)	2.87	0.004*	1.77	4	0%
Hp2-1 & Hp2-2	Hp1-1	360 (300 + 60)	1.96 (0.99, 3.86)	1.94	0.05	5.11	4	22%
Long-term outcome								
Hp2-2	Hp1-1	216 (155 + 61)	1.61 (0.81, 3.16)	1.37	0.17	4.96	2	60%
Hp2-1	Hp1-1	266 (205 + 61)	1.28 (0.65, 2.51)	0.72	0.47	3.58	2	44%
Hp2-2	Hp2-1	360 (155 + 205)	1.34 (0.85, 2.10)	1.27	0.20	0.55	2	0%
Hp2-2	Hp1-1 & Hp2-1	421 (155 + 266)	1.37 (0.89, 2.10)	1.44	0.15	1.55	2	0%
Hp2-1 & Hp2-2	Hp1-1	421 (360 + 61)	1.41 (0.75, 2.65)	1.06	0.29	4.26	2	53%

*$p < 0.05$; *Z*: test for overall effect.

DCI data was used in preference due to greater clinical relevance. Both DCI and CV are features occurring in the initial period after SAH, which have a well-demonstrated impact on short-term morbidity, inpatient stay duration, and economic costs, justifying their joint qualification as short-term outcome. Data for short-term outcome was available from five studies (Borsody et al. [11], Galea et al. [8], Ohnishi et al. [14], Leclerc et al. [12], and Murthy et al. [13]) and was classified as either present or absent. Long-term outcome was defined as dichotomized mRS or GOS, between one and three months after aSAH. mRS and GOS scores of 0–2 and 4-5, respectively, were considered as favourable outcome, with the rest of the scores being unfavourable. Data for long-term outcome was available from three studies at one month (Murthy et al. [13]) or three months (Ohnishi et al. [14], Kantor et al. [6]). Meta-analysis was conducted in Review Manager (RevMan) v5.3.3. The Mantel–Haenszel (M–H) method for calculating the weighted pooled odds ratio in a fixed effects model was used. Significance was accepted to be present at $p < 0.05$.

3. Results

553 aSAH patients were included; short-term and long-term outcome data were available for 360 and 421 patients, respectively. Results are presented in Table 3; forest plots are presented in Figures 2 and 3. The Hp2-2 genotype imparted a worse short-term prognosis compared to Hp1-1 (OR = 2.37, 95% CI = 1.12–5.04, $p = 0.02$). The significance of this relationship was increased by including Hp2-1 with Hp1-1 cases (OR = 2.07, 95% CI = 1.26–3.41, $p = 0.004$) and decreased by including Hp2-1 with Hp2-2 cases (OR = 1.96, 95% CI = 0.99–3.86, $p = 0.05$), suggesting that the outcome of Hp2-1 patients more closely resembled that of Hp1-1 patients. In support of this explanation, the short-term outcome of Hp2-1 patients was significantly different from that of Hp2-2 patients (OR = 1.90, 95% CI = 1.11–3.25, $p = 0.02$), but not that of Hp1-1 patients (OR = 1.53, 95% CI = 0.74–3.14, $p = 0.25$). No effect of Hp genotype on long-term outcome was observed.

4. Discussion

Hp can protect against Hb toxicity in a number of ways. First, Hp lowers the redox potential of Hb by binding it. This is achieved by stabilizing ferryl iron [16] and globin-based amino acid radicals [16, 17], sites within or close to the interface between Hp and Hb [18], preventing these reactive entities from participating in redox reactions. Second, Hp targets Hb for degradation, since Hb-Hp is recognized and cleared by CD163 [1]. Third, Hp induces an anti-inflammatory response (e.g., interleukin-10 secretion [19, 20]), which serves to balance Hb or heme-induced proinflammatory effects (e.g., tumour necrosis factor [21, 22] and interleukin-1 [23] secretion). There is controversy as to whether some of these functions differ between Hp types (Table 1). It is important to note that Hp binding to Hb does not affect its capacity to scavenge nitric oxide [24, 25]. Hence, any nitric oxide-mediated mechanistic basis for differences in vasospasm between Hp genotypes is likely linked to clearance of Hb.

A study of experimental SAH in mice clearly demonstrated differences between Hp1 and Hp2 [26]. Mice only express Hp1, but mice genetically engineered to express Hp2 in place of Hp1 had a poorer outcome after SAH, compared to Hp1 wild-type mice [26]. However, this study did not examine Hp2-1 mice. This meta-analysis has confirmed that the Hp2-2 genotype confers a worse short-term outcome versus the Hp1-1 genotype in humans. Moreover, the short-term outcome of Hp2-1 patients clusters with that of Hp1-1 patients, suggesting that the presence of one Hp1 allele is sufficient to confer protection over Hp2.

The findings in the Hp2-1 heterozygous individuals have mechanistic implications. Functional mechanisms such as lowering Hb redox potential, CD163 uptake, or anti-inflammatory effects would be expected to result in dose-dependent differences in outcome between the three

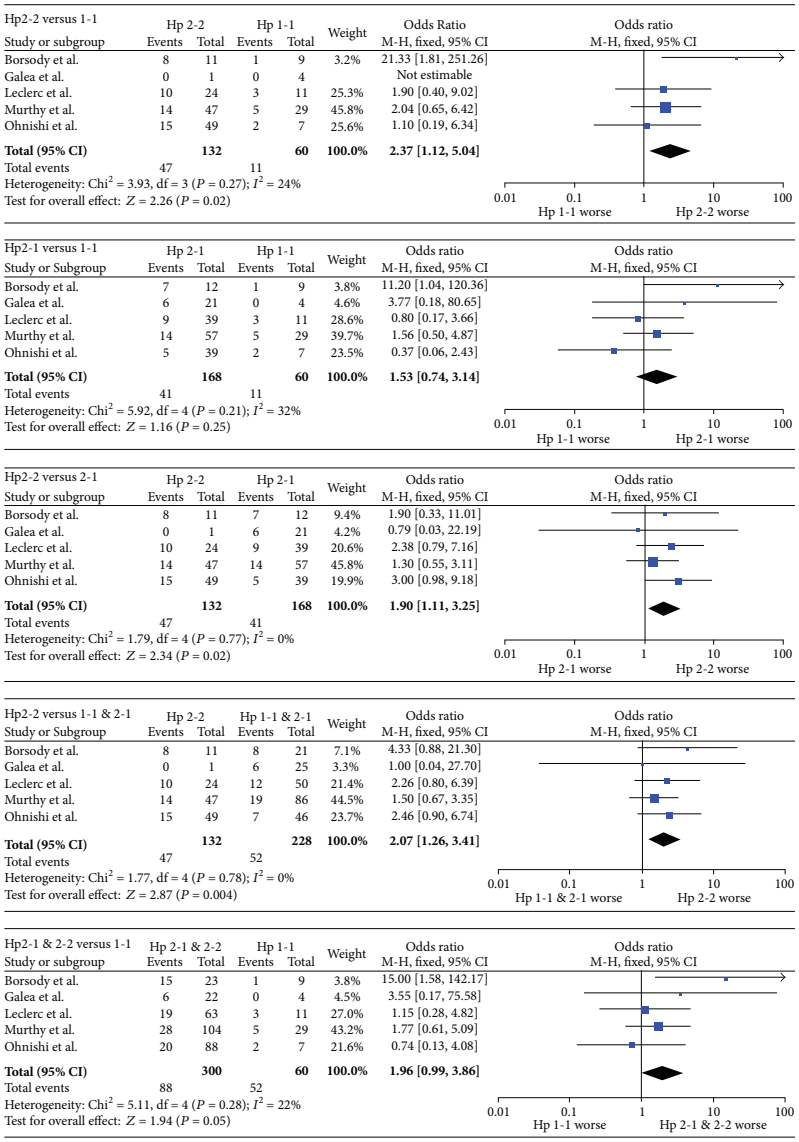

Figure 2: Forest plots for short-term outcome data. Short-term outcome was defined as CV and/or DCI during the inpatient period, as determined by any means, including cerebral angiography, transcranial Doppler ultrasonography, and clinical or radiological evidence of DCI.

genotypes. However, the short-term outcome of Hp2-1 was similar to that of Hp1-1. The common feature amongst Hp1-1 and Hp2-1 individuals is the presence of the Hp dimer, which therefore appears to be important in conferring protection. It is possible that the small size of the dimer facilitates drainage of the Hp-Hb complexes from the brain.

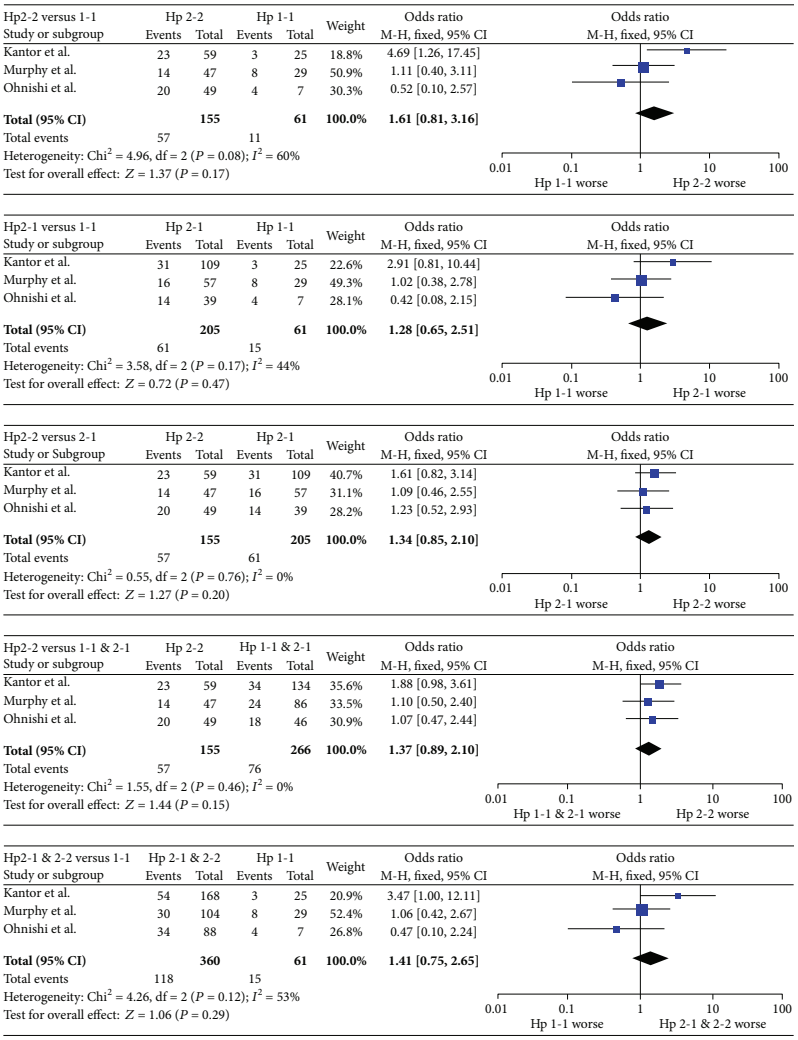

Figure 3: Forest plots for long-term outcome data. Long-term outcome was defined as dichotomized mRS or GOS between one and three months after aSAH.

Although several studies have shown upregulation of CD163 after intracerebral haemorrhage [27–30], CD163 binding sites are limiting after SAH since free Hp-Hb complexes persist in the cerebrospinal fluid [8, 9], possibly compounded by soluble CD163 shedding [8]. For this reason, drainage of Hp-Hb complexes out of the brain via the glymphatic pathway [7] may be important. There is evidence for a size selectivity in the glymphatic pathway [7] so that molecules with a molecular weight above 200 kDa have reduced clearance. The size of the Hp dimer in complex with Hb would be below this threshold (180 kDa), while Hb in complex with Hp polymers of increasing valency would have higher molecular weights. The small size of the Hp dimer also enables it to enter the brain across the blood-brain barrier while higher-order polymers find it more difficult [31]. Hence, amongst all the Hp forms, the Hp dimer would be able to recycle into and out of the brain with greatest ease, clearing Hb from the brain in the process. In keeping with this explanation, a

decrease in serum Hp occurs after aSAH, most marked in individuals with the highest blood-brain barrier disruption [8]. These speculations need to be addressed by experimental work to prove that Hp1-1-Hb complex size impacts on outcome by altering drainage of Hb out of the brain. It is still possible that Hp1/Hp2 differences in lowering Hb redox potential, CD163-mediated Hp-Hb uptake, or anti-inflammatory action could affect outcome in a manner which is not dose dependent.

The findings of this meta-analysis are important for prognostication in the clinical setting, since the Hp2-2 status clearly reflects a group of individuals who may benefit from closer monitoring within a specialist neurointensive care unit. Hp genotype did not affect long-term outcome in this meta-analysis, despite a clear relationship with short-term outcome. This may be due to several reasons. CV may not be related to long-term outcome and this remains controversial [32]. Due to their relatively crude nature, the GOS and mRS scales may not be sufficiently sensitive to detect differences. Recently, two groups have demonstrated upregulation of neuronal CD163 expression after intracranial haemorrhage in nonhuman models [30, 33, 34]—if this finding is confirmed in humans, neurons in Hp1 individuals may accumulate more intracellular heme/iron, which is toxic [35]. It is possible that the short-term beneficial effects of Hp1-1 on vasospasm are balanced by the long-term deleterious effects of Hp1-1 on neuronal iron accumulation, so that long-term outcome is unaffected overall.

This study has a number of limitations. Long-term outcome combined one- and three-month outcomes; however, a sensitivity analysis excluding the one-month study did not change the finding that Hp genotype did not affect the long-term outcome. Short-term outcome was defined as CV and/or DCI, and these phenomena might not necessarily be equivalent [36]; however, a sensitivity analysis excluding CV showed that DCI-only outcome of Hp2-1 patients more closely resembled that of Hp1-1 patients: Hp1-1 and Hp2-1 versus Hp2-2 ($p = 0\,02$, OR (CI) = 1.9 (1.12–3.22)) and Hp1-1 versus Hp2-1 and Hp2-2 ($p = 0\,83$, OR (CI) = 1.08 (0.55–2.14)). We also noted that the majority of participants across all studies had high Fisher grade aSAH, so generalizability of the findings here to other patients with aSAH needs to be approached with caution. Although there was no evidence of significant heterogeneity (Table 3), prognostic factors may still have been distributed asymmetrically amongst studies and/or genotype groups; therefore, an individual patient level data analysis is warranted to weigh up Hp genotype against other prognostic covariates, in determining outcome after aSAH.

5. Conclusions

In conclusion, this study confirms the unfavourable effect of the Hp2 allele on short-term outcome after aSAH. It advances the field by showing that the presence of one Hp1 allele is sufficient to counter the unfavourable effect of Hp2. This suggests that the Hp dimer is the structural determinant of the association of the Hp polymorphism with outcome after SAH. Therapies aimed at augmenting Hp may work best if designed to mainly deliver Hp1, rather than elevate Hp nonspecifically, in Hp2-1 and Hp2-2 individuals. Experimental studies are needed to prove this hypothesis.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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References

[1] B. Gaastra, J. Glazier, D. Bulters, and I. Galea, "Haptoglobin genotype and outcome after subarachnoid haemorrhage: new insights from a meta-analysis," *Oxidative Medicine and Cellular Longevity*, vol. 2017, Article ID 6747940, 9 pages, 2017.

A.4.3 Publication 8: Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis

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This individual patient level data meta-analysis includes data from multiple international cohorts. I collected, coordinated and curated the data used in this study. The primary statistical analysis was performed by Dianxu Ren. I authored the manuscript with input from the co-authors after completion of the first draft.

Specific contribution: Primary role in study design, data collection and curation. Authored first draft of manuscript.

NULL HYPOTHESIS

Haptoglobin genotype and aneurysmal subarachnoid hemorrhage

Individual patient data analysis

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Abstract

Objective

To perform an individual patient–level data (IPLD) analysis and to determine the relationship between haptoglobin (*HP*) genotype and outcomes after aneurysmal subarachnoid hemorrhage (aSAH).

Methods

The primary outcome was favorable outcome on the modified Rankin Scale or Glasgow Outcome Scale up to 12 months after ictus. The secondary outcomes were occurrence of delayed ischemic neurologic deficit, radiologic infarction, angiographic vasospasm, and transcranial Doppler evidence of vasospasm. World Federation of Neurological Surgeons (WFNS) scale, Fisher grade, age, and aneurysmal treatment modality were covariates for both primary and secondary outcomes. As preplanned, a 2-stage IPLD analysis was conducted, followed by these sensitivity analyses: (1) unadjusted; (2) exclusion of unpublished studies; (3) all permutations of *HP* genotypes; (4) sliding dichotomy; (5) ordinal regression; (6) 1-stage analysis; (7) exclusion of studies not in Hardy-Weinberg equilibrium (HWE); (8) inclusion of studies without the essential covariates; (9) inclusion of additional covariates; and (10) including only covariates significant in univariate analysis.

Results

Eleven studies (5 published, 6 unpublished) totaling 939 patients were included. Overall, the study population was in HWE. Follow-up times were 1, 3, and 6 months for 355, 516, and 438 patients. *HP* genotype was not associated with any primary or secondary outcome. No trends were observed. When taken through the same analysis, higher age and WFNS scale were associated with an unfavorable outcome as expected.

Conclusion

This comprehensive IPLD analysis, carefully controlling for covariates, refutes previous studies showing that HP1-1 associates with better outcome after aSAH.

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Glossary

aSAH = aneurysmal subarachnoid hemorrhage; **CI** = confidence interval; **DCI** = delayed cerebral ischemia; **GOS** = Glasgow Outcome Scale; **Hb** = hemoglobin; **Hp** = haptoglobin; ***HP*** = haptoglobin gene; **HWE** = Hardy-Weinberg equilibrium; **IPLD** = individual patient–level data; **mRS** = modified Rankin Scale; **OR** = odds ratio; **PRISMA-IPD** = Preferred Reporting Items for Systematic Reviews and Meta-Analyses–Individual Participant Data; **SAH** = subarachnoid hemorrhage; **TCD** = transcranial Doppler; **WFNS** = World Federation of Neurological Surgeons.

Aneurysmal subarachnoid hemorrhage (aSAH) survivors experience significant morbidity.[1] The strongest predictor of long-term outcome is the World Federation of Neurological Surgeons (WFNS) grade,[2] but it explained only 12% of the variance in outcome as determined by the Glasgow Outcome Scale (GOS)[3] in the largest study of outcome prediction in aSAH.[4] Some studies have suggested that haptoglobin (*HP*) genotype may also influence outcome,[5–9] but results are conflicting.[10] A recent meta-analysis found that the HP2 allele was associated with a worse short-term but not long-term outcome.[11] This meta-analysis had limitations, including small study sizes, heterogeneity in outcome classification, and inability to control for covariates. The relative contribution of *HP* genotype to outcome after aSAH in a large cohort, relative to the WFNS and other covariates, remains unknown.

In humans, there are 2 *HP* alleles, HP1 and HP2, and an individual can be 1 of 3 genotypes: HP1-1, HP2-1, and HP2-2. The main potential mechanism of the *HP* effect is through the function of its protein (Hp) as a scavenger of extracellular hemoglobin (Hb).[10] Because the Hp-Hb scavenging system is active in the CNS after aSAH,[12] there is a strong biological rationale to hypothesize that *HP* genetic variation influences aSAH outcome. We therefore conducted an individual patient–level data (IPLD) analysis of all identified published and unpublished studies to investigate the relationship between *HP* genotype and outcome after aSAH.

Methods

The IPLD analysis was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses–Individual Participant Data (PRISMA-IPD) guidelines.[13] To ensure rigor, we enrolled early professional statistical input, publicly deposited a protocol defining a priori the outcomes and analytic strategy in June 2017,[14] and included all published and unpublished studies identified before analysis. All individual studies had approval from the respective institutions, and the overall IPLD analysis had institutional ethics approval from the University of Southampton.

Search strategy

Published studies were identified by PubMed and Web of Science searches conducted in January 2017 with the key words subarachnoid hemorrhage or subarachnoid hemorrhage and haptoglobin, including reference lists within publications. Abstracts were screened and then full articles were reviewed for eligibility according to the inclusion/exclusion criteria. Two study investigators (B.G. and I.G.) conducted the search. Unpublished studies were identified with the same search terms in Google and via the professional network of the authors in 3 continents (United Kingdom, United States, and Japan).

Inclusion/exclusion criteria

Published and unpublished studies were eligible for inclusion, and there were no restrictions on study design. Inclusion criteria consisted of (1) confirmed aSAH at age >18 years; (2) HP genotype or phenotype available; (3) all essential covariates available (see below); (4) data available and contractual agreement reached by March 31, 2017; and (5) primary outcome measures available at 1 month (±2 weeks) and/or 3 months (±1.5 months) and/or 6 months (4.5–12 months) of aSAH. If >1 outcome was available within each of these time frames, the one closest to 1, 3, or 6 months was used. The only exclusion criterion was non-aSAH.

Data collection and management

The lead authors of all published and unpublished studies identified with the search strategy were contacted verbally or by e-mail and were invited to join the study. IPLD for patients meeting the inclusion/exclusion criteria was requested in spreadsheet format and was stored on a secure server at the University of Southampton, UK. The type of data requested has been published[14] (additional Methods available from Eprints, eprints.soton.ac.uk/426525/). Data were collated according to study center and encoded with study and patient identifiers to blind the statistical team.

Quality control

Studies were assessed for risk of bias with the Newcastle-Ottawa Scale. The data underwent a number of quality control checks. Automated screens were conducted to identify nonsensical values (e.g., out-of-range modified Rankin Scale [mRS] and GOS scores, impossible age) and to check internal consistency (e.g., mRS and GOS scores). Data descriptives were used to compare with the expected norm and thereby identify potential errors. Hardy-Weinberg equilibrium (HWE) was assessed as a marker for case missingness. Manual data checks were performed by 4 authors (B.G., D.O.B, D.R., and I.G.) independently. If any inconsistency or missing data were identified or if further clarification was required, the individual study lead was contacted.

Primary outcome

The per-protocol primary outcome was the mRS[15] or GOS score,[3] dichotomized into favorable and unfavorable. This outcome was selected because it was deemed to be the most clinically relevant outcome and was the most consistently recorded among the studies analyzed. If both mRS and GOS scores were available for the same dataset, the mRS score was used. The GOS score was dichotomized into favorable (GOS score 4–5 [good recovery or moderate disability]) or unfavorable (GOS score 1–3 [severe disability, vegetative state, or death]). The mRS score was dichotomized into favorable (mRS score 0–2 [good recovery, no significant disability, slight disability]) or unfavorable (mRS score 3–6 [moderate disability, moderately severe disability, severe disability, or death]).

Secondary outcomes

The secondary outcomes were delayed cerebral ischemia (DCI), radiologic infarction, angiographic evidence of vasospasm, and transcranial Doppler (TCD) evidence of vasospasm, defined as velocity >200 cm/s. The secondary outcomes could have been at any time during admission for aneurysm rupture. If a secondary outcome variable was not collected in a specific study, that study was not included in the meta-analysis of that secondary outcome.

Covariates

A minimum set of essential covariates was identified a priori to minimize sample size attrition while retaining the strongest known predictors of outcome after aSAH.[16] These essential covariates were used for both primary and secondary outcomes: (1) age; (2) Fisher grade, dichotomized into grades 1 + 2 and 3 + 4; (3) admission WFNS or Hunt and Hess grade dichotomized into good (grades 1–3) and poor (grades 4–5); and (4) treatment, categorized into endovascular and surgical. Aneurysms treated conservatively were excluded due to small numbers. If both WFNS and Hunt and Hess grades were available, the WFNS grade was used. For the primary outcome, follow-up time was used as a covariate using longitudinal modeling. During the analysis, subsequent to publication of the protocol, it became apparent that some of these essential covariates resulted in highly sparse cells, especially in small studies. Because WFNS grade is by far the most influential predictor among the minimum essential covariates,[4] studies in which WFNS grade could not be included as a covariate were excluded from the analysis. For both primary and secondary outcomes when possible, allowing for sample size and data availability (table e-1available from Eprints, eprints.soton.ac.uk/426525/), additional covariates were used and prioritized in the following order: diabetes mellitus, hypertension, race, and aneurysm site. If additional covariates were used, the same covariates were used in all studies.

Data analysis

The primary analytic strategy was a 2-stage IPLD study design; we also planned a secondary 1-stage IPLD analysis because it is thought that both designs have their individual strengths.[17] The primary study statistician (D.R.) mainly conducted the statistical analysis , and a second statistician in the team (T.H.) confirmed the results. The statisticians were blinded to the identification of the studies and patients throughout the analysis. HWE was assessed for all studies included. Two primary comparisons were planned: a binary comparison of HP2-2 vs HP2-1 and HP1-1 (because the previous meta-analysis showed that HP2-1 is similar to HP1-1 with respect to outcome) and a multicategorical comparison of HP1-1 vs HP2-1 vs HP2-2. The primary 2-stage IPLD analysis was performed for the primary and all secondary outcomes as follows. In the first stage, the analysis was conducted for each individual study to estimate the association of outcome with Hp, adjusting for the baseline covariates and time point. Given the binary nature of primary outcomes measured at time points of 1-, 3-, and 6-month follow-up (with studies having 1, 2, or 3 of these time points), generalized estimating equation models with logit link were implemented to account for the correlation between different time points within the same subject for each individual study. The interaction between time point and *HP* was also checked. Binary logistic regression models were used for all the secondary outcomes because they were associated with only 1 time point. Odds ratios (ORs) and 95% confidence intervals (CIs) from each individual study were estimated from the above models. Cases with missing data were excluded; that is, data were not imputed. In the second stage, ORs from the individual studies were combined by the use of random-effects meta-analysis. Results are reported as ORs with their 95% CIs and corresponding p values. Heterogeneity was assessed with the Higgins and Thompson I^2 statistic and Cochrane Q test, and publication bias (small-study effects) was examined with funnel plots and the Egger test. All hypotheses were tested at a nominal significance level of 0.05; that is, the probability of a type I error (α) was 5%. SAS (version 9.4, SAS Institute, Inc, Cary, NC) and STATA (version 14, StataCorp, College Station, TX) were used for all the analyses.

To assess the robustness of results from the above primary analysis, extensive sensitivity and/or subgroup analyses were conducted. We first replicated the analysis using the 1-stage approach with random-effect logistic regression modeling for all outcomes including site as random effect. Next, several subgroup analyses were performed, including exclusion of those studies not in HWE or at high risk of bias, inclusion of studies that do not have all the essential covariates, exclusion of essential covariates, and inclusion of additional covariates as defined above. We also coded GOS or mRS scores in 2 other ways. In the above analysis, we used the traditional approach of dichotomizing of GOS and mRS scores into 2 categories, namely favorable and unfavorable (i.e., GOS scores 1–3 vs 4–5; mRS scores 0–2 vs 3–6), to render statistical analysis and interpretation of results more straightforward and identical to most other published studies. However, this approach poses disadvantages: it discards valuable information of the full ordinal nature of outcome

measures and ignores initial prognostic risk of patients.[18–21] Hence, 2 alternative approaches were used as part of a sensitivity analysis for the primary outcome. First, in the sliding dichotomy method, the cutoff point for binarization of GOS and mRS scores is differentiated by the predicted baseline prognosis risk.[18,21] Instead of defining good or bad outcome for all patients using a single dichotomization point, the sliding dichotomy approach customizes the definition of good outcome according to the baseline prognosis risk of each patient (additional Methods available from Eprints, eprints.soton.ac.uk/426525/). Second, the proportional odds model (also referred to as shift analysis or ordinal logistic regression) was used to analyze the ordinal outcomes. This method is sensitive for detecting a shift of the entire ordinal outcome distribution and estimates a common OR for each of the possible cutoff points of the outcome scale. The common OR is formally valid if the ORs for each cut point are the same (the proportional odds assumption).

Data availability

Anonymized aggregate data will be shared after formal request to the corresponding author in accordance with the University of Southampton's data-sharing policies and contracts with the coauthors and their institutions.

Results

Study inclusion

A PRISMA-IPD flow diagram details how studies were identified (figure 1). Of 18 published studies identified in the literature search, 5 were eligible for inclusion in the meta-analysis[5–9,22] (table e-2 available from Eprints, eprints.soton.ac.uk/426525/). A further 6 unpublished studies were identified through the Haemoglobin after Intracranial Haemorrhage (HATCH) Consortium (www.southampton.ac.uk/hatch). The lead authors of these 11 eligible studies were invited to join the study, and all provided IPLD. The study characteristics of published and

Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses–Individual Patient Data (IPD) flow diagram

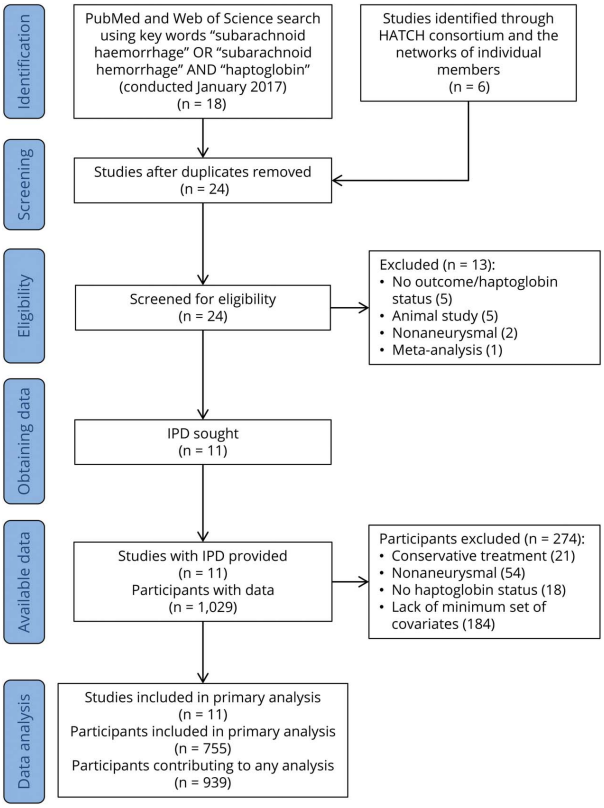


Table 1 Demographic information on studies included in the IPLD, labeled by study identifier

	Study identifier										
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**
Country of origin	UK	Japan	US	US	UK	US	US	US	US	US	US and Italy
Published sample size, n	—	95	74	—	—	133	—	—	193	32	—
Cases excluded from published data, n											
Conservative treatment	—	—	—	—	—	—	—	—	1	2	—
Nonaneurysmal	—	—	2	—	—	—	—	—	—	—	—
Unpublished cases, n	44	—	16	57	214	10	47	59	—	—	55
Cases excluded from unpublished data, n											
Conservative treatment	2	—	2	2	11	—	—	—	—	—	1
Nonaneurysmal	5	—	—	—	26	—	—	21	—	—	—
No HP status	—	—	—	—	8	—	1	1	—	—	5
Sample size for analysis, n	37	95	86	55	169	143	46	37	192	30	49
HP typing	Western blot	Western blot	Western blot	Western blot	Western blot	Western blot	Western blot	Western blot	PCR	Western blot	Western blot
Outcomes available	Primary outcome DCI Radiologic infarction TCD VS[a]	Primary outcome DCI Radiologic infarction Angiographic VS	Primary outcome DCI Radiologic infarction Angiographic VS	Primary outcome DCI Radiologic infarction Angiographic VS TCD VS	Primary outcome TCD VS	Primary outcome DCI[a] Angiographic VS TCD VS	Primary outcome DCI Radiologic infarction Angiographic VS[a] TCD VS	Radiologic infarction TCD VS[a]	Primary outcome DCI Angiographic VS TCD VS	Angiographic VS[b]	DCI[b] Angiographic VS[b]
Outcomes entered into analysis (percentage of sample size available used in analysis)[c]	Primary outcome (100) DCI (100) Radiologic infarction (100)	Primary outcome (100) DCI (100) Radiologic infarction (100) Angiographic VS (99)	Primary outcome (98.8) DCI (97.7) Radiologic infarction (91.9) Angiographic VS (68.6)	Primary outcome (76.4) DCI (100) Radiologic infarction (98.2) Angiographic VS (100) TCD VS (74.6)	Primary outcome (95.3) TCD VS (55)	Primary outcome (77.6) Angiographic VS (81.1) TCD VS (81.1)	Primary outcome (89.1) DCI (93.5) Radiologic infarction (93.5) TCD (89.1) VS	—	Primary outcome (95.3) DCI (95.3) Angiographic VS (55.7) TCD VS (90.6)	—	—
Follow-up time for primary outcome, n											
1 mo	0	95	86	42	0	111	38	—	0	—	—

Continued

Table 1 Demographic information on studies included in the IPLD, labeled by study identifier *(continued)*

	Study identifier										
	A	B	C	D	E	F	G	H	I	J	K
3 mo	37	95	84	42	0	58	25	—	192	—	—
6 mo	34	61	0	23	162	6	2	—	161	—	—
Age, mean (SD), y	59.5 (13.0)	62.1 (13.7)	54.4 (14.3)	54.1 (12.9)	50.8 (11.6)	53.7 (13.9)	53.5 (12.9)	52.5 (15.7)	54.42 (11.2)	51.53 (12.8)	53.20 (11.2)
Fisher grade, n (%)											
1 + 2	0	9 (9.6)	7 (8.1)	12 (21.8)	45 (26.6)	50 (36.5)	3 (6.8)	2 (25)	54 (28.1)	0	0
3 + 4	37 (100)	85 (90.4)	79 (91.9)	43 (78.2)	124 (73.4)	87 (63.5)	41 (93.2)	6 (75)	138 (71.9)	30 (100)	49 (100)
WFNS grade, n (%)											
Good (1–3)	12 (32.4)	57 (60)	55 (64)	34 (61.8)	155 (91.7)	87 (62.6)	31 (70.5)	17 (85)	152 (79.2)	—	—
Poor (4–5)	25 (67.6)	38 (40)	31 (36)	21 (38.2)	14 (8.3)	52 (37.4)	13 (29.5)	3 (15)	40 (20.8)	—	—
Hunt and Hess grade, n (%)											
Good grade (1–3)	—	55 (57.9)	66 (76.7)	35 (63.6)	—	105 (75.5)	30 (66.7)	5 (100)	152 (79.2)	15 (68.2)	—
Poor grade (4–5)	—	40 (42.1)	20 (23.3)	20 (36.4)	—	34 (24.5)	15 (33.3)		40 (20.8)	7 (31.8)	—
Treatment, n (%)											
Endovascular	32 (86.5)	22 (23.2)	41 (47.7)	35 (63.6)	143 (85.1)	25 (20)	31 (67.4)	3 (50)	115 (60)	13 (43.3)	17 (34.7)
Surgical	5 (13.5)	73 (76.8)	45 (52.3)	20 (36.4)	25 (14.9)	100 (80)	15 (32.6)	3 (50)	77 (40)	17 (56.7)	32 (65.3)
Haptoglobin status, n (%)											
HP1-1	8 (21.6)	7 (7.4)	11 (12.8)	13 (23.6)	25 (14.8)	34 (23.8)	15 (32.6)	6 (16.2)	25 (13)	9 (30)	7 (14.3)
HP2-1	16 (43.3)	39 (41)	45 (52.3)	30 (54.6)	80 (47.3)	62 (43.3)	24 (52.2)	22 (59.5)	109 (56.8)	10 (33.3)	26 (53.1)
HP2-2	13 (35.1)	49 (51.6)	30 (34.9)	12 (21.8)	64 (37.9)	47 (32.9)	7 (15.2)	9 (24.3)	58 (30.2)	11 (36.7)	16 (32.6)
HWE, χ^2 (*p* value)	0.52 (0.471)	0.04 (0.841)	0.86 (0.354)	0.46 (0.498)	0 (1)	2.26 (0.133)	0.27 (0.603)	1.44 (0.230)	5.55 (0.018)	3.27 (0.071)	0.47 (0.493)

Abbreviations: DCI = delayed cerebral ischemia; HP = haptoglobin; HWE = Hardy-Weinberg equilibrium; IPLD = individual patient–level data; TCD = transcranial Doppler; VS = vasospasm.
Missing data are signified by —.
[a] Excluded from analysis because of the small number of observations available.
[b] Excluded from analysis because core covariates were not available.
[c] Includes only studies entered into primary and secondary analyses.

unpublished studies are summarized in table 1. For blinding purposes, each study was allocated a study identifier and is referred to by this identifier throughout the article. Across the 11 studies, 939 patients had IPLD available. Published studies were assessed for risk of bias and attained a score of 5 (of a total of 9 maximum points) with the Newcastle-Ottawa Scale.

Demographics

Table 1 summarizes the demographics of the 11 studies included in the meta-analysis. Only 1 study (study I) was significantly out of HWE ($p = 0.018$). Overall, the IPLD was in HWE ($p = 0.663$).

Primary outcome

Eight studies (A–G and I), with a total sample size of 755, were included in the primary outcome analysis due to data availability (table e-1 available from Eprints, eprints.soton.ac.uk/426525/). Age, Fisher grade, WFNS grade, treatment, and hypertension were controlled for in all studies except studies B and G, in which inclusion of Fisher grade resulted in highly sparse cells. Follow-up times were as follows: 1, 3, and 6 months for 355, 516, and 438 patients (table 1 for individual studies). Using the 2-stage approach, we identified no association between *HP* genotype and unfavorable outcome when comparing HP2-2 vs HP2-1 and HP1-1 (OR 0.977, 95% CI 0.672–1.421, $p = 0.905$) (figure 2A). When taken through the same multivariate analysis, higher age (OR 1.05, 95% CI 1.02–1.08) and WFNS grade (OR 8.4, 95% CI 4.4–15.9) were associated with an unfavorable outcome. Higher Fisher grade was associated with poor outcome (OR 2.6, 95% CI 1.3–5.3), and a trend for hypertension was seen (OR 1.5, 95% CI 0.9–2.4) in univariate analysis only. Treatment was not significant (table e-3 available from Eprints, eprints.soton.ac.uk/426525/). No significant association was identified in subgroup analysis of HP2-2 vs HP1-1, HP2-1 vs HP1-1, HP2-2 vs HP2-1, and HP2-2 and 2-1 vs HP1-1 (table 2 and figure e-1 available from Eprints, eprints.soton.ac.uk/426525/).

Because not all patients were included in the primary outcome analysis due to data availability, we assessed selection bias in these patients (i.e., only those included in this analysis). With respect to *HP* genotype, patients were overall in HWE ($p = 0.671$). Compared to a typical hospital population,[23] patients in the primary outcome analysis of this IPLD had similar WFNS grades (grades 4–5, 27.3% vs 24.7%, $p = 0.143$), similar age (median 54.9 vs 55 years), lower coiling rate compared to clipping (55.8% vs 81.4%, $p < 0.001$), and a higher incidence of DCI (37.2% vs 21.7%, $p < 0.001$). Compared to a typical randomized controlled trial population,[24] patients in the primary outcome analysis of this IPLD had similar WFNS grades (grades 4–5, 27.3% vs 22.9%, $p = 0.05$), lower Fisher grade (grades 3–4, 77.3% vs 84.2%, $p = 0.001$), higher age (mean 54.7 vs 50 years, $p < 0.001$), lower coiling rate compared to clipping (55.8% vs 66.9%, $p < 0.001$), higher incidence of DCI (37.2% vs 16%, $p < 0.001$), and similar favorable dichotomized mRS score at 6 months (74% vs 71.7%, $p = 0.353$).

Secondary outcomes

The preplanned secondary outcomes were DCI, radiologic infarction, angiographic vasospasm, and TCD evidence of vasospasm. All analyses were conducted comparing HP2-2 vs HP2-1 and HP1-1, HP2-2 vs HP1-1, HP2-1 vs HP1-1, HP2-2 vs HP2-1, and HP2-2 and 2-1 vs HP1-1. Only results for HP2-2 vs HP2-1 and HP1-1 are detailed in the text below; the other comparisons are summarized in table 2 (and their forest plot data, figures e-2–e-5 available from Eprints, eprints.soton.ac.uk/426525/).

Six studies (A–C, D, G, and I), with a total sample size of 497, were included in the secondary outcome analysis for DCI. Age, Fisher grade, WFNS grade, treatment, and hypertension were controlled for in all studies except studies B, C, and G. Fisher grade was not controlled for in studies B, C, and G due to highly sparse cells. Using the 2-stage approach, we identified no association between *HP* genotype and DCI when comparing HP2-2 vs HP2-1 and HP1-1 (OR 1.171, 95% CI 0.735–1.867, $p = 0.507$) or other permutations of *HP* subgroups (figure 3A and table 2).

Five studies (A–D and G), with a total sample size of 308, were included in the secondary outcome analysis for radiologic infarction. Age, Fisher grade, WFNS grade, treatment, hypertension, and aneurysm location were controlled for in all studies except studies B and C. Fisher grade was not controlled in studies B and C due to highly sparse cells. Using the 2-stage approach, we identified no association between *HP* genotype and radiologic infarction when comparing HP2-2 vs HP2-1 and HP1-1 (OR 1.255, 95% CI 0.632–2.490, $p = 0.516$) (figure 3B and table 2).

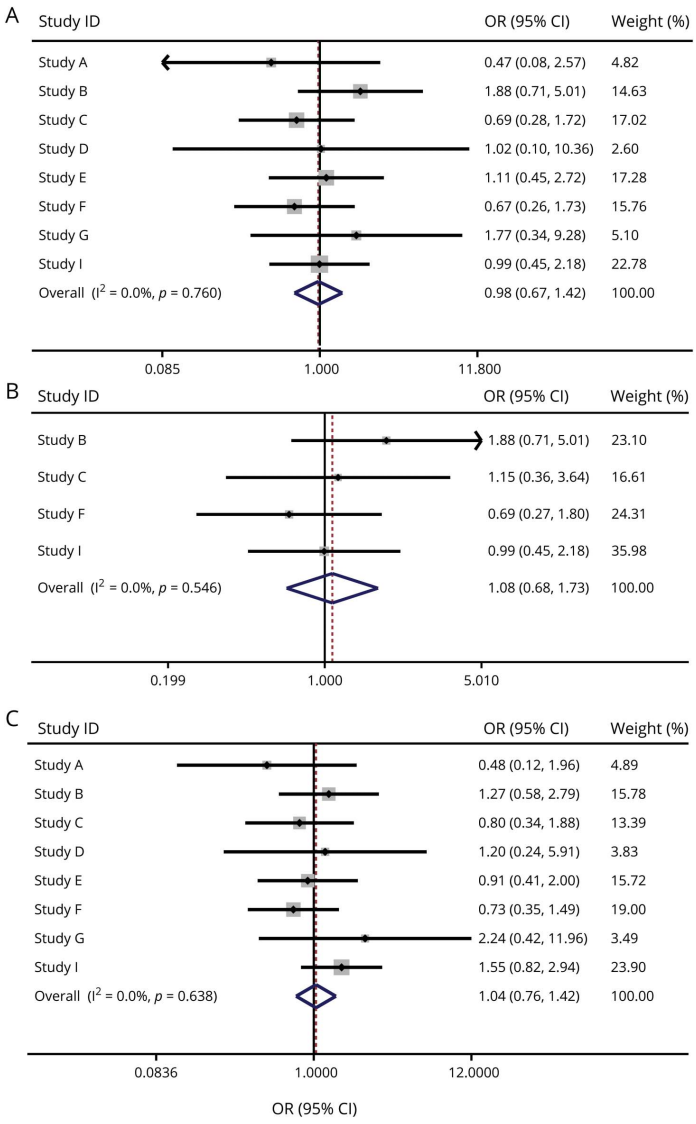
Five studies (B–D, F, and I), with a total sample size of 431, were included in the secondary outcome analysis for the presence of angiographic evidence of vasospasm. Age, Fisher grade, WFNS grade, treatment, and hypertension were controlled for in all studies. Using the 2-stage approach, we identified no association between *HP* genotype and angiographic evidence of vasospasm when comparing HP2-2 vs HP2-1 and HP1-1 (OR 1.130, 95% CI 0.498–2.564, $p = 0.771$) (figure 3C and table 2).

Five studies (D–G and I), with a total sample size of 465, were included in the secondary outcome analysis for the presence of TCD evidence of vasospasm. Age, Fisher grade, WFNS grade, treatment, and hypertension were controlled for in all studies except study G. Fisher grade and treatment were not controlled in study G due to highly sparse cells. Using the 2-stage approach, we identified no association between *HP* genotype and TCD evidence of vasospasm when comparing HP2-2 vs HP2-1 and HP1-1 (OR 0.895, 95% CI 0.557–1.439, $p = 0.648$) (figure 3D and table 2).

Sensitivity analyses

There were 2 main potential reasons to explain the discrepancy between the results here and the data from individual

Figure 2 Forest plots for 2-stage individual patient–level data analysis for primary outcome (dichotomized modified Rankin Scale score in HP2-2 vs HP2-1 and HP1-1)



An odds ratio (OR) >1 denotes a higher probability of poor outcome. (A) Adjusted for covariates, (B) same as A but including published studies only, and (C) unadjusted for covariates. CI = confidence interval; HP = haptoglobin; ID = identifier.

published studies. The first was publication bias; here, we included all studies, and unpublished studies are more likely to be negative. The second was controlling for covariates, because most published studies did not control well for covariates. We proceeded to investigate the relative contribution of these 2 possibilities by repeating the primary analyses for primary (figure 2B) and secondary (figure 4) outcomes using data from published studies alone. We also repeated the primary (i.e., 2-stage IPLD) analyses without adjusting for covariates for both primary (figure 2C) and

Table 2 Summary of the 2-stage IPLD analysis results for all primary and secondary outcomes adjusted for covariates

Outcome	Analysis	OR (95% CI)	*p* Value
Primary	HP2-2 vs HP2-1 and HP1-1	0.997 (0.672–1.421)	0.905
	HP2-2 vs HP1-1	0.752 (0.429–1.321)	0.322
	HP2-1 vs HP1-1	0.814 (0.470–1.410)	0.462
	HP2-2 vs HP2-1	1.021 (0.684–1.524)	0.921
	HP2-2 and 2-1 vs HP1-1	0.776 (0.461–1.305)	0.339
Secondary			
DCI	HP2-2 vs HP2-1 and HP1-1	1.171 (0.735–1.867)	0.507
	HP2-2 vs HP1-1	0.878 (0.437–1.762)	0.713
	HP2-1 vs HP1-1	0.735 (0.393–1.376)	0.336
	HP2-2 vs HP2-1	1.187 (0.707–1.993)	0.517
	HP2-2 and 2-1 vs HP1-1	0.851 (0.476–1.523)	0.587
Radiologic infarction	HP2-2 vs HP2-1 and HP1-1	1.255 (0.632–2.490)	0.516
	HP2-2 vs HP1-1	0.868 (0.314–2.402)	0.785
	HP2-1 vs HP1-1	0.536 (0.218–1.319)	0.174
	HP2-2 vs HP2-1	1.369 (0.662–2.832)	0.397
	HP2-2 and 2-1 vs HP1-1	0.611 (0.259–1.441)	0.260
Angiographic vasospasm	HP2-2 vs HP2-1 and HP1-1	1.130 (0.498–2.564)	0.771
	HP2-2 vs HP1-1	0.942 (0.445–1.993)	0.877
	HP2-1 vs HP1-1	0.862 (0.285–2.602)	0.792
	HP2-2 vs HP2-1	1.184 (0.422–3.321)	0.749
	HP2-2 and 2-1 vs HP1-1	1.015 (0.484–2.127)	0.969
TCD evidence of vasospasm	HP2-2 vs HP2-1 and HP1-1	0.895 (0.557–1.439)	0.648
	HP2-2 vs HP1-1	0.962 (0.496–1.867)	0.909
	HP2-1 vs HP1-1	1.048 (0.566–1.940)	0.881
	HP2-2 vs HP2-1	0.882 (0.534–1.456)	0.662
	HP2-2 and 2-1 vs HP1-1	0.980 (0.550–1.746)	0.945

Abbreviations: CI = confidence interval; HP = haptoglobin; IPLD = individual patient–level data; OR = odds ratio; TCD = transcranial Doppler.
An OR >1 denotes a higher probability of poor outcome.

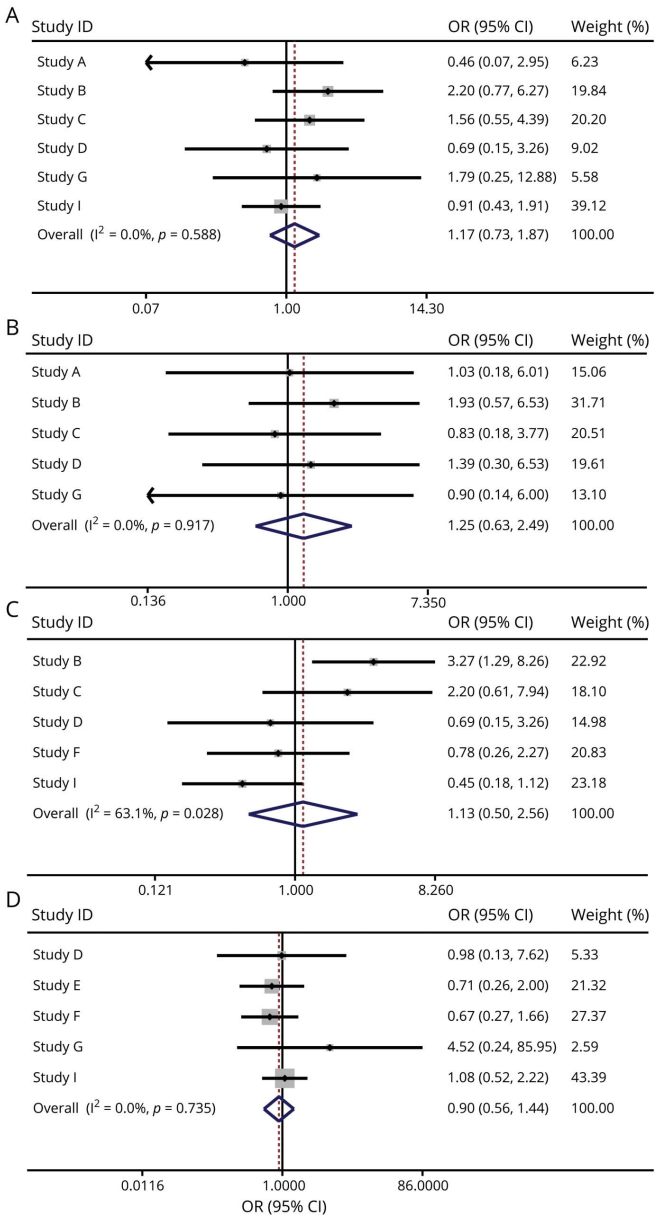
secondary (figure 5) outcomes. None of these analyses showed a significant difference or a trend suggesting an association.

A number of other sensitivity analyses were conducted to evaluate the robustness of the results. These were as follows: (1) exclusion of studies in which patients included in the primary analysis were not in HWE; (2) inclusion of additional covariates; (3) inclusion of studies that did not have all the essential covariates; (4) sliding dichotomy analysis; (5) ordinal regression; (6) 1-stage analysis; (7) unpublished studies only; and (8) including only covariates significant in univariate analysis. For all, the results remained consistent (figures e-6–e-11 and tables e-4–e-6 available from Eprints, eprints.soton.ac.uk/426525/). There were no trends suggesting an association between HP genotype and primary or secondary outcomes in any of these analyses.

Heterogeneity and publication bias

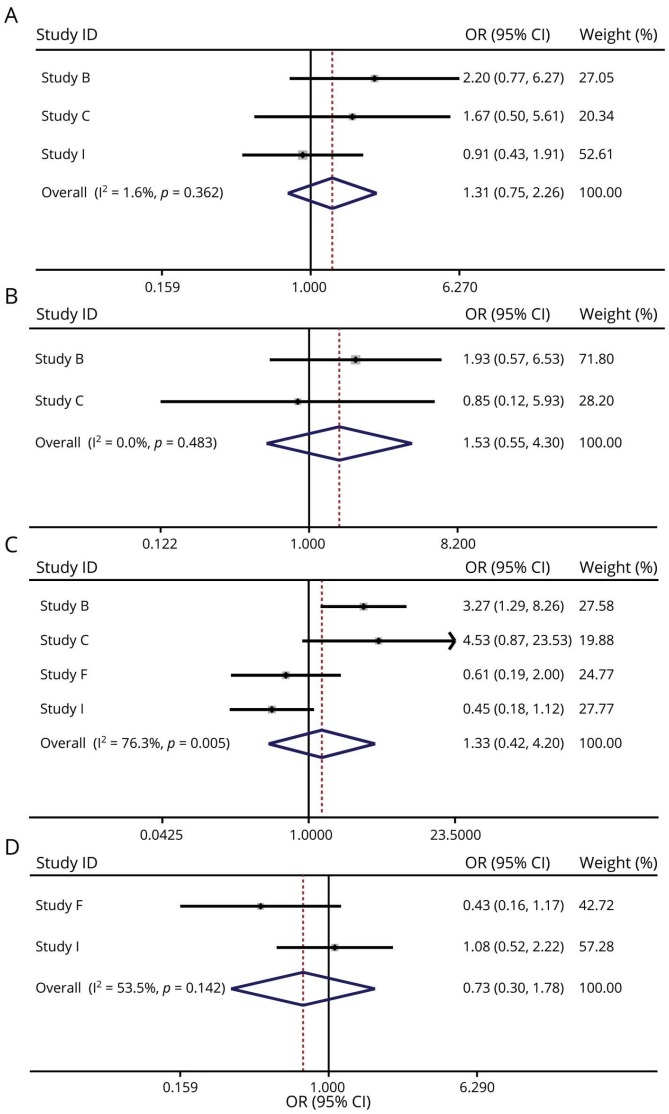
In all analyses, there was little evidence for heterogeneity (I^2 range 0%–35.8%, Cochrane Q tests were not significant, $p > 0.05$) except for angiographic vasospasm (figure 3C and figure e-10C available from Eprints, eprints.soton.ac.uk/426525/). There was no indication of publication bias from funnel plots (Egger regression test, $p > 0.05$ for all the tests) for any analyses (figures e-12–e-36 available from Eprints, eprints.soton.ac.uk/426525/).

Figure 3 Forest plots for 2-stage individual patient–level data analysis for secondary outcomes adjusted for covariates (HP2-2 vs HP2-1 and HP1-1)



An odds ratio (OR) >1 denotes a higher probability of poor outcome. (A) Delayed cerebral ischemia, (B) radiologic infarction, (C) angiographic evidence of vasospasm, and (D) transcranial Doppler evidence of vasospasm. CI = confidence interval; HP = haptoglobin; ID = identifier.

Figure 4 Forest plots for secondary outcomes, adjusted for covariates, published studies only (HP2-2 vs HP2-1 and HP1-1)

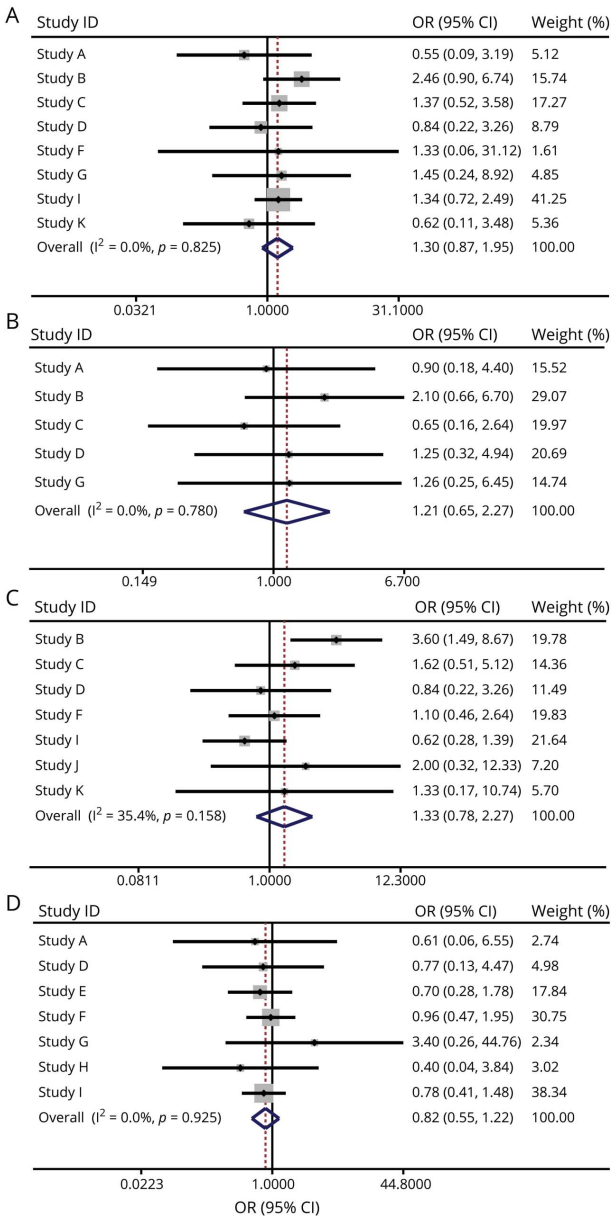


An odds ratio (OR) >1 denotes a higher probability of poor outcome. (A) Delayed cerebral ischemia, (B) radiologic infarction, (C) angiographic evidence of vasospasm, and (D) transcranial Doppler evidence of vasospasm. CI = confidence interval; HP = haptoglobin; ID = identifier.

Discussion

As is normal in IPLD methodology, study size was driven by data availability rather than by a predetermined sample size. Prestudy power, by which we mean a power calculation using estimates from prior studies, was performed on data from the largest published study in the IPLD (study I),[11] which had an effect size of an OR of 1.8 for comparing HP2-2 vs HP2-1 and HP1-1 on the primary outcome. A logistic regression of the binary response variable (mRS score) on the binary independent variable (HP2-2 vs HP2-1 and HP1-1) with a sample size of 755 subjects (of whom 69% were HP2-1 and

Figure 5 Forest plots for secondary outcomes, unadjusted for covariates (HP2-2 vs HP2-1 and HP1-1)



An odds ratio (OR) >1 denotes a higher probability of poor outcome. (A) Delayed cerebral ischemia, (B) radiologic infarction, (C) angiographic evidence of vasospasm, and (D) transcranial Doppler evidence of vasospasm. CI = confidence interval; HP = haptoglobin; ID = identifier.

HP1-1 and 31% were HP2-2) achieved 94% power at a 0.050 significance level to detect a small to medium effect size of an OR of 1.8 with a 2-sided Wald test. Even with the conservative estimate of an OR of 1.6 (10% reduction of OR = 1.8), power was 79% at a 0.050 significance level to detect the association. Therefore, this study has conclusively proven that HP2-2 is not associated with a poor long-term outcome as defined by the mRS score, down to a minimum OR of 1.6.

Although the reason could simply be that there is no difference in the relative protective effects of different *HP* genotypes, there are several possible explanations of how a clinical effect could have been missed. First, the mRS and GOS may be insufficiently sensitive outcome measures to detect subtle yet important outcome variation in patients after aSAH, including cognitive impairment, anxiety, and return to work.[25,26] Subarachnoid hemorrhage (SAH)–specific outcome measures covering these more subtle outcomes such as the SAH outcome tool,[27] may be more sensitive in detecting an association between *HP* genotype and functional outcome. Another possible reason is that the early brain injury takes longer to settle and expose the final residual permanent deficit influenced by *HP* genotype. In this study, outcomes were analyzed 2 weeks to 1 year after aSAH; however, improvements have been demonstrated beyond this time. For example, mRS score has been shown to improve in 19% of patients between 12 and 36 months after aSAH.[28] Hence, future studies should consider longer follow-up periods.

The negative result for all secondary outcomes is not consistent with the recent meta-analysis that provided evidence that the HP2 allele was associated with worse short-term outcome, including DCI and vasospasm.[11] The previous meta-analysis had a number of limitations that may underlie this discrepancy. First, the meta-analysis used a composite definition of short-term outcome grouping DCI or cerebral vasospasm by any definition into 1 binary outcome measure. In comparison, the IPLD analysis here used specific definitions of cerebral vasospasm and DCI, which were analyzed separately. Second, the meta-analysis did not control for covariates known to affect outcome after aSAH, the inclusion of which in the IPLD analysis may explain the different result. The effect sizes observed for the secondary outcomes, besides not achieving statistical significance or showing trends, were extremely small. Taken together, this demonstrates that there is no meaningful, clinically significant difference in these outcomes between *HP* genotypes.

Although this IPLD analysis included a number of unpublished studies, which may have contributed significantly to the negative result, a sensitivity analysis of published studies only was still negative. It has previously been noted that incorporation of unpublished studies does not significantly change the results of most meta-analyses.[29]

The binding of Hp to Hb is thought to confer protection via a number of mechanisms, including limiting the oxidative damage potential of Hb,[30] facilitating its clearance via the CD163 membrane receptor on macrophages/microglia,[31] and generating an anti-inflammatory response.[32] The lack of a clear effect of *HP* genotype on outcome after aSAH in humans contrasts with observations in animal models. Transgenic mice expressing a murine equivalent of human HP2 experienced more vasospasm and functional deficit after experimentally induced SAH compared to wild-type mice.[33] However, there are important biological differences between mice and humans. The influence of Hp on the affinity of CD163 to Hb is markedly different,[34] and CD163 shedding occurs in humans,[12] not mice.[35] These differences suggest that the Hb scavenging system is sufficiently different between the 2 species such that extrapolation of the detrimental effect of HP2 observed in this mouse model to humans should be done with extreme caution.

The basic unit of Hp protein is an Hp monomer consisting of 1 α and 1 β subunit. The HP1 allele codes for an α subunit (called α_1) with 1 cysteine residue that enables dimerization of the Hp monomer by formation of a disulfide bond. The HP2 allele codes for an α_2 subunit that contains an extra cysteine residue compared to α_1 and is therefore able to make multiple disulfide bonds, resulting in several polymers of increasing size in HP2-1 heterozygotes and HP2-2 homozygotes.[10] Whether there is a functional difference between the proteins expressed by different *HP* genotypes is controversial and very much depends on which characteristic of Hp one considers. It is well established that Hp expression is influenced by genotype: HP1-1 > HP1-2 > HP2-2.[36] Some investigators have demonstrated that the Hp1-1 dimer is more effective than the Hp2-2 polymer in reducing the oxidative potential of Hb,[37–39] although other reports suggest that there is no difference.[40–42] Binding affinity to CD163 appears to be higher for Hb in complex with Hp2-2 polymer compared to Hp1-1 dimer.[31,43] However, studies looking at the uptake of Hb-Hp complexes by CD163-expressing cells are less clear, with some reporting no difference[40] and others indicating an increased binding affinity of both Hp1-1 dimer[43] and Hp2-2 polymer,[44] depending on the experimental conditions. Differences may extend to inflammatory effects because binding of Hp1-1-Hb complexes to CD163 results in secretion of the anti-inflammatory cytokine interleukin-10[32,45] at levels several-fold higher compared to Hp2-2-Hb complexes.[45] It is also plausible that differences may be unrelated to Hb scavenging. For example, the HP1-1 genotype appears to decrease endothelial progenitor cell cluster formation.[46] HP2 has also been associated with poorer clinical outcome in people with diabetes mellitus, ischemic heart disease, and infections,[36] suggesting that it may influence outcome after aSAH in individuals with these comorbidities.

HP genotype may not influence outcome after aSAH, even if there are differences between *HP* genotypes in Hb scavenging efficiency. Recently, CD163 expression by neurons has been demonstrated in animal models of cerebral hemorrhage.[47] Because Hb is normally predominantly taken up in the CNS by microglia, it has been proposed that this increased neuronal

CD163 expression may lead to increased neuronal toxicity through uptake of Hb.[48] It is therefore possible that any potential protective effect conferred by different *HP* genotypes may be mitigated by increased neuronal toxicity. To date, CD163 expression by human neurons in situ remains to be demonstrated.

This IPLD analysis provides the most robust evidence to date examining the relationship between *HP* genotype and outcome after aSAH and has a number of strengths. First, this study has the largest sample size to date,[17] although still smaller than usual for genetic studies. Second, the study population was in HWE, excluding significant case missingness or technical problems with genotype/phenotype ascertainment. Third, a number of covariates known to affect outcome after aSAH that have not been consistently controlled for in previous studies were included in the analysis: age, WFNS grade, Fisher grade, and treatment.[23,49] Fourth, this study includes a large number of unpublished studies identified through a network of investigators worldwide. Fifth, it uses IPLD. Sixth, all analyses were preplanned; the protocol was published before the analysis was started; and the statisticians were blinded to the identities of the study and *HP* genotypes (deidentification of the studies was performed at the end, table e-7 available from Eprints, eprints.soton.ac.uk/426525/). Finally, a comprehensive array of statistical approaches was used.

There are several limitations. First, there was minor but significant evidence of selection bias when patients in this study were compared with both a hospital aSAH population[23] and a typical aSAH randomized controlled study,[24] favoring patients with a lower coiling rate compared to clipping and a higher incidence of DCI. Second, this study was retrospective, and despite the collection of IPLD, the available data limited the choice and number of covariates that could be used. It does not control for other covariates known to be important in predicting outcome after aSAH, including need for CSF diversion and preoperative rebleeding,[23] because of a lack of data availability. In addition, although we have controlled for follow-up time, the duration varied significantly between studies. Future studies could examine Hp subunit expression because the Hp α_1 chain band intensity may be prognostic in HP2-1 individuals.[50]

Author contributions

D.O. Bulters and I. Galea conceived the study. B. Gaastra, D. Ren, S. Alexander, D.M. Bielawski, S.L. Blackburn, M.K. Borsody, S. Doré, J. Galea, K. Iihara, Y. Kawamura, P.A. Nyquist, D.O. Bulters, and I. Galea contributed to the design of the study. All authors contributed to different aspects of data acquisition. B. Gaastra, D. Ren, T. He, P.A. Nyquist, D.O. Bulters, and I. Galea analyzed the data. D. Ren and T. He performed statistical analyses; B. Gaastra, D. Ren, and I. Galea wrote the first draft of the manuscript. B. Gaastra, D. Ren, D.O. Bulters, P.A. Nyquist, and I. Galea worked on sequential drafts of the manuscript. All authors revised and approved the final version of the manuscript.

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Disclosure

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References

1. Rinkel GJ, Algra A. Long-term outcomes of patients with aneurysmal subarachnoid haemorrhage. Lancet Neurol 2011;10:349–56.
2. Teasdale GM, Drake CG, Hunt W, Kassell K, Pertuiset B, De Villiers JC. A universal subarachnoid hemorrhage scale: report of a Committee of the World Federation of Neurosurgical Societies. J Neurol Neurosurg Psychiatry 1988;51:1457.
3. Jennett B, Bond M. Assessment of outcome after severe brain damage. Lancet 1975;1:480–484.
4. Jaja BNR, Saposnik G, Lingsma HF, et al. Development and validation of outcome prediction models for aneurysmal subarachnoid haemorrhage: the SAHIT multinational cohort study. BMJ 2018;360:j5745.
5. Borsody M, Burke A, Coplin W, Miller-Lotan R, Levy A. Haptoglobin and the development of cerebral artery vasospasm after subarachnoid hemorrhage. Neurology 2006;66:634–640.
6. Kantor E, Bayır H, Ren D, et al. Haptoglobin genotype and functional outcome after aneurysmal subarachnoid hemorrhage. J Neurosurg 2014;120:386–390.
7. Leclerc JL, Blackburn S, Neal D, Mendez JA, Waters MF, Doré S. Haptoglobin phenotype predicts the development of focal and global cerebral vasospasm and may influence outcomes after aneurysmal subarachnoid hemorrhage. Proc Natl Acad Sci USA 2015;112:1155–1160.
8. Murthy SB, Caplan J, Levy AP, et al. Haptoglobin 2-2 genotype is associated with cerebral salt wasting syndrome in aneurysmal subarachnoid hemorrhage. Neurosurgery 2016;78:71–76.
9. Ohnishi H, Iihara K, Kaku Y, et al. Haptoglobin phenotype predicts cerebral vasospasm and clinical deterioration after aneurysmal subarachnoid hemorrhage. J Stroke Cerebrovasc Dis 2013;22:520–526.
10. Bulters D, Gaastra B, Zolnourian A, et al. Haemoglobin scavenging in intracranial bleeding: biology and clinical implications. Nat Rev Neurol 2018;14:416–432.
11. Gaastra B, Glazier J, Bulters D, Galea I. Corrigendum to haptoglobin genotype and outcome after subarachnoid haemorrhage: new insights from a meta-analysis. Oxid Med Cell Longev 2018;2018:8.
12. Galea J, Cruickshank G, Teeling JL, Boche P, Perry VH, Galea I. The intrathecal CD163-haptoglobin-hemoglobin scavenging system in subarachnoid hemorrhage. J Neurochem 2012;121:785–792.
13. Stewart LA, Clarke M, Rovers M, et al. Preferred reporting items for a systematic review and meta-analysis of individual participant data: the PRISMA-IPD statement. JAMA 2015;313:1657–1665.
14. Gaastra BAS, Bielawski D, Blackburn S, et al. Haptoglobin after subarachnoid haemorrhage: individual patient level data (IPLD) analysis [online]. Available at: http://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=70830&VersionID=108574. Accessed March 25, 2019.

15. Farrell B, Godwin J, Richards S, Warlow C. The United Kingdom Transient Ischaemic Attack (UK-TIA) aspirin trial: final results. J Neurol Neurosurg Psychiatry 1991;54:1044–1054.
16. Jaja BN, Cusimano MD, Etminan N, et al. Clinical prediction models for aneurysmal subarachnoid hemorrhage: a systematic review. Neurocrit Care 2013;18:143–153.
17. Riley RD, Lambert PC, Abo-Zaid G. Meta-analysis of individual participant data: rationale, conduct, and reporting. BMJ 2010;340:c221.
18. Murray GD, Barer D, Choi S, et al. Design and analysis of phase III trials with ordered outcome scales: the concept of the sliding dichotomy. J Neurotrauma 2005;22:511–517.
19. Savitz SI, Lew R, Bluhmki E, Hacke W, Fisher M. Shift analysis versus dichotomization of the modified Rankin Scale outcome scores in the NINDS and ECASS-II trials. Stroke 2007;38:3205–3212.
20. Roozenbeek B, Lingsma HF, Maas AI. New considerations in the design of clinical trials for traumatic brain injury. Clin Investig (Lond) 2012;2:153–162.
21. Ilodigwe D, Murray GD, Kassell NF, Torner RS, Molyneux AJ, Macdonald RL. Sliding dichotomy compared with fixed dichotomization of ordinal outcome scales in subarachnoid hemorrhage trials. J Neurosurg 2013;118:3–12.
22. Vergouwen MD, Vermeulen M, van Gijn J, et al. Definition of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage as an outcome event in clinical trials and observational studies: proposal of a multidisciplinary research group. Stroke 2010;41:2391–2395.
23. Galea JP, Dulhanty L, Patel HC; UK and Ireland Subarachnoid Hemorrhage Database Collaborators. Predictors of outcome in aneurysmal subarachnoid hemorrhage patients: observations from a multicenter data set. Stroke 2017;48:2958–2963.
24. Kirkpatrick PJ, Turner CL, Smith C, Hutchinson PJ, Murray GD. Simvastatin in Aneurysmal Subarachnoid Haemorrhage (STASH): a multicentre randomised phase 3 trial. Lancet Neurol 2014;13:666–675.
25. Macdonald RL, Jaja B, Cusimano MD, et al. SAHIT Investigators: on the outcome of some subarachnoid hemorrhage clinical trials. Transl Stroke Res 2013;4:286–296.
26. Hutter BO, Gilsbach JM. Which neuropsychological deficits are hidden behind a good outcome (Glasgow = 1) after aneurysmal subarachnoid hemorrhage? Neurosurgery 1993;33:999–1005.
27. Pace A, Mitchell S, Casselden E, et al. A subarachnoid haemorrhage-specific outcome tool. Brain 2018;141:1111–1121.
28. Wilson DA, Nakaji P, Albuquerque FC, McDougall CG, Zabramski JM, Spetzler RF. Time course of recovery following poor-grade SAH: the incidence of delayed improvement and implications for SAH outcome study design. J Neurosurg 2013;119:606–612.
29. Schmucker CM, Blümle A, Schell LK, et al. Systematic review finds that study data not published in full text articles have unclear impact on meta-analyses results in medical research. PLoS One 2017;12:e0176210.
30. Cooper CE, Schaer DJ, Buehler PW, et al. Haptoglobin binding stabilizes hemoglobin ferryl iron and the globin radical on tyrosine beta145. Antioxid Redox Signal 2013;18:2264–2273.
31. Kristiansen M, Graversen JH, Jacobsen C, Sonne HJ, Law SK, Moestrup SK. Identification of the haemoglobin scavenger receptor. Nature 2001;409:198–201.
32. Philippidis P, Mason JC, Evans BJ, Nadra KM, Haskard DO, Landis RC. Hemoglobin scavenger receptor CD163 mediates interleukin-10 release and heme oxygenase-1 synthesis: antiinflammatory monocyte-macrophage responses in vitro, in resolving skin blisters in vivo, and after cardiopulmonary bypass surgery. Circ Res 2004;94:119–126.
33. Chaichana KL, Levy AP, Miller-Lotan R, Shakur S, Tamargo RJ. Haptoglobin 2-2 genotype determines chronic vasospasm after experimental subarachnoid hemorrhage. Stroke 2007;38:3266–3271.
34. Etzerodt A, Kjolby M, Nielsen MJ, Maniecki M, Svendsen P, Moestrup SK. Plasma clearance of hemoglobin and haptoglobin in mice and effect of CD163 gene targeting disruption. Antioxid Redox Signal 2013;18:2254–2263.
35. Etzerodt A, Rasmussen MR, Svendsen P, et al. Structural basis for inflammation-driven shedding of CD163 ectodomain and tumor necrosis factor-alpha in macrophages. J Biol Chem 2014;289:778–788.
36. Sadrzadeh SM, Bozorgmehr J. Haptoglobin phenotypes in health and disorders. Am J Clin Pathol 2004;121(suppl):S97–S104.
37. Asleh R, Guetta J, Kalet-Litman S, Miller-Lotan R, Levy AP. Haptoglobin genotype- and diabetes-dependent differences in iron-mediated oxidative stress in vitro and in vivo. Circ Res 2005;96:435–441.
38. Bamm VV, Tsemakhovich VA, Shaklai M, Shaklai N. Haptoglobin phenotypes differ in their ability to inhibit heme transfer from hemoglobin to LDL. Biochemistry 2004;43:3899–906.
39. Melamed-Frank M, Lache O, Enav BI, et al. Structure-function analysis of the antioxidant properties of haptoglobin. Blood 2001;98:3693–3698.
40. Lipiski M, Deuel JW, Baek JH, Engelsberger WR, Buehler PW, Schaer DJ. Human HP1-1 and HP2-2 phenotype-specific haptoglobin therapeutics are both effective in vitro and in guinea pigs to attenuate hemoglobin toxicity. Antioxid Redox Signal 2013;19:1619–1633.
41. Mollan TL, Jia Y, Banerjee S, et al. Redox properties of human hemoglobin in complex with fractionated dimeric and polymeric human haptoglobin. Free Radic Biol Med 2014;69:265–277.
42. Pimenova T, Pereira CP, Gehrig P, Buehler PW, Schaer DJ, Zenobi R. Quantitative mass spectrometry defines an oxidative hotspot in hemoglobin that is specifically protected by haptoglobin. J Proteome Res 2010;9:4061–4070.
43. Asleh R, Marsh S, Shilkrut M, et al. Genetically determined heterogeneity in hemoglobin scavenging and susceptibility to diabetic cardiovascular disease. Circ Res 2003;92:1193–1200.
44. Na N, Ouyang J, Taes YE, Delanghe JR. Serum free hemoglobin concentrations in healthy individuals are related to haptoglobin type. Clin Chem 2005;51:1754–1755.
45. Guetta J, Strauss M, Levy NS, Fahoum L, Levy AP. Haptoglobin genotype modulates the balance of Th1/Th2 cytokines produced by macrophages exposed to free hemoglobin. Atherosclerosis 2007;191:48–53.
46. Rouhl RP, van Oostenbrugge RJ, Damoiseaux JG, et al. Haptoglobin phenotype may alter endothelial progenitor cell cluster formation in cerebral small vessel disease. Curr Neurovasc Res 2009;6:32–41.
47. Liu R, Cao S, Hua Y, Keep RF, Huang Y, Xi G. CD163 expression in neurons after experimental intracerebral hemorrhage. Stroke 2017;48:1369–1375.
48. Chen-Roetling J, Regan RF. Haptoglobin increases the vulnerability of CD163-expressing neurons to hemoglobin. J Neurochem 2016;139:586–595.
49. Pegoli M, Mandrekar J, Rabinstein AA, Lanzino G. Predictors of excellent functional outcome in aneurysmal subarachnoid hemorrhage. J Neurosurg 2015;122:414–418.
50. Kim BJ, Kim Y, Kim S-E, Jeon JP. Study of correlation between Hp α1 expression of haptoglobin 2-1 and clinical course in aneurysmal subarachnoid hemorrhage. World Neurosurg 2018;117:e221–e227.

A.4.4 Publication 9: Genetic variation in NFE2L2 is associated with outcome following aneurysmal subarachnoid haemorrhage

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ORIGINAL ARTICLE

Genetic variation in *NFE2L2* is associated with outcome following aneurysmal subarachnoid haemorrhage

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Abstract

Background and purpose: Nuclear factor erythroid 2-related factor 2 (NRF2; encoded by the *NFE2L2* gene) has been implicated in outcome following aneurysmal subarachnoid haemorrhage (aSAH) through its activity as a regulator of inflammation, oxidative injury and blood breakdown product clearance. The aim of this study was to identify whether genetic variation in *NFE2L2* is associated with clinical outcome following aSAH.

Methods: Ten tagging single nucleotide polymorphisms (SNPs) in *NFE2L2* were genotyped and tested for association with dichotomized clinical outcome, assessed by the modified Rankin scale, in both a discovery and a validation cohort. *In silico* functional analysis was performed using a range of bioinformatic tools.

Results: One SNP, rs10183914, was significantly associated with outcome following aSAH in both the discovery ($n = 1007$) and validation cohorts ($n = 466$). The risk of poor outcome was estimated to be 1.33-fold (95% confidence interval 1.12–1.58) higher in individuals with the T allele of rs10183914 ($p_{\text{meta-analysis}} = 0.001$). *In silico* functional analysis identified rs10183914 as a potentially regulatory variant with effects on transcription factor binding in addition to alternative splicing with the T allele, associated with a significant reduction in the *NFE2L2* intron excision ratio ($p_{\text{sQTL}} = 1.3 \times 10^{-7}$).

Conclusions: The *NFE2L2* SNP, rs10183914, is significantly associated with outcome following aSAH. This is consistent with a clinically relevant pathophysiological role for oxidative and inflammatory brain injury due to blood and its breakdown products in aSAH. Furthermore, our findings support NRF2 as a potential therapeutic target following aSAH and other forms of intracranial haemorrhage.

KEYWORDS
NF-E2-related factor 2, polymorphism, single nucleotide, subarachnoid haemorrhage

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INTRODUCTION

Aneurysmal subarachnoid haemorrhage (aSAH) is a devastating form of stroke associated with significant morbidity and mortality [1]. It has worse outcomes and occurs in younger people compared with other forms of stroke, meaning aSAH results in the greatest socioeconomic burden of all stroke types [2].

The initial surge in intracranial pressure caused by aneurysm rupture instigates early brain injury [3]. The toxic cascades initiated in this early brain injury, together with blood and its breakdown products, which are gradually released into the cerebrospinal fluid (CSF) as the clot lyses, lead to delayed brain injury characterized by cerebral vasospasm, inflammation, oxidative injury and cortical spreading depression [4–7]. Oxidative injury is thought to play a key role in the pathophysiology of neurological injury following aSAH [8]. There is significant evidence of oxidative stress within human CSF following aSAH, with a higher oxidative burden associated with complications and worse outcomes [9–11].

Nuclear factor erythroid 2-related factor 2 (NRF2) has been demonstrated in humans and animals to play an important role in oxidative and neurological injury following aSAH. NRF2, encoded by the *NFE2L2* gene in humans, is a transcription factor which responds to oxidative stress by upregulating multiple antioxidants including glutathione S-transferase and peroxiredoxin [12]. It also regulates transcription of blood breakdown product scavenging molecules such as heme oxygenase-1 (HO-1) [12] and haptoglobin (Hp) [13]. NRF2 has also been shown to have anti-inflammatory properties [14]. It is activated following aSAH in response to oxidative stress and by blood breakdown products released into the CSF following haemorrhage [5]. Consequently, NRF2 plays a potentially pivotal role in protection from neurological injury and outcome following aSAH, not only by upregulating antioxidants and dampening inflammation but also by promoting the clearance of blood breakdown products which drive the oxidative and inflammatory response to aSAH [15, 16].

Nrf2 has been implicated in the pathophysiology of aSAH in multiple animal studies. Nrf2 is upregulated in the cerebral vasculature and cortex following experimental subarachnoid haemorrhage (SAH) in rodents [17, 18]. Nrf2 knockout mice exposed to experimental SAH have increased brain injury and neurological deficits compared to wild-type mice [19]. Numerous studies have demonstrated that pharmacological upregulation of Nrf2 is associated with reduced oxidative and neurological injury, with improved outcomes in rodent SAH models [20, 21]. Sulforaphane is a pharmacological upregulator of Nrf2 activity, which in rodent SAH models reduces neurological injury, cerebral vasospasm and inflammatory cytokines [18, 22].

NFE2L2 is a highly polymorphic gene for which genetic variation has been associated with human disease risk including in respiratory, gastrointestinal and haematological conditions [23]. In neurological disease, genetic variation in *NFE2L2* has been associated with Parkinson's disease and amyotrophic lateral sclerosis incidence and age of onset [24, 25]. In the context of human aSAH, genetic variation in *NFE2L2* has not been specifically studied.

Given its pivotal role in both oxidative and inflammatory injury and its association with the pathophysiology of a wide spectrum of diseases, we hypothesized that genetic variation in *NFE2L2* influences outcome following aSAH. The aims of this study were to investigate whether genetic variation within *NFE2L2* is associated with outcome following aSAH and to validate the findings in an external cohort.

METHODS

This was an *NFE2L2* candidate gene study to test for an association between single nucleotide polymorphisms (SNPs) and outcome following aSAH in a discovery and a validation cohort.

This study had both national (REC 19/SC/0485) and local (ERGO 49253) ethical approval. Patients, or next of kin if patients lacked capacity, gave written informed consent. The study is reported according to the STREGA recommendations [26].

Discovery analysis

Subjects

DNA and phenotype information were obtained from patients with aSAH recruited to the Genetics and Observational Subarachnoid Haemorrhage (GOSH) study. The GOSH study recruited patients from 22 neurosurgical centres in the United Kingdom between 2011 and 2014, and was designed to study the genetic characteristics of aSAH, the details of which have previously been reported [27]. All individuals with available DNA, confirmed aSAH and suitable data on clinical outcome were eligible for inclusion in this study.

Outcomes and covariates

The primary outcome was the dichotomized modified Rankin scale (mRS) score at follow-up. An mRS score of 0–1 was defined as good outcome and an mRS score of 2–6 as poor outcome. This high threshold for good outcome was prespecified in view of the known long follow-up periods (up to 8 years) and consequently good outcomes in this cohort [28, 29]. The following prespecified covariates were included in the analysis: World Federation of Neurological Surgeons (WFNS) grade; Fisher grade [30]; treatment (conservative, endovascular, surgical); time to follow-up; sex; and age. All covariates were tested as categorical apart from age and time to follow-up, which were treated as continuous variables. Multilevel categorical data were converted to a set of binary dummy variables for analysis. Missing covariate data were imputed using a method of polytomous regression for multilevel categorical data and predictive mean matching for continuous data [31].

SNP selection and genotyping

Tagging SNPs within and surrounding the *NFE2L2* gene were identified using a linkage-disequilibrium-based mapping approach [25] to efficiently capture as much common variation as possible. Eight tagging SNPs were identified from HapMap data (release 28) [32] including regions 5 kb downstream to 10 kb upstream of *NFE2L2* (minor allele frequency >5%, $r^2 = 0.9$). A further two tagging SNPs were identified from the upstream regulatory region (see Table 1 for details of tagging SNPs). These SNPs were genotyped by Kompetitive Allele-Specific PCR (KASP) at LGC Genomics.

Quality control

Candidate SNPs were excluded if the genotyping rate was <90% for the cohort, minor allele frequency was <0.05 or there was significant deviation from Hardy–Weinberg equilibrium ($p < 0.0001$).

Association analysis

Individual SNPs were tested for association with dichotomized outcome under an additive model with logistic regression controlling for all covariates. Haplotypes including all 10 SNPs and a frequency ≥0.01 were tested for association using the same logistic regression model. As this was an exploratory analysis, correction for multiple testing was not performed.

Validation analysis

The SNPs and haplotypes associated with outcome ($p < 0.05$) were assessed for replication in an independent cohort of Dutch aSAH patients who were recruited from the University Medical Centre Utrecht, The Netherlands. mRS scores for all individuals were determined at 3 months after aSAH. DNA was extracted from blood and genotyped on either Illumina GSA or CNV370-duo platforms. Additional SNPs were imputed on the Michigan Imputation Server. Further details about the genotyping and their quality control have been published elsewhere [33]. Array genotype data underwent quality control including exclusion of individuals with >10% missingness; SNPs were excluded if minor allele frequency was <0.05, there was extreme deviation from the Hardy–Weinberg equilibrium ($p < 0.0001$) or the SNP call rate was <90%.

In the validation cohort an mRS score of 0–2 was defined as good outcome and an mRS score of 3–6 as poor outcome. This was a different threshold from that used in the discovery cohort in view of the fact that follow-up in this cohort was at 3 months. This is a more common assessment time in aSAH studies and, consequently, the threshold for good outcome most often employed in aSAH studies was applied. This approach ensured comparable numbers of patients with poor outcome in the discovery and validation cohorts for statistical analysis. To explore the effect of these mRS score thresholds, the primary analysis was repeated using the same threshold as that used in the validation cohort (see sensitivity analysis).

The same logistic regression model was used to validate the association between significant SNPs and haplotypes with outcome following aSAH. Age, sex and WFNS grade were included as covariates in the model. All individuals were followed up at the same time point and, consequently, follow-up time was not required as a covariate in the model. Fisher grade and treatment status were not available for this dataset. The Bonferroni method for multiple corrections was applied based on the number of SNPs and haplotypes tested in the replication cohort.

The final effect size for statistically significant validated SNPs was identified by a fixed effects meta-analysis of summary statistics from the discovery and validation cohorts.

Functional analysis

Genetic variants which replicated in the validation cohort underwent *in silico* functional analysis to assess their biological relevance. The RegulomeDB probability score was used to assess the likelihood of any variant being regulatory [34]. UCSC Genome Browser [35–37] and HaploReg (version 4.1) [38] were used to annotate against the 15-state chromatin model [39] to identify whether relevant variants and their proxies ($r^2 > 0.85$) had significant chromatin interactions. GTEx (version 8) [40] was used to assess whether variants or their proxies ($r^2 > 0.85$) were associated with gene expression levels (eQTL) and/or alternative splicing (sQTL).

Sensitivity analyses

The primary analysis was also repeated in the GOSH cohort using mRS score 0–2 to define good outcome, the same dichotomization threshold as used in the validation cohort. As Fisher grade and treatment status were not available in the validation cohort, the primary analysis was repeated in the GOSH cohort excluding these covariates.

All analyses were performed in STATA (StataCorp. 2011. Stata Statistical Software: Release 16. College Station, TX: StataCorp LP) and PLINK versions 1.07 and 1.9.

Data availability

Study data will be available from the authors subject to institutional agreements and ethical approvals.

RESULTS

Discovery cohort

A total of 1202 patients with aSAH were identified from the GOSH dataset, of whom 116 individuals were excluded due to missing outcome data. Of the remaining 1086 samples, 17 (1.6%), 57 (5.2%), 10 (0.9%) and 18 (1.7%) were missing WFNS grade, Fisher grade, treatment status and time to follow-up, respectively, and were imputed. Genetic data were available on 1078 patients, of whom 1069 passed quality control thresholds. After dichotomization on mRS score, 68% of patients ($n = 732$) were defined as having a good outcome (mRS score 0–1) and 32% ($n = 337$) had a poor outcome (mRS score 2–6). The mean (range) follow-up time was 24.7 (0–96) months (Figure S1). See Figure 1 for flow chart of sample inclusion and Table 2 for demographics of included patients.

Association analysis

Multivariable logistic regression identified two candidate SNPs significantly associated with outcome following aSAH and adjusting for known prognostically relevant covariates (age, Fisher grade, time to follow-up, sex, treatment and WFNS grade): the rs10183914 T allele was associated with poor outcome with an odds ratio (OR) of 1.27 (95% confidence interval [CI] 1.04–1.55; $p = 0.021$, $n = 1007$) and the rs6433657 A allele was associated with poor outcome with an OR of 1.24 (95% CI 1.02–1.50; $p = 0.034$, $n = 1002$ [Table 3 and Tables S1–S3]). Two haplotypes were associated with outcome using the same multivariable analysis to adjust for covariates (Table S4). Of these two haplotypes the haplotype containing the rs10183914 T and rs6433657 A risk alleles was associated with an increased risk of poor outcome (OR 1.39), whereas the haplotype containing

TABLE 1 Tagging single nucleotide polymorphisms included in analysis

Chromosome	SNP	Base pair	Location	Major allele	Minor allele	Minor allele frequency
2	rs13035806	177,227,094	Intergenic	G	A	0.18
2	rs2706110	177,227,434	Intergenic	C	T	0.20
2	rs10183914	177,232,938	Intronic	C	T	0.34
2	rs6726395	177,238,501	Intronic	G	A	0.45
2	rs10930781	177,249,904	Intronic	G	A	0.09
2	rs1806649	177,253,424	Intronic	C	T	0.23
2	rs2364723	177,261,818	Intronic	G	C	0.31
2	rs6706649	177,265,343	Upstream	C	T	0.13
2	rs35652124	177,265,345	Upstream	T	C	0.31
2	rs6433657	177,269,949	Intergenic	G	A	0.45

Note: Base pair is reported in reference to hg38. Minor allele frequency is reported for the Genetics and Observational Subarachnoid Haemorrhage (GOSH) study cohort.

Abbreviation: SNP, single nucleotide polymorphism.

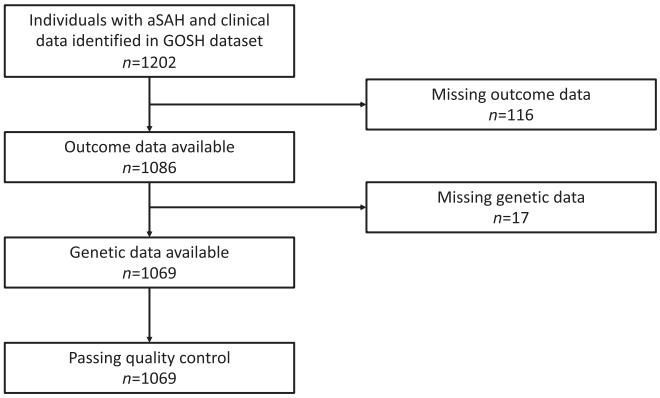

FIGURE 1 Flow chart for Genetics and Observational Subarachnoid Haemorrhage (GOSH) study sample inclusion. aSAH, aneurysmal subarachnoid haemorrhage

TABLE 2 Demographics of patients included from discovery and validation cohort

Cohort	Discovery: GOSH cohort	Validation: Utrecht cohort
Sample size	1069	466
Mean (range) age, years	53.6 (19–92)	57.3 (23–92)
WFNS grade, *n* (%)		
1	602 (56)	216 (46)
2	237 (22)	126 (27)
3	53 (5)	23 (5)
4	104 (10)	57 (12)
5	73 (7)	44 (9)
Fisher grade, *n* (%)		
1	83 (8)	Not available
2	321 (30)	
3	241 (23)	
4	424 (40)	
Mean (range) time to follow-up, months	24.7 (0–96)	3
Treatment, *n* (%)		
Conservative	9 (1)	Not available
Endovascular	846 (79)	
Surgical	214 (20)	
Sex, *n* (%)		
Female	754 (71)	339 (73)
Male	315 (29)	127 (27)
Outcome, *n* (%)		
mRS score 0	354 (33)	5 (1)
mRS score 1	378 (35)	25 (5)
mRS score 2	158 (15)	318 (68)
mRS score 3	85 (8)	19 (4)
mRS score 4	36 (3)	32 (7)
mRS score 5	15 (1)	30 (6)
mRS score 6	43 (4)	37 (8)
Good outcome[a]	732 (68)	348 (75)
Poor outcome	337 (32)	118 (25)

Note: Good outcome was defined as mRS score 0–1 and mRS score 0–2 in the discovery and validation cohorts, respectively. Discovery demographics are reported following imputation.

Abbreviations: GOSH, Genetics and Observational Subarachnoid Haemorrhage; mRS, modified Rankin scale; WFNS, World Federation of Neurological Surgeons.

the rs10183914 C and rs6433657 G alleles was protective, with a reduced risk of poor outcome (OR 0.59).

Validation analysis

A total of 466 patients were identified from the University Medical Centre Utrecht dataset, all of whom passed quality control parameters (see Table 2 for demographics). In all, 348 patients (75%) had a good outcome (mRS score 0–2) and 118 (25%) a poor outcome (mRS score 3–6).

The two statistically significant associated SNPs (rs10183914 and rs6433657) were tested for replication in the Utrecht cohort using a logistic regression model controlling for age, sex and WFNS grade. Using Bonferroni correction (corrected p value <0.025), the rs10183914 T allele replicated with a statistically significant association with outcome in the Utrecht cohort ($p = 0.019$). The rs6433657 A allele did not replicate in the Utrecht cohort ($p = 0.693$) (Table 3). In a fixed-effects meta-analysis of the discovery and validation cohorts the odds of a poor outcome were estimated to be 1.33 times higher per copy of the risk T allele (95% CI 1.12–1.58; $p = 0.001$), with no evidence of heterogeneity ($I^2 = 0\%$; Figure 2).

The two significant haplotypes were also tested for validation in the Utrecht cohort using the same multivariable logistic regression model. Neither of the haplotypes replicated in the Utrecht dataset.

Functional analysis

rs10183914 has a RegulomeDB probability score of 0.609, suggesting this is a putative regulatory variant with evidence of transcription and enhancer activity in brain tissue. Interrogation of HaploReg epigenomic information highlighted a cluster of enhancers, defined by the 15-state chromatin model [39], within the brain, associated with two SNPs (rs13001694 and rs36030784) in strong linkage disequilibrium with rs10183914 ($r^2 > 0.85$, $D' = 0.95$; Figure 3). HaploReg and RegulomeDB also identified that rs10183914 alters regulatory motifs for transcription factors Foxc1, CEBPB and PBX2. rs10183914 is also associated with significant splicing quantitative trait loci changes in brain cortex, with the T allele associated with a significantly reduced *NFE2L2* intron excision ratio ($p = 1.3 \times 10^{-7}$) as analysed by LeafCutter [41] (Figure 3). Finally, rs10183914 is associated with significantly altered expression of *NFE2L2* using eQTL analysis, for example, in cultured fibroblasts, although not in brain tissue [40].

Sensitivity analyses

When an mRS score of 0–2 was used to define good outcome in the GOSH study cohort, only 17% of individuals were classified as having poor outcome. Using this definition the rs10183914 T allele had an OR of poor outcome of 1.27 (95% CI 0.98–1.62; $p = 0.076$, $n = 1007$) and the rs6433657 A allele had an OR of poor outcome of 1.24 (95% CI 0.85–1.40; $p = 0.480$, $n = 1002$). A fixed-effects meta-analysis of rs10183914 in the discovery and validation cohorts identified a highly significant estimated OR of 1.34 (95% CI 1.09–1.64; $p = 0.005$). Exclusion of Fisher grade and treatment status as covariates had minimal effect on the significance of the relationship between genotype and outcome in the GOSH study cohort (excluding Fisher grade: rs10183914, $p = 0.029$; rs6433657, $p = 0.046$; excluding treatment status: rs10183914, $p = 0.017$; rs6433657, $p = 0.043$).

DISCUSSION

In this *NFE2L2* candidate gene study we identified and externally validated the novel finding that the rs10183914 T allele is associated with poor clinical outcome following aSAH in humans (OR 1.33; 95% CI 1.12–1.58).

NRF2 has been proposed to play an integral role in neurological injury and outcome following aSAH by promoting clearance of blood and its breakdown products and protecting against oxidative and inflammatory injury [15, 16]. At present, however, there is only rodent data supporting a role for Nrf2 in SAH [19, 20]. By identifying genetic variation within *NFE2L2* associated with outcome, this study demonstrates the relevance of NRF2 to functional outcome in humans following aSAH. This is consistent with a clinically relevant pathophysiological role for oxidative and inflammatory brain injury due to blood and its breakdown products following aSAH in humans. It also highlights NRF2 as a strong potential therapeutic target to mitigate the devastating consequences of this condition. At present there is an ongoing clinical trial to assess the impact of sulforaphane, a NRF2 stabilizer, in aSAH [42].

In this study the presence of the rs10183914 T allele was associated with poor outcome following aSAH (OR 1.33; 95% CI 1.12–1.58). The functional effect of the intronic rs10183914 is, however, unknown. The alternate T allele has been associated with later age

TABLE 3 Multivariable logistic regression of significant single nucleotide polymorphisms for the Genetics and Observational Subarachnoid Haemorrhage (GOSH) and Utrecht study cohorts.

			GOSH cohort			Utrecht cohort		
SNP	**Base pair**	**Minor allele**	**Number of samples**	**OR (95% CI)**	***p* value**	**Number of samples**	**OR (95% CI)**	***p* value**
rs10183914	177,232,938	T	1007	1.27 (1.04–1.55)	0.021[a]	466	1.5 (1.07–2.10)	0.019[b]
rs6433657	177,269,949	A	1002	1.24 (1.02–1.50)	0.034[a]	466	1.07 (0.77–1.48)	0.693

Note: ORs are reported with respect to the minor allele. Base pair reported in reference to hg38. Genotypes for rs10183914 in the GOSH cohort: CC 451, CT 418, TT 138; Utrecht cohort: CC 177, CT 211, TT 78; for rs6433657 in the GOSH cohort: GG 327, AG 455, AA 220; Utrecht cohort: GG 121, AG 223, AA 122.

Abbreviations: OR, odds ratio; SNP, single-nucleotide polymorphism.

[a]Signifies <0.05. In the validation cohort.

[b]Represents significance following Bonferroni correction (<0.025).

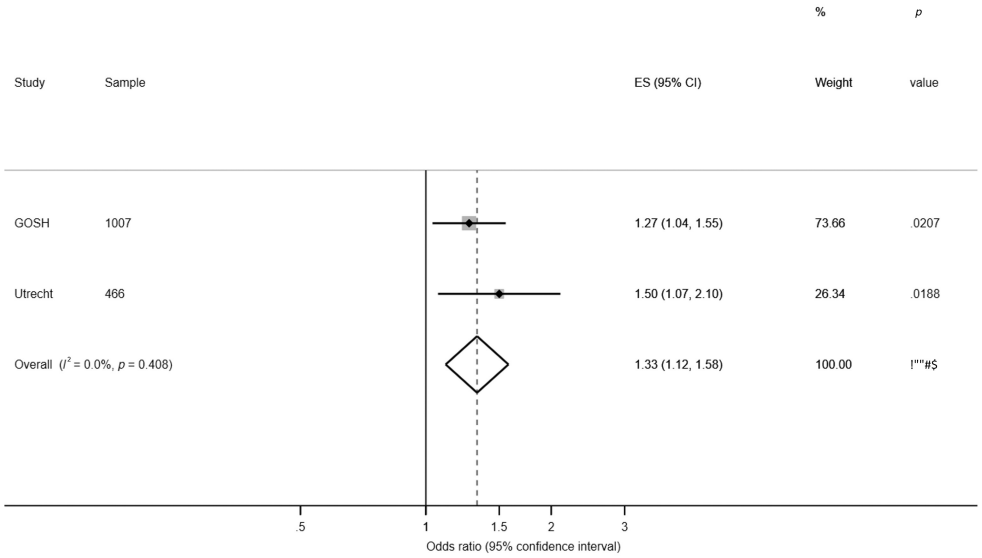

FIGURE 2 Forest plot displaying summary results for rs10183914 in the Genetics and Observational Subarachnoid Haemorrhage (GOSH) study (discovery) and Utrecht (validation) cohorts

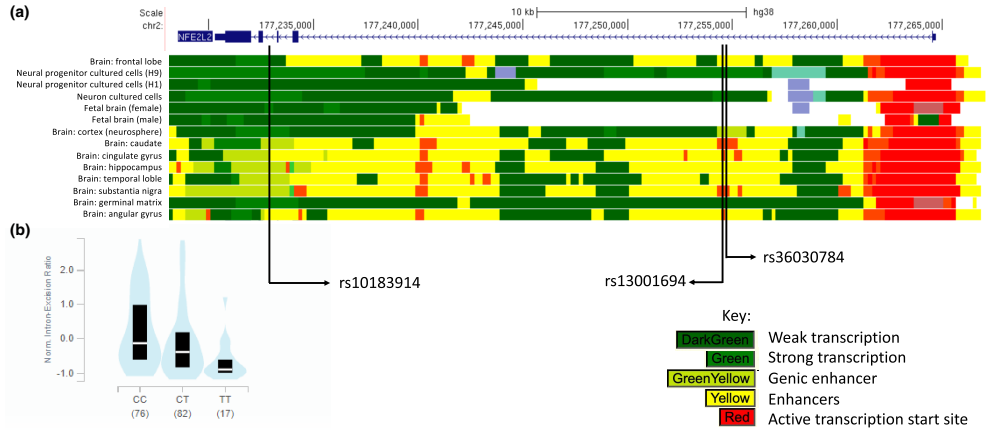

FIGURE 3 *In silico* functional analysis showing relevant SNPs. (a) 15-state chromatin model in relevant tissue (http://genome.ucsc.edu). (b) *NFE2L2* splicing QTL analysis for rs10183914 (http://gtexportal.org).

of onset in Parkinson's disease, although the effect of the genetic variant is unknown, with no change in expression of *NFE2L2* in human olfactory neurosphere-derived cell lines [25]. *In silico* functional analysis links both rs10183914 and SNPs in strong linkage disequilibrium, rs13001694 and rs36030784, to gene regulation, suggesting a significant functional role for this variant. Splicing QTL analysis identified that rs10183914 significantly alters the intron-excision ratio of NFE2L2 specifically in brain tissue ($p = 1.3 \times 10^{-7}$), not only supporting a functional effect of the variant but also highlighting it specifically in neural tissue. Splicing significantly contributes to transcriptome and subsequent proteome diversity [43] and the risk rs10183914 T allele is associated with a reduced intron-excision ratio, leading to lower transcription diversity in the brain and potentially a longer protein, which is likely to be dysfunctional. rs10183914 also significantly influences expression of NRF2 although not specifically in brain tissue. Further *in vitro* analysis is required to characterize the functional effect of this variant and inform future studies.

The findings of this study also have implications for other haemorrhagic stroke conditions such as intracerebral haemorrhage, where Nrf2 activity has also been shown to be neuroprotective in rodent models [44]. As genetic variation influences outcome after aSAH in humans this may translate to other conditions, such as intracerebral haemorrhage, where NRF2 may also be a promising therapeutic target to improve outcome, building on the body of animal evidence available [45].

In this study, only 10 tagging SNPs were genotyped, all of which are common, with a minor allele frequency >0.05. Despite this small number of variants, a significant association with outcome following aSAH was identified. Future studies including a wider range of common and rare genetic variants may identify stronger relationships to outcome, further supporting an integral role for *NFE2L2* in aSAH recovery.

This finding adds to the growing body of evidence that genetic variation plays an important role in outcome following aSAH [46, 47] and supports the ongoing effort to perform a genome-wide association study of outcome following aSAH [48].

A strength of this study is the long-term follow-up duration in the GOSH cohort, ranging from 0 to 96 months. This suggests that the effect of NRF2 on outcome is not simply an acute phenomenon but persists in the long term. The wide range of follow-up in the GOSH dataset is complemented by the fixed shorter-term follow-up time in the Utrecht dataset (3 months), both of which identify a significant association between rs10183914 and outcome, supporting the significance of this variant in both the short and long term.

The difference in follow-up duration between the two cohorts, however, results in a higher proportion of individuals with mRS score 0–1 in the GOSH study cohort (Table 2) due to longer recovery time before outcome assessment. For this reason, we prespecified different mRS dichotomization thresholds for good versus poor outcome in the discovery (GOSH; mRS 0–1 vs. 2–6), and validation (Utrecht; mRS 0–2 vs. 3–6) cohorts. Both these dichotomization thresholds are used in the literature in the context of outcome following aSAH [27, 49]. An mRS score of 0–2 is more commonly used to define good outcome in studies with follow-up at 3–6 months (as in the Utrecht cohort), whereas the mRS score 0–1 dichotomy has been applied to longer-term follow-up cohorts such as that of the GOSH study. The differing dichotomies were used in this study to ensure equivalent proportions of good/poor outcome individuals in the two cohorts and to allow robust statistical analysis (Table 2). A sensitivity analysis was performed using mRS scores of 0–2 to define good outcome in

the GOSH study cohort and showed the same direction of effect (OR 1.24 compared to 1.27), with a suggestive p value of 0.076, and meta-analysis of the discovery and validation cohorts remained highly significant (OR 1.34, 95% CI 1.09–1.64; $p = 0.005$), supporting the findings of the primary analysis.

Fisher grade and treatment status were missing in the Utrecht cohort, preventing their inclusion in the multivariable model for the validation cohort. However, this is very unlikely to have influenced the significance of the results. Firstly, Fisher grade and treatment status have minimal influence on outcome, explaining only 0.65% and 1.3% of outcome variation, respectively, in the largest predictive modelling study to date [50] and 4.6% and 0.4% variation in outcome, respectively, in the GOSH dataset. Secondly, exclusion of Fisher grade and treatment status from the GOSH analysis only had a minimal impact on the significance of rs10183914, suggesting that their inclusion would be unlikely to influence the results. In this study only the original Fisher grade [30] was available; the updated modified Fisher grade [51] has been shown to be more predictive of outcome [52] and should be used in future studies when available.

In conclusion, in this study we identify and validate the *NFE2L2* SNP rs10183914 as associated with outcome in humans following aSAH and provide *in silico* functional evidence of the potential functional effect of this variant. This provides insight into the pathophysiological mechanisms of injury following aSAH and emphasizes the importance of oxidative stress. The data presented here further support NRF2 as a therapeutic target following aSAH and have implications for the management of other haemorrhagic stroke conditions.

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CONFLICTS OF INTEREST

Diederik Bulters conceived and was chief investigator for the SFX-01 after SAH (SAS) randomized controlled multicentre trial, sponsored by Evgen Pharma. Diederik Bulters and Ian Galea have consulted for Evgen Pharma and Bio Products Laboratory Limited, and have received research support from Bio Products Laboratory Limited.

DATA AVAILABILITY STATEMENT

Study data will be available from the authors subject to institutional agreements and ethical approvals.

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REFERENCES

1. Schatlo B, Fung C, Stienen MN, et al. Incidence and outcome of aneurysmal subarachnoid hemorrhage: the swiss study on subarachnoid hemorrhage (swiss SOS). *Stroke*. 2021;52(1):344-347.
2. Taylor TN, Davis PH, Torner JC, Holmes J, Meyer JW, Jacobson MF. Lifetime cost of stroke in the United States. *Stroke*. 1996;27(9):1459-1466.
3. Cahill J, Cahill WJ, Calvert JW, Calvert JH, Zhang JH. Mechanisms of early brain injury after subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. 2006;26(11):1341-1353.
4. Bulters D, Gaastra B, Zolnourian A, et al. Haemoglobin scavenging in intracranial bleeding: biology and clinical implications. *Nat Rev Neurol*. 2018;14(7):416-432.
5. Zolnourian A, Galea I, Bulters D. Neuroprotective role of the Nrf2 pathway in subarachnoid Haemorrhage and its therapeutic potential. *Oxid Med Cell Longev*. 2019;2019:6218239.
6. D'Souza S. Aneurysmal Subarachnoid Hemorrhage. *J Neurosurg Anesthesiol*. 2015;27(3):222-240.
7. Macdonald RL, Pluta RM, Zhang JH. Cerebral vasospasm after subarachnoid hemorrhage: the emerging revolution. *Nat Clin Pract Neurol*. 2007;3(5):256-263.
8. Gaetani P, Pasqualin AR, Baena R, Borasio E, Marzatico F. Oxidative stress in the human brain after subarachnoid hemorrhage. *J Neurosurg*. 1998;89(5):748-754.
9. Jorgensen A, Staalsoe JM, Simonsen AH, et al. Progressive DNA and RNA damage from oxidation after aneurysmal subarachnoid haemorrhage in humans. *Free Radic Res*. 2018;52(1):51-56.
10. Pyne-Geithman GJ, Morgan CJ, Wagner K, et al. Bilirubin production and oxidation in CSF of patients with cerebral vasospasm after subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. 2005;25(8):1070-1077.
11. Hsieh YP, Lin CL, Shiue AL, et al. Correlation of F4-neuroprostanes levels in cerebrospinal fluid with outcome of aneurysmal subarachnoid hemorrhage in humans. *Free Radic Biol Med*. 2009;47(6):814-824.
12. Zhang M, An C, Gao Y, Leak RK, Chen J, Zhang F. Emerging roles of Nrf2 and phase II antioxidant enzymes in neuroprotection. *Prog Neurobiol*. 2013;100:30-47.
13. Zhao X, Song S, Sun G, et al. Neuroprotective role of haptoglobin after intracerebral hemorrhage. *J Neurosci*. 2009;29(50):15819-15827.
14. Baird L, Dinkova-Kostova AT. The cytoprotective role of the Keap1-Nrf2 pathway. *Arch Toxicol*. 2011;85(4):241-272.
15. Reeder BJ, Sharpe MA, Kay AD, Kerr M, Moore K, Wilson MT. Toxicity of myoglobin and haemoglobin: oxidative stress in patients with rhabdomyolysis and subarachnoid haemorrhage. *Biochem Soc Trans*. 2002;30(4):745-748.

16. Dutra FF, Bozza MT. Heme on innate immunity and inflammation. *Front Pharmacol*. 2014;5:115.
17. Wang Z, Chen G, Zhu WW, Zhou D. Activation of nuclear factor-erythroid 2-related factor 2 (Nrf2) in the basilar artery after subarachnoid hemorrhage in rats. *Ann Clin Lab Sci*. 2010;40(3):233-239.
18. Chen G, Fang Q, Zhang J, Zhou D, Wang Z. Role of the Nrf2-ARE pathway in early brain injury after experimental subarachnoid hemorrhage. *J Neurosci Res*. 2011;89(4):515-523.
19. Li T, Wang H, Ding Y, et al. Genetic elimination of Nrf2 aggravates secondary complications except for vasospasm after experimental subarachnoid hemorrhage in mice. *Brain Res*. 2014;1558:90-99.
20. Wang T, Xu L, Gao L, et al. Paeoniflorin attenuates early brain injury through reducing oxidative stress and neuronal apoptosis after subarachnoid hemorrhage in rats. *Metab Brain Dis*. 2020;35(6):959-970.
21. Wu L, Su Z, Zha L, et al. Tetramethylpyrazine nitrone reduces oxidative stress to alleviate cerebral vasospasm in experimental subarachnoid hemorrhage models. *Neuromolecular Med*. 2019;21(3):262-274.
22. Zhao X, Wen L, Dong M, Lu X. Sulforaphane activates the cerebral vascular Nrf2-ARE pathway and suppresses inflammation to attenuate cerebral vasospasm in rat with subarachnoid hemorrhage. *Brain Res*. 2016;1653:1-7.
23. Cho HY. Genomic structure and variation of nuclear factor (erythroid-derived 2)-like 2. *Oxid Med Cell Longev*. 2013;2013:286524.
24. Bergström P, von Otter M, Nilsson S, et al. Association of NFE2L2 and KEAP1 haplotypes with amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener*. 2014;15(1–2):130-137.
25. Todorovic M, Newman JR, Shan J, et al. Comprehensive assessment of genetic sequence variants in the antioxidant 'master regulator' NRF2 in idiopathic Parkinson's disease. *PLoS One*. 2015;10(5):e0128030.
26. Little J, Higgins JP, Ioannidis JP, et al. STrengthening the REporting of genetic association studies (STREGA)—an extension of the STROBE statement. *Genet Epidemiol*. 2009;33(7):581-598.
27. Morton MJ, Hostettler IC, Kazmi N, et al. Haptoglobin genotype and outcome after aneurysmal subarachnoid haemorrhage. *J Neurol Neurosurg Psychiatry*. 2020;91(3):305-313.
28. Rankin J. Cerebral vascular accidents in patients over the age of 60. II Prognosis. *Scott Med J*. 1957;2(5):200-215.
29. Farrell B, Godwin J, Richards S, Warlow C. The United Kingdom transient ischaemic attack (UK-TIA) aspirin trial: final results. *J Neurol Neurosurg Psychiatry*. 1991;54(12):1044-1054.
30. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. *Neurosurgery*. 1980;6(1):1-9.
31. Ho D, Imai K, King G, Stuart EA. MatchIt: Nonparametric preprocessing for parametric causal inference. *J Stat Softw*. 2011;42(8):1-28.
32. Consortium IH. The international HapMap project. *Nature*. 2003;426(6968):789-796.
33. Bakker MK, van der Spek RAA, van Rheenen W, et al. Genome-wide association study of intracranial aneurysms identifies 17 risk loci and genetic overlap with clinical risk factors. *Nat Genet*. 2020;52(12):1303-1313.
34. Boyle AP, Hong EL, Hariharan M, et al. Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res*. 2012;22(9):1790-1797.
35. Navarro Gonzalez J, Zweig AS, Speir ML, et al. The UCSC genome browser database: 2021 update. *Nucleic Acids Res*. 2021;49(D1):D1046-D1057.
36. Rosenbloom KR, Sloan CA, Malladi VS, et al. ENCODE data in the UCSC genome browser: year 5 update. *Nucleic Acids Res*. 2013;41(Database issue):D56-D63.
37. Kent WJ, Sugnet CW, Furey TS, et al. The human genome browser at UCSC. *Genome Res*. 2002;12(6):996-1006.
38. Ward LD, Kellis M. HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. *Nucleic Acids Res*. 2016;44(D1):D877-D881.
39. Kundaje A, Meuleman W, Ernst J, et al. Integrative analysis of 111 reference human epigenomes. *Nature*. 2015;518(7539):317-330.
40. Consortium G. Human genomics. The genotype-tissue expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science*. 2015;348(6235):648-660.
41. Li YI, Knowles DA, Humphrey J, et al. Annotation-free quantification of RNA splicing using LeafCutter. *Nat Genet*. 2018;50(1):151-158.
42. Zolnourian AH, Franklin S, Galea I, Bulters DO. Study protocol for SFX-01 after subarachnoid haemorrhage (SAS): a multicentre randomised double-blinded, placebo controlled trial. *BMJ Open*. 2020;10(3):e028514.
43. Wu E, Nance T, Montgomery SB. SplicePlot: a utility for visualizing splicing quantitative trait loci. *Bioinformatics*. 2014;30(7):1025-1026.
44. Wang J, Fields J, Zhao C, et al. Role of Nrf2 in protection against intracerebral hemorrhage injury in mice. *Free Radic Biol Med*. 2007;43(3):408-414.
45. Imai T, Matsubara H, Hara H. Potential therapeutic effects of Nrf2 activators on intracranial hemorrhage. *J Cereb Blood Flow Metab*. 2021;41(7):1483-1500.
46. Ruigrok YM, Slooter AJ, Bardoel A, Frijns CJ, Rinkel GJ, Wijmenga C. Genes and outcome after aneurysmal subarachnoid haemorrhage. *J Neurol*. 2005;252(4):417-422.
47. Ducruet AF, Gigante PR, Hickman ZL, et al. Genetic determinants of cerebral vasospasm, delayed cerebral ischemia, and outcome after aneurysmal subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. 2010;30(4):676-688.
48. Gaastra B, Alexander S, Bakker MK, et al. Genome-wide association study of clinical outcome after aneurysmal subarachnoid Haemorrhage: protocol. *Transl Stroke Res*. 2022;13:565-576.
49. Gaastra B, Ren D, Alexander S, et al. Haptoglobin genotype and aneurysmal subarachnoid hemorrhage: individual patient data analysis. *Neurology*. 2019;92(18):e2150-e2164.
50. Jaja BNR, Saposnik G, Lingsma HF, et al. Development and validation of outcome prediction models for aneurysmal subarachnoid haemorrhage: the SAHIT multinational cohort study. *BMJ*. 2018;360:j5745.
51. Frontera JA, Claassen J, Schmidt JM, et al. Prediction of symptomatic vasospasm after subarachnoid hemorrhage: the modified fisher scale. *Neurosurgery*. 2006;59(1):21-27. discussion -7.
52. van der Steen WE, Leemans EL, van den Berg R, et al. Radiological scales predicting delayed cerebral ischemia in subarachnoid hemorrhage: systematic review and meta-analysis. *Neuroradiology*. 2019;61(3):247-256.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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A.5 Chapter 5

A.5.1 Publication 10: Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol

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I wrote the protocol with guidance from Will Tapper, Ian Galea and Diederik Bulters. All co-authors then input for subsequent drafts.

Specific contribution: Primary role in manuscript authorship.

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METHODS PAPER

Genome-Wide Association Study of Clinical Outcome After Aneurysmal Subarachnoid Haemorrhage: Protocol

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Abstract
Aneurysmal subarachnoid haemorrhage (aSAH) results in persistent clinical deficits which prevent survivors from returning to normal daily functioning. Only a small fraction of the variation in clinical outcome following aSAH is explained by known clinical, demographic and imaging variables; meaning additional unknown factors must play a key role in clinical outcome. There is a growing body of evidence that genetic variation is important in determining outcome following aSAH. Understanding genetic determinants of outcome will help to improve prognostic modelling, stratify patients in clinical trials and target novel strategies to treat this devastating disease. This protocol details a two-stage genome-wide association study to identify susceptibility loci for clinical outcome after aSAH using individual patient-level data from multiple international cohorts. Clinical outcome will be assessed using the modified Rankin Scale or Glasgow Outcome Scale at 1–24 months. The stage 1 discovery will involve meta-analysis of individual-level genotypes from different cohorts, controlling for key covariates. Based on statistical significance, supplemented by biological relevance, top single nucleotide polymorphisms will be selected for replication at stage 2. The study has national and local ethical approval. The results of this study will be rapidly communicated to clinicians, researchers and patients through open-access publication(s), presentation(s) at international conferences and via our patient and public network.

Keywords Subarachnoid haemorrhage · Stroke · Outcome assessment · Health care · Genetics · Medical

Introduction

Aneurysmal subarachnoid haemorrhage (aSAH) has a severe socioeconomic burden [1] as it affects people of young age and survivors suffer persisting physical, cognitive, auditory and psychosocial morbidity [2], leading to unemployment [3]. It is a striking clinical observation that individuals with similar bleeds, clinical characteristics and comorbidities experience widely different outcomes. Dozens of modelling studies, including the most recent enrolling over 10,000 patients [4], have consistently found that only up to a third of the variance in clinical outcome can be explained using a combination of demographic, clinical and imaging characteristics. Consequently, unknown additional factors play a key role in clinical outcome.

The mechanism of neurological injury following aSAH can be divided into an early brain injury (EBI) occurring within 72 h of ictus and a delayed brain injury occurring in the subsequent days to weeks after haemorrhage [5]. EBI is caused by a rapid rise in intracranial pressure and concomitant fall in cerebral blood flow at the time of haemorrhage [6]. Toxic cascades initiated by EBI and the presence of blood and its breakdown products within the cerebrospinal fluid are thought to lead to delayed brain injury, characterised by a range of pathological processes including cerebral vasospasm, inflammation, oxidative damage and cortical spreading depression [7–9]. However, the relative

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significance of each of these pathological pathways is currently unknown, impairing the development of pharmacological and therapeutic strategies to prevent or reduce neurological injury.

Previous studies have indicated that clinical outcome may be influenced by the genetic background of individual patients [10]. For example, we have shown that haptoglobin genotype influences clinical outcome [11] and others have implicated endothelial nitric oxide synthase [12], apolipoprotein E [13], brain-derived neurotrophic factor [14] and genes associated with fibrinolysis [15] and inflammation [16]. An automated search of the literature using GLAD4U, a PubMed gene retrieval and prioritisation tool, identified 324 genes associated with aSAH outcome [17, 18]. These genes were derived from candidate gene studies that rely on a priori knowledge of the genes to make the link with clinical outcome. However, as our understanding of the molecular mechanisms underlying outcome after aSAH is incomplete, these targeted approaches may overlook significant genes and are unlikely to deliver novel findings. A systematic genome-wide analysis will overcome this limitation. While there have been several genome-wide association studies (GWAS) comparing patients with and without aneurysms, or comparing patients with ruptured versus unruptured aneurysms, a GWAS of clinical outcome after aSAH has never been performed to date [19, 20]. This is primarily because of the logistic difficulties associated with collecting clinical outcome in a large number of patients when aSAH has a relatively low incidence of around 6 per 100,000 person-years [21]. In order to address the challenge of adequate case ascertainment, one method is to perform an individual patient level data analysis of retrospective data from multiple collaborators [22].

A greater understanding of the genetic variants associated with outcome following aSAH will provide valuable insight into the pathophysiological mechanisms of outcome after aSAH highlighting the pathways which underlie neurological injury, with the potential to improve patient care. Moreover, genetic variants could be used to improve current prognostic models identifying patients at risk of deterioration who may benefit from increased observation, early intervention or access to rehabilitation [23]. Improved prognostication will also allow patients and carers to scale their expectations and forward plan their work and personal life. Additionally, knowledge of genetic variants associated with clinical outcome could be used to adjust for patient heterogeneity in aSAH clinical trials allowing for patient stratification and decreasing required sample sizes [24]. Finally, genome-wide analysis of genetic variation associated with outcome may highlight genes and pathways that were previously not considered pharmaceutical targets to improve outcome. This will allow drug target analysis for the development of novel and/or repositioning of known therapies to mitigate the devastating consequences of this condition [25]. New treatments are desperately needed in aSAH as at present there is only a single drug (nimodipine) to improve outcome, the effect of which is modest [26, 27].

In this study protocol, we detail the methodology for the first GWAS of clinical outcome following aSAH. Our primary aim is to identify genetic variants which influence clinical outcome, irrespective of whether this is via a direct or indirect path. Consequently, genetic variants associated with outcome identified in this study may not directly influence outcome but rather mediate their effect through the pathological processes of EBI and delayed brain injury. We estimate the study power and describe the statistical methods, including quality control and adjustment for necessary covariates. This study will entail a major international collaboration to identify genes associated with clinical outcome following aSAH. This study will have three main impacts: (1) the development of improved prognostic models for aSAH outcome; (2) the identification of novel therapeutic targets; and (3) the building of the foundations of knowledge needed for future studies on clinical outcome after aSAH.

Materials and Methods

This study will be reported in accordance with the "STrengthening the REporting of Genetic Association studies" (STREGA) statement [28]. This study was initially conceived in 2019, with patient recruitment commencing in January 2021. The authorship includes a statistician (DR) who has advised on study design and analysis.

Study Design

International two-stage multi-centre individual patient-level data GWAS. Case-only analysis comparing good and poor outcome aSAH individuals.

Case Ascertainment Sources

Cases for inclusion in this study are being identified from two sources:

Haemoglobin after intracranial haemorrhage (HATCH) consortium

The HATCH consortium is an international consortium with a focus on outcome following brain haemorrhage, including members from Asia, the Americas and Europe. The consortium is identifying adult aSAH patients by contacting investigators worldwide, identified through clinical trial registries and PubMed searches.

A trial registry search was performed (Table 1) using search conditions: "subarachnoid h(a)emorrhage" AND registration of trial in the last 10 years. The principal investigators were emailed in their native language, inviting them to participate in the study. In January 2019, 498 studies were identified, which were manually screened for relevance to biosample and/or genetic data availability, resulting in 148 contacts who were emailed. This search will be repeated prior to commencing the stage 2 validation analysis (see below) to ensure all available cases are identified.

In collaboration with the International Stroke Genetics Consortium (ISGC), further study sites are being identified through peer networks and presentations at ISGC workshops. In addition, an international campaign, including online advertisements and multiple oral and poster presentations at several international workshops and conferences, has also been commenced.

The UK Biobank

The UK Biobank is an ongoing population-based cohort study that aims to improve the prevention, diagnosis and treatment of a wide range of diseases. Extensive genetic and clinical data have been collected on around half a million participants across the UK that were aged between 40 and 69 at the time of recruitment from 2006 to 2010. The design, data collection and processing are described in detail elsewhere [29]. The UK Biobank includes a substantial cohort of aSAH patients with follow-up data. The UK Biobank has approved this project proposal under application ID 49305.

Inclusion/Exclusion Criteria

HATCH dataset

Adult (≥ 18 years) aSAH cases with aneurysmal cause of bleed confirmed by any angiographic method and genome-wide genotype information available will be eligible for inclusion. Individuals will be excluded if no aneurysm can be identified or if a non-aneurysmal cause for subarachnoid haemorrhage, including vascular malformation and trauma, is present.

UK Biobank dataset

aSAH cases will be identified from the UK Biobank using the following data fields (Supplementary Table 1):

- ICD-9 (data field 41271) and ICD-10 (data field 41270) codes from hospital inpatient data
- Read code information from primary care data (data field 42040)
- Self-reported medical conditions (data field 20002) reported at baseline or subsequent assessment centre visits

Cases identified from the UK Biobank will be cross-checked against the algorithmically generated subarachnoid haemorrhage diagnosis (data field 42012) and first occurrence database for ICD-10 code I60 (data field 131360). Genotyped aSAH cases will be included if they have outcome data available subsequent to the date of diagnosis. Cases will be excluded if there is evidence that subarachnoid haemorrhage is secondary to non-aneurysmal pathologies such as vascular malformation or trauma. Non-aneurysmal causes for subarachnoid haemorrhage will be identified using ICD-9 and ICD-10 codes indicative of non-aneurysmal SAH from hospital inpatient and primary care data (Supplementary Table 1) and individuals with such a code will be excluded regardless of the time interval between diagnosis of subarachnoid haemorrhage and potential non-aneurysmal cause. With respect to traumatic event codes, cases will only be excluded if the date of these events indicates that trauma occurred within 30 days before or after the diagnosis of subarachnoid haemorrhage.

Primary Outcomes and Covariates

1. HATCH dataset

The primary outcome will be dichotomised clinical outcome (assessed at 1–24 months), based on the modified Rankin Scale (mRS) [30–32] or Glasgow Outcome Scale (GOS) [33, 34], which correlate highly with each other ($R_S = -0.90$, $p < 0.001$, manuscript under review). Outcome will be dichotomised into favourable (mRS=0–2, GOS=4–5) and

Table 1 Table of trial registries searched to identify cases for inclusion in the study

Trial registry	Website
WHO International Clinical Trials Registry Platform	http://apps.who.int/trialsearch/
ClinicalTrials.gov	https://clinicaltrials.gov/
European Clinical Trials Database	https://www.clinicaltrialsregister.eu/ctr-search/search
International Standard Registered Clinical/Social Study Number (ISRCTN) registry	http://www.isrctn.com/

unfavourable (mRS=3–6, GOS=1–3), enabling both scales to be used [4]. If both mRS and GOS data are available from a single study only, the variable with the greatest data availability will be used, i.e. mixed mRS or GOS data from individual study sites will not be used.

2. UK Biobank dataset

The primary outcomes will be employment status (data field 6142) and cognition, as measured by reaction time (data field 20023) following aSAH. These measures have been chosen as they have been shown to detect differences in outcome between aSAH cases and controls within the UK Biobank cohort [35].

Employment status will be dichotomised into good and poor outcome with poor outcome defined as "unable to work because of sickness or disability" or "unemployed". Reaction times will be ranked and then dichotomised so that the lower scoring poor outcome group is equivalent, in terms of percentage of the total UK Biobank aSAH cohort, to the poor outcome group in the HATCH dataset. Cognitive outcome and mRS after aSAH are highly correlated [36].

Essential Covariates

The primary aim is not to explain maximum variance as is conventionally done in predictive modelling but to detect associations between genetic variation and outcome. We have limited covariates to confounding variables since this is essential in establishing causality; confounding variables are defined by a forward path linking the variable to both exposure and outcome. Directed acyclic graph theory has been used to rationalise the choice of covariates. Age and genetic ancestry [37, 38] are the only known variables satisfying this definition and will be included as essential covariates (Fig. 1A).

Population stratification will be assessed by principal component analysis, using reference populations from the 1000 Genomes Project [39], and the top five genetic ancestry eigenvectors will be used as covariates.

Additional Covariates

In addition to age and genetic ancestry, a number of variables have been shown to predict outcome following aSAH. These predictors include baseline characteristics such as the World Federation of Neurological Surgeons (WFNS) score [4] and features of the patient's clinical course, for example, aneurysm treatment modality [40], rebleed and delayed cerebral ischemia (DCI) [41]. As these variables are expected to influence outcome but not an individual's genetic profile, they are not considered as confounding variables (Fig. 1B). However, one or more of these variables may mediate a proportion of the effect of genetic variation on outcome, and hence can be viewed as "mechanistic" variables linking gene to outcome. This is more likely for mechanistic variables with proven links to outcome such as DCI and aneurysm rebleed, both of which also happen to have a proposed genetic component [10, 19]. By focussing on the gene → outcome pathway irrespective of mediating variables, this study will detect the genes that matter as potential biological targets for new treatments. In addition, this approach has the added advantage of maximising sample size since it is not dependent on the availability of additional covariates.

As an exploratory endpoint where data availability allows, regression-based mediation analysis will be used to explore whether potential mechanistic variables (WFNS score or Hunt and Hess (H&H) grade, aneurysm rebleed

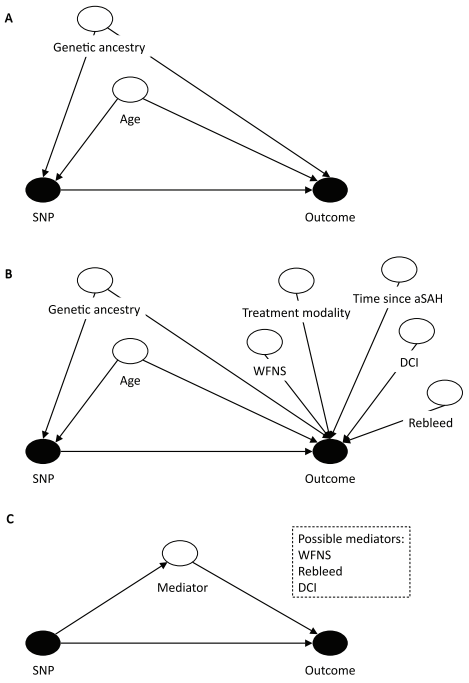

Fig. 1 **A** Directed acyclic graph (DAG) demonstrating confounding covariate interaction with exposure (SNP) and outcome — primary analysis. **B** DAG demonstrating both confounding and selected non-confounding covariates — predictive modelling in cases with available data, for genetic variants confirmed in **A**. **C** Pathway diagram demonstrating possible mediation of a gene → outcome effect by WFNS, rebleed or DCI, for genetic variants confirmed in **A**. SNP, single nucleotide polymorphism; WFNS, World Federation of Neurological Surgeons; aSAH, aneurysmal subarachnoid haemorrhage; DCI, delayed cerebral ischemia

and presence of DCI) mediate any proportion of the genetic effect on outcome following aSAH, for genetic variants confirmed in the primary analysis (Fig. 1C). If data availability allows, an attempt will be made to construct a predictive model to explain maximum variance in outcome, including the covariates: genetic variants confirmed in the primary analysis, age, genetic ancestry, time since aSAH, WFNS score or H&H grade, treatment modality categorised as conservative, endovascular and surgical, aneurysm rebleed and presence of DCI (Fig. 1B). This will be performed as a sensitivity analysis as data is not available for all samples.

In this study, clinical outcome in the HATCH cohort is assessed over a 1- to 24-month time period. This range is broad to maximise patient inclusion in the study. The UK Biobank time to follow-up after aSAH is also broad (1 to 662 months). We have not restricted the time period over which outcome can be assessed in the UK Biobank as we have shown that cognitive and employment outcomes differ between cases and controls over this time period [35], allowing maximum patient inclusion in the study. Outcome is expected to be associated with time since aSAH (Fig. 1B) [42]. Hence, time to follow-up will be included as an additional covariate in a sensitivity analysis of significant genetic variants identified in the primary analysis. We will also conduct a sensitivity analysis including only individuals with follow-up at 3 to 6 months.

In the UK Biobank WFNS is not available, so the length of stay will be used instead since this has a strong association with WFNS [43]. As length of stay data has high missingness (around 40%) and as it is a surrogate of the strongest predictor of clinical outcome [4], it will be imputed using a method of mean imputation to allow for inclusion in the analysis. As the UK Biobank cohort uses cognitive performance and employment as surrogate measures of outcome, the additional covariates Townsend deprivation score [44] (data field 189) and education status dichotomised into individuals holding a college or university degree at the time of initial assessment in the UK Biobank or not (data field 6138) will be included in a sensitivity analysis. For the reaction time analysis, the presence of medications known to influence reaction time in the UK Biobank [45] will also be used.

Genotype Quality Control

Where possible, single nucleotide polymorphism (SNP) data will be sought from collaborators with genotype calls relative to the positive strand. Datasets that are not genotyped on the positive strand will be identified and flipped to the positive strand using SNPFLIP. Genome-wide genotype data will be subjected to standard quality control methods. Patients with gender mismatch, individual missingness >10%, heterozygosity rates ±3 standard deviations from the samples' heterozygosity rate mean and cryptic relatedness (proportional identity by descent > 0.1875) will be excluded. SNPs with extreme deviation from Hardy-Weinberg equilibrium, minor allele frequency (MAF) of <1% and SNP call rate <90% will be excluded. In preparation for imputation and to resolve any residual strand issues, SNPs will be excluded if they are absent from the haplotype reference consortium (HRC), their alleles disagree with HRC, their MAF differ by greater than 0.2 versus HRC or they are palindromic and have MAF greater than 0.4.

Imputation

In the HATCH dataset, imputation will be needed since the genetic data has been obtained on different platforms; it will also increase the density of coverage to enable fine mapping around significant loci. If data has already been imputed by the contributing study teams, this imputation will be used; otherwise, imputation will be performed using the Sanger Imputation Service [46]. Haplotypes will be pre-phased using EAGLE2 [47] into the Haplotype Reference Consortium (r1.1) [46] and imputed using the positional Burrows-Wheeler transform [48]. Imputed genotypes will be quality controlled by excluding SNPs with a posterior genotype probability less than 0.8, a MAF less than 5%, greater than 10% missing genotypes within the cohort or extreme deviation from Hardy-Weinberg equilibrium ($p \leq 1 \times 10^{-10}$).

For datasets already quality controlled and imputed by the contributing team, the above metrics will be reapplied to ensure harmonisation with the exception of heterozygosity which relies on standard deviation of the mean and will therefore be manually reviewed, and imputation quality for which we will report the threshold for each imputed dataset.

Data Analysis

The GWAS will be performed in two stages: discovery and validation (Fig. 2).

Stage 1: Discovery

In the first stage, available cohorts from the HATCH consortium will be analysed separately. Genetic variants will be tested for association using multivariable logistic regression analyses with dichotomised clinical outcome as dependent, genetic variant as predictor, and including the essential covariates specified for the analysis, as detailed above. Residual population stratification will be monitored using lambda from QQ plots and corrected if required. Fixed effects inverse variance weighted meta-analysis of the individual studies will be performed to determine the overall significance and effect size of individual genetic variants in the HATCH consortium. Finally, heterogeneity between cohorts will be examined using Cochrane's Q and I^2 statistics.

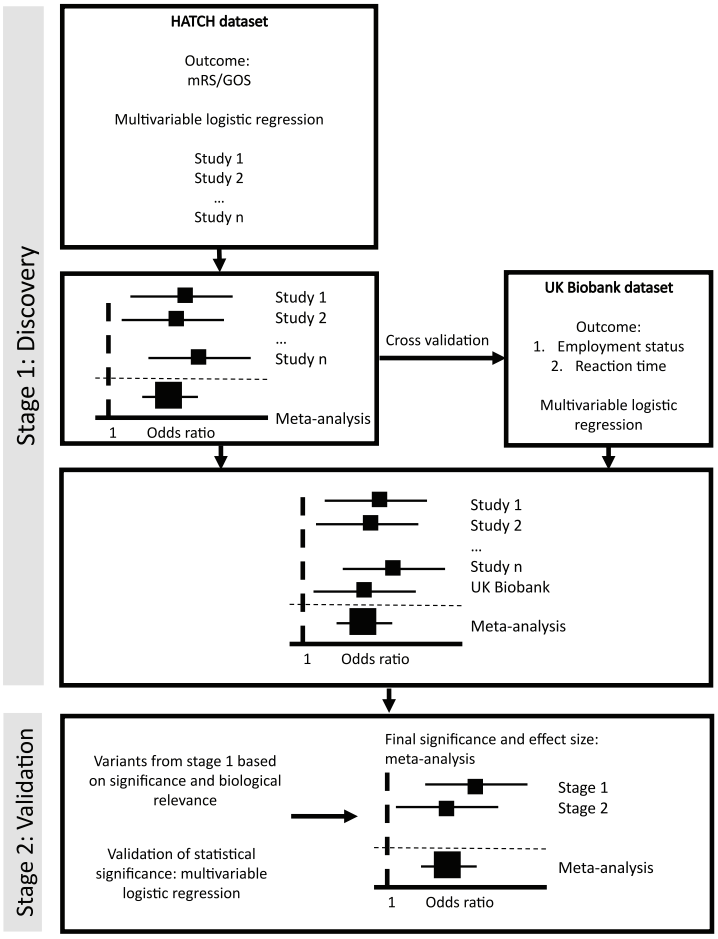

Fig. 2 Analysis plan based on two stages: discovery and validation

The UK Biobank will be considered a single study site and undergo the same multivariable logistic regression analysis as the HATCH dataset. Within the UK Biobank dataset, dichotomised employment status and reaction time outcomes will be considered as separate analyses. Significant findings within the HATCH dataset will be cross-validated in the UK Biobank cohort. As cognitive and traditional (mRS/GOS) outcome measures are correlated after SAH [36], a meta-analysis of summary statistics from both HATCH and UK Biobank datasets will also be performed as the final output of the stage 1 discovery analysis.

To explore the functional relevance of regions associated with clinical outcome, FUMA [49] will be used to determine if the risk SNPs and their proxies ($r^2 \geq 0.6$, within 1Mb and nominally significant) are located within putative functional elements such as active histone marks or transcription factor binding motifs. Additionally, FUMA will be used to annotate these SNPs with respect to evidence of regulatory function using Regulome DB scores [50], combined annotation-dependent depletion scores [51] and gene expression using eQTL analysis [52]. Furthermore, genes functionally related to the risk SNPs will be explored as drug targets using individual gene and network-based approaches. Finally, phenome-wide association study techniques will be used to identify SNPs with pleiotropic effects.

Stage 2: Validation

Genetic variants with the greatest significance in stage 1 will be identified for replication in the stage 2 validation analysis. Variants from stage 1 will be selected for stage 2 based on statistical significance, with all variants with $p<1\times10^{-4}$ considered for replication. The top variants, as ranked by *p*-value, will be included in the validation study. In addition, to maximise the identification of replicable variants in stage 2 [53], variants from stage 1 with $p<1\times10^{-4}$ and evidence of biological/functional relevance will also be included in the validation study. The validation study will use the same multivariable logistic regression analysis as stage 1. Only variants that replicate in the validation study will be considered to be truly associated with outcome with the final significance and effect size determined by a fixed effects meta-analysis of stages 1 and 2.

Sensitivity Analyses

In order to account for time since aSAH, it will be included as an additional covariate with significant genetic variants from the primary analysis retested, incorporating this variable to ensure an independent genetic effect (Fig. 1B). In addition, a further sensitivity analysis will be performed, including only individuals with follow-up at 3 to 6 months.

As the UK Biobank cohort uses employment and cognition as surrogate measures of outcome, a sensitivity analysis including the additional covariates described above will be performed to ensure independent genetic effect. Finally, the UK Biobank cohort will be excluded to ensure no change to the significance of the results.

Mediation Analyses

Regression-based mediation analysis will be used to explore whether potential mechanistic variables (WFNS score or H&H grade, aneurysm rebleed and presence of DCI) mediate any proportion of the genetic effect on outcome following aSAH, for genetic variants confirmed in the primary analysis.

Sample Size and Power Calculation

A recent study demonstrated that in high Fisher grade (III–IV) individuals, the haptoglobin 2-2 genotype was associated with good clinical outcome (mRS 0–1) following aSAH with an odds ratio of 2.6 (95% confidence interval 1.4–4.9) [11]. Based on this finding, we aim to power this study to detect common genetic variation with an effect size of >1.4, the lower end of the 95% confidence interval. After aSAH, 30% are expected to have an unfavourable outcome [54].

At present, approximately 2500 retrospective samples have been identified for inclusion in the stage 1 (discovery) analysis. See www.southampton.ac.uk/hatch/studies/gwas.page for a live tally of sample number and study sites. Recruitment is ongoing for the stage 2 (validation) study with multiple international collaborators. The co-authors of this study have either provided data for stage 1 or are providing samples or data for the stage 2 analysis.

Based on the current stage 1 discovery analysis sample size of 2500, according to the above event rate and assuming an additive model, the stage 1 analysis will have 80% power to detect common SNPs (MAF=0.4) with an effect size of 1.48 and rare SNPs (MAF=0.1) with an effect size of 1.86 at a genome-wide level of significance (Fig. 3A). In the final meta-analysis combining stage 1 and stage 2, a sample size of 5000 is predicted, which using the same assumptions will have 80% power to detect common SNPs (MAF=0.4) with an effect size of 1.33 and rare SNPs (MAF=0.1) with an effect size of 1.56 at a genome-wide level of significance (Fig. 3B).

Limitations

This study does not include individuals who died prior to hospital admission. In addition, it is possible that recruitment is biased towards better outcome individuals as poor prognosis individuals who die in the first few days after aSAH are less likely to be recruited. We will assess this bias by comparing the proportion of good/poor outcome patients in this study with that in contemporary observational studies of outcome and case fatality [55]. Even if such a bias is identified, it will not be a significant limitation to this study as the ultimate aim is to identify pathophysiological mechanisms which can act as therapeutic targets to improve outcome. After aSAH, the time window over which individuals deteriorate following a bleed is relatively long (days to weeks) and during this time patients usually remain in hospital. This means that there is a window of opportunity to administer treatments to prevent deterioration and poor outcome following haemorrhage. Unfortunately, individuals who die prior to admission or in the first few days after aSAH are unlikely to benefit from such interventions. This means that although our study population may be biased away from individuals who die in the first few days after aSAH, it includes the individuals who realistically will benefit from the output of this study.

The UK Biobank has different outcome measures (cognition and employment) than the HATCH datasets that employ the mRS or GOS. This limits the comparability of the UK Biobank and HATCH datasets. To address this limitation, we have separated the UK Biobank and HATCH datasets, and the UK Biobank will be used to cross-validate findings from the HATCH datasets. This allows significant findings

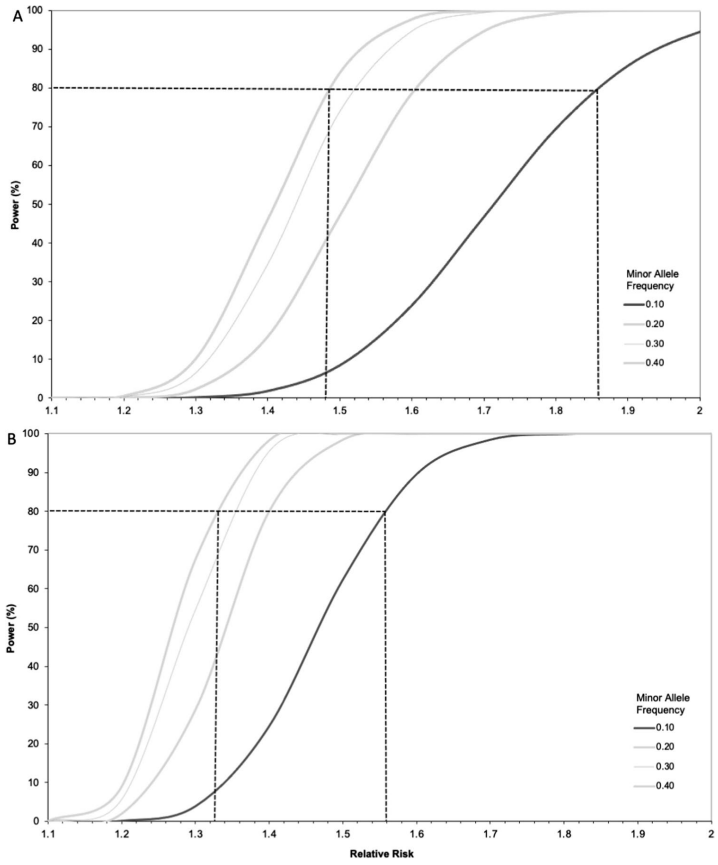

Fig. 3 Graph of power versus SNP effect size for a range of minor allele frequencies at genome-wide significance. Dashed line identifies effect size at 80% power. **A** Sample size = 2500 (stage 1); **B** sample size = 5000 (final meta-analysis: stage 1 + stage 2)

in the HATCH data to be further validated using outcome metrics more sensitive to the nuances of outcome and more relevant to patients than mRS/GOS. We also combine the UK Biobank and HATCH datasets to maximise study power. Within the UK Biobank, only 2% of individuals have psychomotor reaction time and/or employment status recorded both before and after aSAH. Nevertheless, a detailed analysis of employment and cognition in the UK Biobank has shown that psychomotor reaction time and employment are significantly impaired compared to matched controls and therefore constitute valid outcome measures [35].

Patients and Public Involvement

We have worked with the Wessex Subarachnoid Haemorrhage Support Group and participants from previous research studies of patients with aSAH to prioritise research questions important to them and their carers since 2012, holding regular meetings and workshops. The group identified maximising use of samples obtained from prior studies as an important principle and understanding mechanisms of poor outcome to develop new treatments as a priority.

Ethics

For this study, national (REC 19 SC 0485) and local (ERGO 49253) ethical approvals are already in place.

Dissemination

The output of this discovery study will be published in relevant open-access peer-reviewed journals to ensure rapid

dissemination to the target audience. All contributors to the study will be co-authors on manuscripts alphabetised between first and senior authors. Results of the study will also be presented at national and international stroke and aSAH meetings. In addition, through our links with the Wessex SAH support group, we will promote the output of this study to patients and the public along with presentation of results on the HATCH, local hospital and university websites, and social media.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12975-021-00978-2.

Code Availability Not applicable

Authors Contribution All authors contributed to the study's conception and design. The first draft of the manuscript was written by BG, WT, IG and DB; all authors commented on previous versions of the manuscript. Finally, all authors read and approved the final manuscript.

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Data Availability Study data will be available from the authors subject to institutional agreements and ethical approvals.

Declarations

Ethics Approvals National (REC 19 SC 0485) and local (ERGO 49253) ethical approval in place.

Consent to Participate Informed consent was obtained from all individual participants included in the study.

Consent for Publication Not applicable

Conflict of Interest The authors declare no competing interests.

References

1. O. Rivero-Arias, A. Gray, and J. Wolstenholme, "Burden of disease and costs of aneurysmal subarachnoid haemorrhage (aSAH) in the United Kingdom," *Cost Eff Resour Alloc,* vol. 8, p. 6, Apr 27 2010, 10.1186/1478-7547-8-6.
2. A. Pace *et al.*, "A subarachnoid haemorrhage-specific outcome tool," *Brain,* vol. 141, no. 4, pp. 1111-1121, Apr 1 2018, 10.1093/brain/awy003.
3. S. Wallmark, E. Ronne-Engstrom, and E. Lundstrom, "Predicting return to work after subarachnoid hemorrhage using the Montreal Cognitive Assessment (MoCA)," *Acta Neurochir (Wien),* vol. 158, no. 2, pp. 233-239, Feb 2016, https://doi.org/10.1007/s00701-015-2665-4.
4. Jaja BNR, et al. Development and validation of outcome prediction models for aneurysmal subarachnoid haemorrhage: the SAHIT multinational cohort study. *BMJ*. 360:j5745. https://doi.org/10.1136/bmj.j5745.
5. D. Bulters *et al.*, "Haemoglobin scavenging in intracranial bleeding: biology and clinical implications," *Nat Rev Neurol,* vol. 14, no. 7, pp. 416-432, Jul 2018, https://doi.org/10.1038/s41582-018-0020-0.
6. Cahill J, Cahill WJ, Calvert JW, Calvert JH, Zhang JH. Mechanisms of early brain injury after subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. 2006;26(11):1341–53. https://doi.org/10.1038/sj.jcbfm.9600283.
7. Zolnourian A, Galea I, Bulters D. Neuroprotective role of the Nrf2 pathway in subarachnoid haemorrhage and its therapeutic potential. *Oxid Med Cell Longev*. 2019;2019:6218239. https://doi.org/10.1155/2019/6218239.
8. S. D'Souza, "Aneurysmal subarachnoid hemorrhage," *J Neurosurg Anesthesiol,* vol. 27, no. 3, pp. 222-240, Jul 2015, https://doi.org/10.1097/ANA.0000000000000130.
9. R. L. Macdonald, R. M. Pluta, and J. H. Zhang, "Cerebral vasospasm after subarachnoid hemorrhage: the emerging revolution," *Nat Clin Pract Neurol,* vol. 3, no. 5, pp. 256-263, May 2007, https://doi.org/10.1038/ncpneuro0490.
10. A. F. Ducruet *et al.*, "Genetic determinants of cerebral vasospasm, delayed cerebral ischemia, and outcome after aneurysmal subarachnoid hemorrhage," *J Cereb Blood Flow Metab,* vol. 30, no. 4, pp. 676-688, Apr 2010, https://doi.org/10.1038/jcbfm.2009.278.
11. Morton MJ, et al. Haptoglobin genotype and outcome after aneurysmal subarachnoid haemorrhage. *J Neurol Neurosurg Psychiatry*. 91(3):305–13. https://doi.org/10.1136/jnnp-2019-321697.
12. R. M. Starke *et al.*, "Endothelial nitric oxide synthase gene single-nucleotide polymorphism predicts cerebral vasospasm after aneurysmal subarachnoid hemorrhage," *J Cereb Blood Flow Metab,* vol. 28, no. 6, pp. 1204-1211, Jun 2008, https://doi.org/10.1038/jcbfm.2008.11.
13. M. J. Gallek, Y. P. Conley, P. R. Sherwood, M. B. Horowitz, A. Kassam, and S. A. Alexander, "APOE genotype and functional outcome following aneurysmal subarachnoid hemorrhage," *Biol Res Nurs,* vol. 10, no. 3, pp. 205-212, Jan 2009, https://doi.org/10.1177/1099800408323221.

14. Vilkki J, Lappalainen J, Juvela S, Kanarek K, Hernesniemi JA, Siironen J. Relationship of the Met allele of the brain-derived neurotrophic factor Val66Met polymorphism to memory after aneurysmal subarachnoid hemorrhage. *Neurosurgery*. 2008;63(2):198–203; discussion 203. https://doi.org/10.1227/01.NEU.0000320382.21577.8E.
15. M. D. Vergouwen, C. J. Frijns, Y. B. Roos, G. J. Rinkel, F. Baas, and M. Vermeulen, "Plasminogen activator inhibitor-1 4G allele in the 4G/5G promoter polymorphism increases the occurrence of cerebral ischemia after aneurysmal subarachnoid hemorrhage," *Stroke,* vol. 35, no. 6, pp. 1280-1283, Jun 2004, https://doi.org/10.1161/01.STR.0000128707.48644.7e.
16. Y. M. Ruigrok, A. J. Slooter, A. Bardoel, C. J. Frijns, G. J. Rinkel, and C. Wijmenga, "Genes and outcome after aneurysmal subarachnoid haemorrhage," *J Neurol,* vol. 252, no. 4, pp. 417-422, Apr 2005, https://doi.org/10.1007/s00415-005-0661-y.
17. Jourquin J, Duncan D, Shi Z, Zhang B. GLAD4U: deriving and prioritizing gene lists from PubMed literature. *BMC Genomics*. 2012;13(Suppl 8):S20. https://doi.org/10.1186/1471-2164-13-S8-S20.
18. B. Gaastra and I. Galea, "Candidate molecular predictors of outcome after aneurysmal subarachnoid haemorrhage: a systematic review of haemoglobin metabolism, inflammation and oxidative injury pathways," *medRxiv,* p. 19004853, 2019, https://doi.org/10.1101/19004853.
19. Bakker MK, et al. Genome-wide association study of intracranial aneurysms identifies 17 risk loci and genetic overlap with clinical risk factors. *Nat Genet*. 2020;52(12):1303–13. https://doi.org/10.1038/s41588-020-00725-7.
20. Y. M. Ruigrok and G. J. Rinkel, "From GWAS to the clinic: risk factors for intracranial aneurysms," *Genome Med,* vol. 2, no. 9, p. 61, Sep 2010, https://doi.org/10.1186/gm182.
21. Etminan N, et al. Worldwide incidence of aneurysmal subarachnoid hemorrhage according to region, time period, blood pressure, and smoking prevalence in the population: a systematic review and meta-analysis. *Jama Neurol*. 2019;76(5):588–97. https://doi.org/10.1001/jamaneurol.2019.0006.
22. B. Gaastra *et al.*, "Haptoglobin genotype and aneurysmal subarachnoid hemorrhage: individual patient data analysis," *Neurology,* vol. 92, no. 18, pp. e2150-e2164, Apr 2019, https://doi.org/10.1212/WNL.0000000000007397.
23. Lewis CM, Vassos E. Polygenic risk scores: from research tools to clinical instruments. *Genome Med*. 2020;12(1):44. https://doi.org/10.1186/s13073-020-00742-5.
24. E. Hughes *et al.*, "Ancestrally unbiased polygenic breast cancer (BC) risk assessment," *Journal of Clinical Oncology,* vol. 39, no. 15_suppl, pp. 10502-10502, 2021, https://doi.org/10.1200/JCO.2021.39.15_suppl.10502.
25. Cao C, Moult J. GWAS and drug targets. *BMC Genomics*. 2014;15(Suppl 4):S5. https://doi.org/10.1186/1471-2164-15-S4-S5.
26. J. D. Pickard *et al.*, "Effect of oral nimodipine on cerebral infarction and outcome after subarachnoid haemorrhage: British aneurysm nimodipine trial," *BMJ,* vol. 298, no. 6674, pp. 636-642, 1989. [Online]. Available: https://doi.org/10.1136/bmj.298.6674.636.
27. S. M. Dorhout Mees *et al.*, "Calcium antagonists for aneurysmal subarachnoid haemorrhage," *Cochrane Database Syst Rev,* no. 3, p. CD000277, 2007, https://doi.org/10.1002/14651858.CD000277.pub3.
28. J. Little *et al.*, "STrengthening the REporting of Genetic Association Studies (STREGA)--an extension of the STROBE statement," *Genet Epidemiol,* vol. 33, no. 7, pp. 581-598, Nov 2009, https://doi.org/10.1002/gepi.20410.
29. Sudlow C, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*. 2015;12(3):e1001779. https://doi.org/10.1371/journal.pmed.1001779.
30. B. Farrell, J. Godwin, S. Richards, and C. Warlow, "The United Kingdom transient ischaemic attack (UK-TIA) aspirin trial: final results," *J Neurol Neurosurg Psychiatry,* vol. 54, no. 12, pp. 1044-1054, Dec 1991. [Online]. Available: https://doi.org/10.1136/jnnp.54.12.1044.
31. T. J. Quinn, J. Dawson, M. R. Walters, and K. R. Lees, "Reliability of the modified Rankin Scale: a systematic review," *Stroke,* vol. 40, no. 10, pp. 3393-3395, Oct 2009, https://doi.org/10.1161/STROKEAHA.109.557256.
32. Rankin J. Cerebral vascular accidents in patients over the age of 60. II. Prognosis. *Scott Med J*. 1957;2(5):200–15. https://doi.org/10.1177/003693305700200504.
33. B. Jennett and M. Bond, "Assessment of outcome after severe brain damage," *Lancet,* vol. 1, no. 7905, pp. 480-4, 1975. [Online]. Available: https://doi.org/10.1016/S0140-6736(75)92830-5.
34. Jennett B, Snoek J, Bond MR, Brooks N. Disability after severe head injury: observations on the use of the Glasgow Outcome Scale. *J Neurol Neurosurg Psychiatry*. 1981;44(4):285–93. https://doi.org/10.1136/jnnp.44.4.285.
35. Gaastra B, Ewbank F, Tapper W, Bulters D, Galea I. Long-term cognitive outcome following aneurysmal subarachnoid haemorrhage. *Journal of Stroke and Cerebrovascular Diseases*. 2022;31(1):106184. https://doi.org/10.1016/j.jstrokecerebrovasdis.2021.106184.
36. Mayer SA, et al. Global and domain-specific cognitive impairment and outcome after subarachnoid hemorrhage. *Neurology*. 2002;59(11):1750–8. https://doi.org/10.1212/01.wnl.0000035748.91128.c2.
37. Zacharia BE, Grobelny BT, Komotar RJ, Sander Connolly E, Mocco J. The influence of race on outcome following subarachnoid hemorrhage. *J Clin Neurosci*. 2010;17(1):34–7. https://doi.org/10.1016/j.jocn.2009.05.015.
38. Eden SV, et al. The role of race in time to treatment after subarachnoid hemorrhage. *Neurosurgery*. 2007;60(5):837–43; discussion 837-43. https://doi.org/10.1227/01.NEU.0000255451.82483.50.
39. Auton A, et al. A global reference for human genetic variation. *Nature*. 2015;526(7571):68–74. https://doi.org/10.1038/nature15393.
40. Gaastra B, et al. CRP (C-reactive protein) in outcome prediction after subarachnoid hemorrhage and the role of machine learning. *Stroke*. 2021;52(10):3276–85. https://doi.org/10.1161/STROKEAHA.120.030950.
41. Galea JP, Dulhanty L, Patel HC, U. A. I. S. H. D. Collaborators. Predictors of outcome in aneurysmal subarachnoid hemorrhage patients: observations from a multicenter data set. *Stroke*. 2017;48(11):2958–63. https://doi.org/10.1161/STROKEAHA.117.017777.
42. Haug T, Sorteberg A, Sorteberg W, Lindegaard KF, Lundar T, Finset A. Cognitive outcome after aneurysmal subarachnoid hemorrhage: time course of recovery and relationship to clinical, radiological, and management parameters. *Neurosurgery*. 2007;60(4):649–56; discussion 656-7. https://doi.org/10.1227/01.NEU.0000255414.70807.A0.
43. Yousef K, Crago E, Fisher A, Mahmoud K, Lagattuta T, Hravnak M. Grading scales in subarachnoid hemorrhage: which scale to control for when studying outcomes. *Critical Care Medicine*. 2019;47(1):S2. https://doi.org/10.1097/01.ccm.0000550795.32974.8f.
44. J. P. Mackenbach, "Health and deprivation. Inequality and the North: by P. Townsend, P. Phillimore and A. Beattie (eds.) Croom Helm Ltd, London, 1987 221 pp., ISBN 0-7099-4352-0, [pound sign]8.95," *Health Policy,* vol. 10, no. 2, pp. 207-206,

1988. [Online]. Available: https://doi.org/10.1016/0168-8510(88)90006-1.
45. Nevado-Holgado AJ, Kim CH, Winchester L, Gallacher J, Lovestone S. Commonly prescribed drugs associate with cognitive function: a cross-sectional study in UK Biobank. *BMJ Open*. 2016;6(11):e012177. https://doi.org/10.1136/bmjopen-2016-012177.
46. McCarthy S, et al. A reference panel of 64,976 haplotypes for genotype imputation. *Nat Genet*. 2016;48(10):1279–83. https://doi.org/10.1038/ng.3643.
47. Loh PR, et al. Reference-based phasing using the Haplotype Reference Consortium panel. *Nat Genet*. 2016;48(11):1443–8. https://doi.org/10.1038/ng.3679.
48. Durbin R. Efficient haplotype matching and storage using the positional Burrows-Wheeler transform (PBWT). *Bioinformatics*. 2014;30(9):1266–72. https://doi.org/10.1093/bioinformatics/btu014.
49. Watanabe K, Taskesen E, van Bochoven A, Posthuma D. Functional mapping and annotation of genetic associations with FUMA. *Nat Commun*. 1826;8(1):2017. https://doi.org/10.1038/s41467-017-01261-5.
50. Boyle AP, et al. Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res*. 2012;22(9):1790–7. https://doi.org/10.1101/gr.137323.112.
51. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet*. 2014;46(3):310–5. https://doi.org/10.1038/ng.2892.
52. Consortium GT. Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science*. 2015;348(6235):648–60. https://doi.org/10.1126/science.1262110.
53. Huffman JE. Examining the current standards for genetic discovery and replication in the era of mega-biobanks. *Nat Commun*. 2018;9(1):5054. https://doi.org/10.1038/s41467-018-07348-x.
54. Kirkpatrick PJ, Turner CL, Smith C, Hutchinson PJ, Murray GD, Collaborators S. Simvastatin in aneurysmal subarachnoid haemorrhage (STASH): a multicentre randomised phase 3 trial. *Lancet Neurol*. 2014;13(7):666–75. https://doi.org/10.1016/S1474-4422(14)70084-5.
55. Schatlo B, et al. Incidence and outcome of aneurysmal subarachnoid hemorrhage: the Swiss Study on Subarachnoid Hemorrhage (Swiss SOS). *Stroke*. 2021;52(1):344–7. https://doi.org/10.1161/STROKEAHA.120.029538.

A.5.2 Publication 11: A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis

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This discovery analysis uses data from seven retrospective datasets. I identified, collected and curated these datasets. I performed the primary analysis under the guidance of Will Tapper. I authored the manuscript with input from the co-authors after completion of the first draft.

Specific contribution: Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.

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RESEARCH

A Genome-Wide Association Study of Outcome After Aneurysmal Subarachnoid Haemorrhage: Discovery Analysis

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Abstract
Candidate gene studies have identified genetic variants associated with clinical outcomes following aneurysmal subarachnoid haemorrhage (aSAH), but no genome-wide association studies have been performed to date. Here we report the results of the discovery phase of a two-stage genome-wide meta-analysis of outcome after aSAH. We identified 157 independent loci harbouring 756 genetic variants associated with outcome after aSAH ($p < 1 \times 10^{-4}$), which require validation. A single variant (rs12949158), in *SPNS2*, achieved genome-wide significance ($p = 4.29 \times 10^{-8}$) implicating sphingosine-1-phosphate signalling in outcome after aSAH. A large multicentre international effort to recruit samples for validation is required and ongoing. Validation of these findings will provide significant insight into the pathophysiology of outcomes after aSAH with potential implications for treatment.

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Introduction

Aneurysmal subarachnoid haemorrhage (aSAH) is a devastating form of stroke with the worst outcomes and highest socioeconomic burden of any stroke type [1]. The pathophysiology of neurological injury following aSAH is incompletely understood. The mechanism is thought to be multifactorial with the initial surge in intracranial pressure following haemorrhage combined with the presence of blood breakdown products in the cerebrospinal fluid leading to a pattern of injury characterised by inflammation, cerebral vasospasm, microthrombosis, oxidative injury and cortical spreading depression [2–4]. Despite multiple clinical trials, nimodipine is the only therapeutic agent to improve outcomes [5]. It is our incomplete understanding of the mechanisms underlying neurological injury that is, at least in part, responsible for the lack of therapeutic innovation to improve outcomes.

The best outcome prediction model after aSAH, utilising clinical, demographic and imaging characteristics only explains up to 31% of the variation in outcome following aSAH [6]. Consequently, a large proportion of variation in outcome following aSAH is unexplained. There is a growing body of evidence from candidate gene studies that genetic background accounts for a proportion of this unexplained variation [7, 8]. However, no genome-wide analysis has been performed. Such a study would have the potential to provide valuable insights into the mechanisms underlying neurological injury following aSAH by identifying, as yet unstudied, genes associated with outcome and thus novel targets for therapeutic intervention.

In 2018, the HATCH consortium highlighted the need to better understand the pathophysiological mechanisms underlying outcome and proposed a large multicentre genetic analysis of outcome following aSAH [2]. The HATCH consortium has developed this proposal into an international collaboration to undertake a two-stage (discovery and validation) genome-wide association (GWA) study of outcome following aSAH, the protocol for which was published in this journal [9].

The aim of this manuscript is to (1) report the completion of the discovery stage of the study including preliminary results and (2) raise awareness of the study to recruit further samples for the validation stage.

Methods

This is a two-stage (discovery and validation) GWA meta-analysis of outcome following aSAH. The results of the discovery analysis are reported in this manuscript. All analyses were performed according to the published protocol [9]. The study has both national ethical (REC 19 SC 0485) and institutional (ERGO 49253) approval.

For the discovery analysis, individuals were identified from (1) six studies from the HATCH consortium network and (2) the UK Biobank, a major biomedical database with extensive genetic and clinical data, previously described in detail[10] (application number 49305).

In the HATCH dataset, the primary outcome was the modified Rankin Scale (mRS) [11, 12] or Glasgow Outcome Scale (GOS) [13, 14] dichotomised into good (mRS 0–2, GOS 4–5) and poor (mRS 3–6, GOS 1–3) outcomes in the first two years following aSAH. The mRS and/or GOS are not available in the UK Biobank and, therefore, a measure of cognitive performance, psychomotor reaction time, was used since cognition is highly correlated with mRS/GOS following aSAH [15] and reaction time is significantly slower in aSAH cases compared to controls in the UK Biobank [16]. Reaction times were ranked from fastest to slowest and then the UK Biobank was dichotomised into good (faster) and poor (slower) outcomes, generating an equivalent proportion of good outcome individuals to the HATCH dataset.

Genotype information from eligible patients underwent quality control and imputation as required (see protocol for details [9]). Within individual cohorts, genetic variants were tested for association with dichotomised outcome using multivariable logistic regression under an additive model, controlling for confounding variables (age and genetic ancestry). A fixed effects meta-analysis was performed to determine each genetic variant's overall effect size and significance. The meta-analysis was performed on all datasets and repeated in the HATCH dataset alone (i.e. excluding the UK Biobank given its alternative outcome metric). Independent loci were identified for validation using a clumping procedure to group single nucleotide polymorphisms (SNPs) in linkage disequilibrium (LD) ($R^2 > 0.2$) and within 250 kb of each other. Index SNPs with suggestive significance ($p < 1 \times 10^{-4}$) were selected for validation. The threshold for genome-wide significance was $p \leq 5 \times 10^{-8}$ and all analyses were performed using PLINK, STATA (StataCorp. 2011. Stata Statistical Software: Release 16. College Station, TX: StataCorp LP), wANNOVAR [17] and FUMA [18].

Appendix A

Table 1 Demographics and outcome data for included samples divided by dataset. If both mRS and GOS were available for a single study, the scale with greater data availability was used. If data availability was equal for mRS and GOS then mRS was used as it is the preferred outcome scale in stroke research. *WFNS*, World Federation of Neurological Surgeons; *SD*: standard deviation; ms: millisecond

	HATCH dataset						UK Biobank
Dataset	1	2	3	4	5	6	7
Origin	GOSH, UK	Utrecht, Netherlands	Geneva, Switzerland	Hallym, South Korea	Pittsburgh, USA	Geisinger, USA	UK Biobank
Selected publication detailing dataset	Bakker et al	Bakker et al	Bakker et al	Hong et al	Kim et al	Li et al	www.ukbiobank.ac.uk/enable-your-research/about-our-data/genetic-data
Sample size (*n*)	817	470	63	89	180	66	804
Age							
Mean (±SD)	54.4 (±12.4)	57.2 (±12.9)	53.6 (±13.0)	58.7 (±11.6)	54.9 (±11.1)	57.7 (±13.2)	47.6 (±11.5)
Sex							
Male (*n*, %)	230 (28.2%)	129 (27.4%)	15 (23.8%)	32 (36.0%)	55 (30.6%)	21 (31.8%)	336 (41.8%)
Female (*n*, %)	587 (71.8%)	341 (72.6%)	48 (76.2%)	57 (64.0%)	125 (69.4%	45 (68.2%)	468 (58.2%)
WFNS grade (*n*, %)					*Hunt and Hess*		
1	473 (57.9%)	216 (46.0%)	39 (61.9%)	48 (53.9%)	27 (15.0%)	30 (45.5%)	-
2	171 (20.9%)	127 (27.0%)	7 (11.1%)	1 (1.1%)	56 (31.1%)	12 (18.2%)	-
3	36 (4.4%)	23 (4.9%)	5 (7.9%)	10 (11.2%)	61 (33.9%)	4 (6.1%)	-
4	88 (10.8%)	57 (12.1%)	5 (7.9%)	20 (22.5%)	23 (12.8%)	8 (12.1%)	-
5	42 (5.1%)	44 (9.4%)	7 (11.1%)	10 (11.2%)	13 (7.2%)	12 (18.2%)	-
Missing	7 (0.9%)	3 (0.6%)	-	-	-	-	804 (100.0%)
Time to follow-up (months)							
Mean (±SD)	9.4 (±6.8)	3.0 (±0)	6.5 (±2.5)	5.6 (±1.2)	6.0 (±2.2)	3.0 (±0)	129.3 (±122.1)
Missing (*n*, %)	89 (10.9%)	-	-	-	-		-
Outcome							
mRS							
0 (*n*, %)	260 (31.8%)	5 (1.1%)	6 (9.5%)	0 (0.0%)	43 (23.9%)	5 (7.6%)	-
1 (*n*, %)	300 (36.9%)	26 (5.5%)	19 (30.2%)	48 (53.9%)	64 (35.6%)	22 (33.3%)	-
2 (*n*, %)	134 (16.5%)	319 (67.9%)	18 (28.6%)	9 (10.1%)	27 (15.0%)	18 (27.3%)	-
3 (*n*, %)	59 (7.2%)	20 (4.3%)	12 (19.0%)	15 (16.9%)	9 (5.0%)	6 (9.1%)	-
4 (*n*, %)	24 (2.9%)	33 (7.0%)	1 (1.6%)	7 (7.9%)	3 (1.7%)	4 (6.1%)	-
5 (*n*, %)	11 (1.4%)	30 (6.4%)	0 (0.0%)	3 (3.4%)	5 (2.8%)	12 (18.2%)	-
6 (*n*, %)	0 (0.0%)	37 (7.9%)	7 (11.1%)	7 (7.9%)	29 (16.1%)	0	-
Missing (*n*, %)	29 (3.6%)	-	-	-	-	-	-
GOS							
1 (*n*, %)	31 (3.8%)	37 (7.9%)	-	7 (7.9%)	29 (16.1%)	-	-
2 (*n*, %)	0 (0.0%)	1 (0.2%)	-	2 (2.2%)	0 (0.0%)	-	-
3 (*n*, %)	33 (4.1%)	62 (13.2%)	-	11 (12.4%)	10 (5.6%)	-	-
4 (*n*, %)	124 (15.2%)	123 (26.2%)	-	13 (14.6%)	42 (23.3%)	-	-
5 (*n*, %)	629 (77.3%)	247 (52.6%)	-	56 (62.9%)	99 (55.0%)	-	-
Missing (*n*, %)	-	-	-	-	-	-	-
Reaction time (ms)							
Mean (±SD)	-	-	-	-	-	-	587.2 (±138.3)
Missing	-	-	-	-	-	-	-
Metric defining outcome	*GOS*	*mRS*	*mRS*	*mRS*	*mRS*	*mRS*	*Reaction time*
Good (*n*, %)	753 (92.5%)	350 (74.0%)	43 (68.3%)	57 (64.0%)	134 (74.4%)	45 (68.1%)	653 (81.2%)
Poor (*n*, %)	64 (7.9%)	120 (26.0%)	20 (31.7%)	32 (36.0%)	46 (25.6%)	21 (31.8%)	151 (18.8%)

Results

A total of 2489 samples were used for the discovery analysis following quality control. These samples were drawn from six datasets from the HATCH consortium [19–22] ($n=1685$ patients) and 804 individuals from the UK Biobank. After dichotomisation of mRS/GOS within the HATCH consortium data, 1382 (82.0%) patients were classified as good outcome and 303 (18.0%) as poor outcome. Based on reaction times in the UK Biobank, 653 (81.2%) individuals were classified as good outcome and 151 (18.8%) as poor outcome. Table 1 details the demographics and other characteristics of the included datasets.

Analysis of samples from the HATCH consortium ($n=1685$) identified 403 SNPs associated with clinical outcome ($p<1\times10^{-4}$) within 97 independent loci after LD-based SNP clumping (Fig. 1A and Supplementary Table 1A and B). No genetic variants reached genome-wide significance.

Including all seven datasets ($n=2489$) 85 independent loci were identified from 406 SNPs, associated with clinical outcome ($p<1\times10^{-4}$) (Fig. 1B and Supplementary Table 2A and B). A single variant, rs12949158, reached genome-wide significance ($p=4.29\times10^{-8}$). rs12949158 is located on chromosome 17 in an intronic region of the sphingolipid transporter 2 (*SPNS2*) gene, which codes for the major transporter of sphingosine-1-phosphate (S1P) (Supplementary Table 2A). The rs12949158 SNP was only genotyped in two datasets [UK Biobank ($n=744$) and Korean datasets ($n=89$)] (Fig. 1C). Arrays used in the other datasets did not include rs12949158 or any other SNPs with sufficient LD to allow reliable imputation. The rs12949158 alternate A allele was associated with an increased risk of poor outcome with an odds ratio of 2.15 (95% confidence interval 1.63–2.82). The association of rs12949158 with psychomotor reaction time was specific to aSAH, since in a previously published UK Biobank control cohort matched to the same aSAH population[16], this relationship was absent ($p=0.55$).

Including both analyses, a total of 157 independent loci were identified from 756 unique SNPs and these will be taken forward for validation.

Discussion

In this discovery genome-wide meta-analysis, we identified 157 independent loci from 756 unique SNPs associated with outcome following aSAH ($p<1\times10^{-4}$) for validation. We also report that the rs12949158 alternate A allele, located within the *SPNS2* gene, was associated with an increased risk of poor outcome after aSAH (OR 2.15 95% CI 1.63–2.82) with genome-wide significance ($p=4.29\times10^{-8}$). Although one possible alternative explanation is that rs12949158 associates with psychomotor reaction time independent of aSAH, this association was not observed in control individuals in the UK Biobank.

The genome-wide significant rs12949158 finding is not conclusive and requires validation. Firstly, the rs12949158 genotype was only typed in a subset of the discovery cohort ($n=833$). Secondly, the finding is primarily driven by the UK Biobank, which uses an outcome measure that is different from the other datasets (psychomotor reaction time). In the second stage, a customised genotyping array will be used to directly capture all variants targeted for validation including rs12949158.

The rs12949158 variant is intronic, located within the gene *SPNS2*, a member of the S1P signalling pathway. While variation in a gene intron does not guarantee that the same gene is involved, intron-mediated enhancement of gene expression is increasingly recognised and the intronic variation is most likely to regulate the closest gene [23]. Moreover, the S1P signalling pathway is a biologically plausible candidate to influence outcomes after aSAH since S1P has been implicated in neurological injury following stroke via activation of S1P receptors (S1PR) leading to microglial activation, neuronal death, inflammation and blood–brain barrier disruption [24, 25]. Specifically, after human aSAH, S1P was found to be elevated in the cerebrospinal fluid where its concentration correlated with haemorrhage volume [26] and worse neurological outcome [26]. A possible mechanism linking S1P to clinical outcome is provided by studies showing that S1P induces cerebral vasospasm in canine basilar artery in vitro and in vivo [27] and in murine basilar artery in vitro via S1PR3 [28]. This pathway is of particular interest since S1PR-modulating drugs, already licensed in other neurological conditions (e.g. fingolimod) have been shown to be neuroprotective in ischemic stroke [29] and intracerebral haemorrhage [30] in humans and could be re-purposed for aSAH if this finding is validated.

The study's population was biased towards participants with a good outcome almost certainly because individuals with a poor prognosis were less likely to be recruited. However, this study's aim was to better understand the pathophysiological mechanisms underlying outcomes in survivors with a view to developing treatments to improve outcomes. Individuals dying in the acute phase or early after admission will unfortunately be unlikely to benefit from such interventions. Hence, while this study was biased towards participants with a good outcome, these are the individuals most likely to benefit from the findings.

This discovery study achieved its prespecified sample size in a timely manner and represents a highly successful international collaboration generating interesting results to take forward to validation. The total target sample size including the validation cohort is 5000 [9] which would be

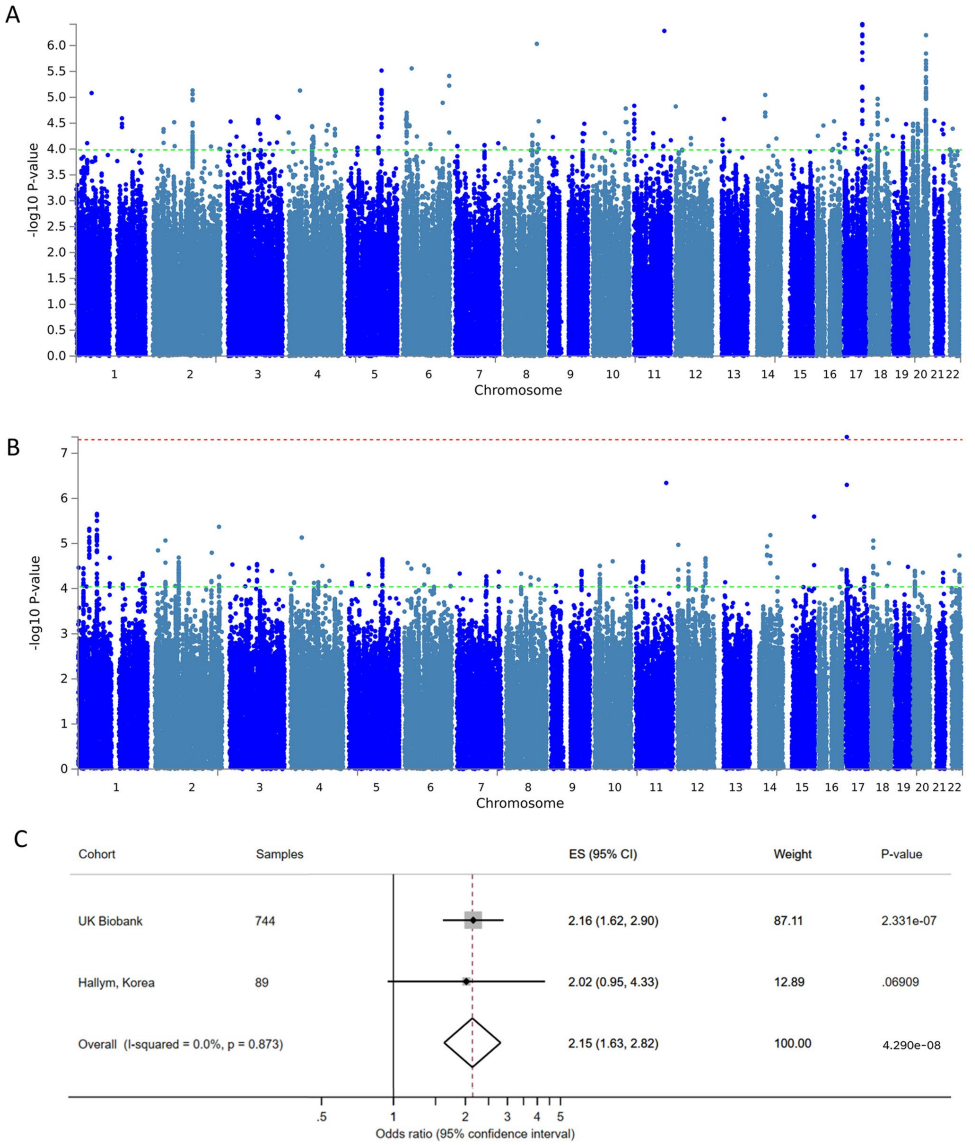

Fig. 1 **A** Manhattan plot from the meta-analysis that includes HATCH datasets alone. **B** Manhattan plot from the meta-analysis that includes UK Biobank and HATCH datasets. The red dotted line signifies genome-wide significance ($p < 5 \times 10^{-8}$), green dotted line signifies suggestive significance ($p < 1 \times 10.^{-4}$). Manhattan plots generated using FUMA. **C** Forest plot for genome-wide significant SNP rs12949158. rs12949158 genotypes in: UK Biobank: AA 252, AG 346, GG 146; Korean dataset: AA 22, AG 42, GG 25

powered to detect common variation (minor allele frequency (MAF = 0.4) with an effect size of 1.39 and rare variation (MAF = 0.1) with an effect size of 1.66 at genome-wide significance. Recruitment is ongoing for validation and investigators wishing to collaborate using either retrospective or prospective data can find further information on the study website. The study has been designed to maximise inclusivity. It is open to any investigator able to provide the following de-identified biosamples or data from patients with aSAH: genome-wide genotype information (or DNA/cellular sample for genotyping), mRS/GOS within two years of haemorrhage, age, sex and evidence of institutional review board approval. In addition, funding will be provided to facilitate genotyping where local funding is not already in place. Where data availability allows we will also explore whether significant genetic variants mediate the effect of other factors, known to influence outcome after aSAH such as clinical and radiological features.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12975-022-01095-4.

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Author Contribution All authors contributed to the study conception and design. BG and WT performed the analysis. The first draft of the manuscript was written by BG, WT, IG and DB, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability Study data will be available from the authors subject to institutional agreements and ethical approvals.

Declarations

Ethics Approvals National (REC 19 SC 0485) and local (ERGO 49253) ethical approval in place.

Consent to Participate Informed consent was obtained from all individual participants included in the study.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

References

1. Taylor TN, Davis PH, Torner JC, Holmes J, Meyer JW, Jacobson MF. Lifetime cost of stroke in the United States, (in eng). Stroke. 1996;27(9):1459–66.
2. Bulters D, et al. Haemoglobin scavenging in intracranial bleeding: biology and clinical implications, (in eng). Nat Rev Neurol. 2018;14(7):416–32. https://doi.org/10.1038/s41582-018-0020-0.
3. Zolnourian A, Galea I, Bulters D. Neuroprotective Role of the Nrf2 Pathway in Subarachnoid Haemorrhage and Its Therapeutic Potential, (in eng). Oxid Med Cell Longev. 2019;2019:6218239. https://doi.org/10.1155/2019/6218239.
4. Macdonald RL, Pluta RM, Zhang JH. Cerebral vasospasm after subarachnoid hemorrhage: the emerging revolution, (in eng). Nat Clin Pract Neurol. 2007;3(5):256–63. https://doi.org/10.1038/ncpneuro0490.
5. Pickard JD, et al. Effect of oral nimodipine on cerebral infarction and outcome after subarachnoid haemorrhage: British aneurysm nimodipine trial, (in eng). BMJ. 1989;298(6674):636–42.
6. Jaja BNR, et al. Development and validation of outcome prediction models for aneurysmal subarachnoid haemorrhage: the SAHIT multinational cohort study. BMJ. 2018;360:j5745. https://doi.org/10.1136/bmj.j5745.
7. Morton MJ, et al. Haptoglobin genotype and outcome after aneurysmal subarachnoid haemorrhage," (in eng). J Neurol Neurosurg Psychiatry. 2020;91(3):305–13. https://doi.org/10.1136/jnnp-2019-321697.
8. Gallek MJ, Conley YP, Sherwood PR, Horowitz MB, Kassam A, Alexander SA. APOE genotype and functional outcome following aneurysmal subarachnoid hemorrhage, (in eng). Biol Res Nurs. 2009;10(3):205–12. https://doi.org/10.1177/1099800408323221.
9. Gaastra B, et al. Genome-Wide Association Study of Clinical Outcome After Aneurysmal Subarachnoid Haemorrhage: Protocol, (in eng). Transl Stroke Res. 2022. https://doi.org/10.1007/s12975-021-00978-2.
10. Sudlow C, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age, (in eng). PLoS Med. 2015;12(3):e1001779. https://doi.org/10.1371/journal.pmed.1001779.
11. Farrell B, Godwin J, Richards S, Warlow C. The United Kingdom transient ischaemic attack (UK-TIA) aspirin trial: final results. J Neurol Neurosurg Psychiatry. 1991;54(12):1044–54.

12. Rankin J. Cerebral vascular accidents in patients over the age of 60. II. Prognosis. Scott Med J. 1957;2(5):200–15. https://doi.org/10.1177/003693305700200504.
13. Jennett B, Bond M. Assessment of outcome after severe brain damage. Lancet. 1975;1(7905):480–4.
14. Jennett B, Snoek J, Bond MR, Brooks N. Disability after severe head injury: observations on the use of the Glasgow Outcome Scale, (in eng). J Neurol Neurosurg Psychiatry. 1981;44(4):285–93. https://doi.org/10.1136/jnnp.44.4.285.
15. Mayer SA, et al. Global and domain-specific cognitive impairment and outcome after subarachnoid hemorrhage, (in eng). Neurology. 2002;59(11):1750–8. https://doi.org/10.1212/01.wnl.0000035748.91128.c2.
16 Gaastra B, Ewbank F, Tapper W, Bulters D, Galea I. Long-Term Cognitive Outcome following Aneurysmal Subarachnoid Haemorrhage. J Stroke Cerebrovasc Dis. 2022;31(1):106184. https://doi.org/10.1016/j.jstrokecerebrovasdis.2021.106184.
17. Yang H, Wang K. Genomic variant annotation and prioritization with ANNOVAR and wANNOVAR, (in eng). Nat Protoc. 2015;10(10):1556–66. https://doi.org/10.1038/nprot.2015.105.
18 Watanabe K, Taskesen E, van Bochoven A, Posthuma D. Functional mapping and annotation of genetic associations with FUMA, (in eng). Nat Commun. 2017;8(1):1826. https://doi.org/10.1038/s41467-017-01261-5.
19. Bakker MK, et al. Genome-wide association study of intracranial aneurysms identifies 17 risk loci and genetic overlap with clinical risk factors, (in eng). Nat Genet. 2020;52(12):1303–13. https://doi.org/10.1038/s41588-020-00725-7.
20. Kim H, et al. Cerebral vasospasm after sub-arachnoid hemorrhage as a clinical predictor and phenotype for genetic association study, (in eng). Int J Stroke. 2013;8(8):620–5. https://doi.org/10.1111/j.1747-4949.2012.00823.x.
21. Li J, et al. Polygenic Risk Scores Augment Stroke Subtyping, (in eng). Neurol Genet. 2021;7(2):e560. https://doi.org/10.1212/NXG.0000000000000560.
22. Hong EP et al. Genomic Variations in Susceptibility to Intracranial Aneurysm in the Korean Population," (in eng), J Clin Med, 2019;8(2). https://doi.org/10.3390/jcm8020275.
23. Rose AB. Introns as Gene Regulators: A Brick on the Accelerator, (in eng). Front Genet. 2018;9:672. https://doi.org/10.3389/fgene.2018.00672.
24 Gaire BP, Choi JW. Sphingosine 1-Phosphate Receptors in Cerebral Ischemia, (in eng). Neuromolecular Med. 2021;2(1):211–23. https://doi.org/10.1007/s12017-020-08614-2.
25 Sun N, Keep RF, Hua Y, Xi G. Critical Role of the Sphingolipid Pathway in Stroke: a Review of Current Utility and Potential Therapeutic Targets, (in eng). Transl Stroke Res. 2016;7(5):420–38. https://doi.org/10.1007/s12975-016-0477-3.
26. Männer A, et al. Sphingosine 1-phosphate levels in cerebrospinal fluid after subarachnoid hemorrhage, (in eng). Neurol Res Pract. 2020;2:49. https://doi.org/10.1186/s42466-020-00093-x.
27. Tosaka M, et al. Sphingosine 1-phosphate contracts canine basilar arteries in vitro and in vivo: possible role in pathogenesis of cerebral vasospasm, (in eng). Stroke. 2001;32(12):2913–9. https://doi.org/10.1161/hs1201.099525.
28. Salomone S, et al. Analysis of sphingosine 1-phosphate receptors involved in constriction of isolated cerebral arteries with receptor null mice and pharmacological tools, (in eng). Br J Pharmacol. 2008;153(1):140–7. https://doi.org/10.1038/sj.bjp.0707581.
29. Fu Y, et al. Impact of an immune modulator fingolimod on acute ischemic stroke, (in eng). Proc Natl Acad Sci U S A. 2014;111(51):18315–20. https://doi.org/10.1073/pnas.1416166111.
30. Fu Y, et al. Fingolimod for the treatment of intracerebral hemorrhage: a 2-arm proof-of-concept study, (in eng). JAMA Neurol. 2014;71(9):1092–101. https://doi.org/10.1001/jamaneurol.2014.1065.

A.5.3 Publication 12: Evidence-based interconversion of the Glasgow Outcome and modified Rankin scales: pitfalls and best practices

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This study used data from my individual patient level data analysis of haptoglobin genotype after aneurysmal subarachnoid haemorrhage and two clinical trials (CLEAR III and MISTIE III). I curated the data. The primary statistical analysis was performed by Dianxu Ren. I performed the comparison of empirical cumulative distributions. I authored the manuscript with input from the co-authors after completion of the first draft.

Specific contribution: Primary role in study design, data collection and curation. Secondary role in data analysis. Authored first draft of manuscript.

Evidence-based interconversion of the Glasgow Outcome and modified Rankin scales: pitfalls and best practices

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Objective: The aim of this study was to provide the evidence base to guide interconversion of the modified Rankin Scale (mRS) and Glasgow Outcome Scale (GOS) in neurological research. *Methods:* A retrospective analysis of paired mRS and GOS recordings was conducted using datasets with the following selection criteria: (1) patients had haemorrhagic stroke, (2) simultaneous mRS and GOS measurements were available, and (3) data sharing was possible. The relationship between mRS and GOS was assessed using correlation analysis. The optimum dichotomisation thresholds for agreement between the mRS and GOS were identified using Cohen's kappa coefficient. Two-way conversion tables between mRS and GOS were developed based on the highest agreement between scores. Finally, to identify which direction of conversion (mRS to GOS or vice versa) was better, the Kolmogorov-Smirnov D statistic was calculated. *Results:* Using 3474 paired recordings the mRS and GOS were shown to be highly correlated ($\rho = 0.90$, $p < 0.0001$). The greatest agreement between the two scoring systems occurred when mRS=0-2 and GOS=4-5 was used to define good outcome (κ=0.83, 95% confidence interval: 0.81−0.85). Converting from mRS to GOS was better than the reverse direction as evidenced by a lower Kolmogorov-Smirnov statistic (D=0.054 compared to D=0.157). *Conclusions:* This study demonstrates that the mRS and GOS are highly correlated, establishes the optimum dichotomisation threshold for agreement, provides a method for interconversion and shows that mRS to GOS conversion is superior to the reverse direction if a choice is available.

Keywords: Stroke—Outcome—Modified Rankin scale—Glasgow outcome scale

Abbreviations: modified Rankin Scale, mRS; Glasgow Outcome Scale, GOS; Intracerebral Haemorrhage, ICH; aneurysmal Subarachnoid Haemorrhage, aSAH; Intraventricular Haemorrhage, IVH; National Institute of Neurological Disorders and Stroke, NINDS; Subarachnoid Haemorrhage Outcome Tool, SAHOT

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Introduction

The modified Rankin (mRS)[1,2] and Glasgow Outcome Scales (GOS)[3] are commonly used clinical outcome assessment tools in neurological research, especially in stroke. Both scales grade individuals according to functional recovery with the mRS ranging from 0 (no symptoms) to 6 (death) and the GOS from 1 (death) to 5 (good recovery) (Fig. 1).

Haemorrhagic stroke, including intracerebral haemorrhage (ICH) and aneurysmal subarachnoid haemorrhage (aSAH), only represents a small fraction of all strokes (15%). Consequently, observational studies of outcome following haemorrhagic stroke often rely on pooling data from multiple centres to increase sample size. As individual centres may adopt either the mRS or GOS, but rarely both, researchers commonly encounter the need to interconvert the two scales to allow pooled analysis. This interconversion is typically conducted by dichotomising the individual mRS and GOS scores into "good" and "poor" outcome and then pooling.

Modified Rankin Scale (mRS)	0 – No symptoms 1 – No significant disability 2 – Slight disability 3 – Moderate disability 4 – Moderately severe disability 5 – Severe disability 6 – Dead
Glasgow Outcome Scale (GOS)	1 – Dead 2 – Vegetative state 3 – Severe disability 4 – Moderate disability 5 – Good recovery

Fig. 1. *The modified Rankin Scale (mRS) and Glasgow Outcome Scale (GOS).*

A single National Institute of Neurological Disorders and Stroke (NINDS) workshop reported strong agreement between the mRS and GOS in the context of ischaemic stroke[4] and this is also likely to be the case for haemorrhagic stroke. There is, however, no evidence base in the literature guiding interconversion of the two scales. Specifically, the precise dichotomisation thresholds defining "good/poor" outcome in the mRS and GOS, which result in the best agreement between the two scales, has not been established. This information is essential to facilitate rigorous retrospective meta-analyses of individual patient level data from multiple studies employing both scales. We set out to address this unmet need, taking advantage of available datasets and adopting a rigorous statistical approach. Specifically in the context of haemorrhagic stroke our aims were to: (1) assess the correlation between the mRS and GOS, (2) identify which dichotomisation thresholds for "good/poor" outcome result in the best agreement between the two scales, and (3) develop the evidence base to enable conversion between the two scales.

Methods

This study used published data with institutional approval (ERGO 31523.A1). Patients with haemorrhagic stroke were identified from three international multicentre studies: (1) a retrospective observational analysis of outcome following aSAH[5], (2) CLEAR III, a randomised controlled trial of thrombolytic therapy for intraventricular haemorrhage (IVH)[6], and (3) MISTIE III, a randomised controlled trial of minimally invasive surgery with thrombolysis for ICH.[7] All paired mRS and GOS values, collected at the same timepoint, between 1 and 12 months were eligible for inclusion, meaning individuals could contribute multiple paired values.

Spearman's rank correlation coefficient, rho (ρ), was used to assess the relationship between scales. Cohen's kappa coefficient was used to assess which mRS and GOS dichotomisation thresholds defining "good/poor" outcome showed the best agreement. A higher kappa value indicates stronger agreement. For this analysis, death and severe disability (GOS=1-3 and mRS=4-6) were excluded from the definition of "good" outcome because these are universally regarded as "poor" outcome.

To develop a conversion table between the mRS and GOS, individual scores were cross-tabulated, and two-way conversions assigned based on the highest agreement between scores. At this level of agreement, we computed the probability that an individual's true score was the same as the converted score. Finally, to identify which direction of conversion (mRS to GOS or vice versa) was better, the Kolmogorov-Smirnov statistic, describing the maximum vertical distance between empirical cumulative distribution functions of directly acquired and converted scores, was calculated. Kuiper, Cramer-von Mises and Anderson-Darling statistical tests were also performed as sensitivity analyses to support the findings. A smaller statistic reflects more similar distributions for all of these tests.

To test the interconversion methods proposed in this study we used published data from the INTERACT2 trial.[8] INTERACT2 was a randomised controlled trial designed to assess the benefit of intensive blood pressure lowering compared to guideline-recommended treatment following spontaneous ICH. The primary outcome was the proportion of participants with poor outcome, defined as mRS=3-6, at 90 days. To assess performance of the proposed mRS/GOS interconversion methods we converted the mRS scores from INTERACT2 to GOS to see if interconversion would influence the primary study outcome.

Statistical analyses were performed in SAS (SAS Institute) and R (version 4.1.2, R Foundation for Statistical Computing). Data are available from the authors subject to institutional agreements and ethical approvals.

Results

A total of 1495 individuals with 3474 paired mRS and GOS recordings between 1 and 12 months post haemorrhagic stroke were identified for inclusion (see Table 1 for demographics, sample size and number of paired mRS/GOS values in each study).

The mRS and GOS were strongly correlated (ρ = 0.90, $p < 0.001$). When analysed individually, all three datasets showed the same direction and significance of results (aSAH: ρ = -0.88, $p < 0.001$; IVH: ρ = -0.84, $p < 0.001$; ICH: ρ = -0.82, $p < 0.001$).

The kappa statistic, commonly used to assess agreement on a nominal scale, was employed to establish the optimum dichotomisation threshold between the mRS and GOS. The greatest agreement (highest kappa) was seen when a dichotomisation threshold of mRS=0-2 and GOS=4-5 was used to define good outcome (κ=0.83, Table 2).

In order to provide the research community with a tool to convert from one scale to the other, we cross-tabulated the mRS and GOS to find the highest levels of agreement, and based on this, we provide two-way conversion tables in Table 3. The probability that an individual's true score was the same as their converted score is also shown.

When both the mRS and GOS are available from studies within an individual patient level data meta-analysis, it is

Table 1. *Demographics of patients included in the study. Two individuals were excluded from the study as they were coded as dead according to the GOS but as severe disability on the mRS. A sensitivity analysis including these individuals made no difference to the direction and significance of the results. SD: standard deviation, IPLD: individual patient level data.*

Study	Haptoglobin IPLD[3]	CLEAR III[4]	MISTIE III[5]
Stroke type	Aneurysmal subarachnoid haemorrhage	Intraventricular haemorrhage	Intracerebral haemorrhage
Sample size	496	499	500
Number of paired mRS and GOS values	1030	968	1476
Age, mean (SD)	54.6 (12.7)	58.6 (11.2)	61.2 (12.4)
Sex, n (%)			
Male	140 (28)	277 (56)	307 (61)
Female	352 (71)	222 (44)	193 (39)
Missing	4 (1)	-	-

important to know whether one conversion direction is superior to the other. To this end we used the D statistic in a two sample Kolmogorov-Smirnov test, comparing the empirical cumulative distribution functions of the scale before and after conversion. Converting from mRS to GOS was better than the reverse direction since the empirical cumulative distribution functions of directly acquired and converted scores were closer to each other in the mRS to GOS direction (D=0.054 compared to D=0.157, Table 4, Fig. 2). The direction of conversion is further supported by similar results using the Kuiper, Cramer-von Mises and Anderson Darling tests (Table 4).

As an exemplar, we converted the directly acquired mRS scores from the INTERACT2 study to GOS using the proposed conversion methodology (Table 3), and results are presented in Table 5. The primary outcome in the INTERACT2 study was based on the mRS, with poor outcome defined as mRS=3-6. Using the dichotomisation threshold agreements from Table 2 this definition of poor outcome equates to GOS=1-3. When the primary outcome of the INTERACT2 study was re-analysed using the converted GOS scores, the proportion of participants with poor outcome was identical to that of the original analysis using directly acquired mRS scores (Table 5).

Table 2. *Kappa statistics (95% confidence interval) and number of samples included in analysis (n, %) of agreement between dichotomised modified Rankin Scale (mRS) and Glasgow Outcome Scale (GOS) at different thresholds to separate "good" and "poor" outcome. Higher kappa values indicate better agreement. Death and severe disability (GOS 1−3 and mRS 4−6) were excluded from the definition of "good" outcome because these are universally regarded as "poor" outcome.*

		GOS	
		5	4-5
mRS	0	0.41 (0.37, 0.45) *n* = 3041, 87.5%	0.24 (0.21, 0.27) *n* = 2627, 75.6%
	0-1	0.81 (0.79, 0.84) *n* = 3291, 94.7%	0.62 (0.59, 0.65) *n* = 2995, 86.2%
	0-2	0.70 (0.67, 0.73) *n* = 3110, 89.5%	0.83 (0.81, 0.85) *n* = 3230, 93.0%
	0-3	0.42 (0.40, 0.45) *n* = 2552, 73.5%	0.67 (0.65, 0.70) *n* = 2932, 84.4%

Discussion

In this study we show that mRS and GOS are highly correlated following haemorrhagic stroke and provide the evidence base to allow interconversion of the two scales. Moreover, we test the interconversion methodology in an external dataset, with excellent results.

Table 3. *Conversion table between mRS and GOS. To develop the conversion table, directly acquired mRS and GOS scores were cross-tabulated and the best conversion is displayed, based on the highest probability of agreement between the two scores. When mRS is converted to GOS the best performing conversion does not include GOS=2, since the probability of agreement for this category was lower (P [mRS 5 → GOS 2] = 0.2558). When GOS is converted to mRS, the best performing conversion does not include mRS=3, since the probability of agreement for this category was lower (P [GOS 4 → mRS 3] = 0.3023).*

mRS	Converted GOS	Probability of agreement
0	5	0.9581
1	5	0.8272
2	4	0.5517
3	3	0.7578
4	3	0.9612
5	3	0.7401
6	1	1.0000
GOS	**Converted mRS**	**Probability of agreement**
1	6	1.0000
2	5	0.9947
3	4	0.3591
4	2	0.4837
5	1	0.5197

Table 4. *The Kolmogorov-Smirnov statistic D, or maximum vertical distance between empirical cumulative distribution functions of directly acquired and converted scores, was computed. Conversions were performed using the data presented in Table 3. As indicated by a smaller D statistic, the direction mRS → GOS was better than GOS → mRS. Kuiper, Cramer-von Mises and Anderson-Darling test statistics are also shown as sensitivity analyses to demonstrate robustness of findings; a smaller statistic reflects more similar distributions.*

Distribution comparison	Kolmogorov-Smirnov statistic (D)	Kuiper test statistic	Cramer-von Mises test statistic	Anderson-Darling test statistic
Directly acquired mRS & GOS converted to mRS	0.157	0.298	0.048	4.593×10^{-5}
Directly acquired GOS & mRS converted to GOS	0.054	0.080	0.004	3.239×10^{-6}

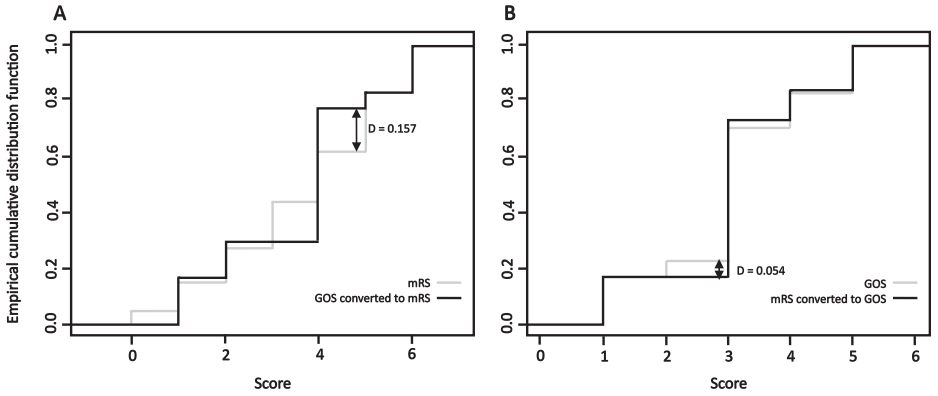

Fig. 2. *Empirical cumulative distribution functions comparing A: mRS directly acquired from patients and GOS converted to mRS; and B: GOS directly acquired from patients and mRS converted to GOS. The arrows signify the maximum vertical distance between the two empirical distribution functions with a smaller value representing more similar data distributions (Kolmogorov-Smirnov statistic (D)).*

Table 5. *The directly acquired mRS and converted GOS for the two arms of the INTERACT2 study including the proportion of individuals with poor outcome (the primary INTERACT2 study outcome). The scores were converted using the conversion tables in Table 3.*

	Intensive blood-pressure lowering (*n*=1382)	Guideline-recommended blood pressure lowering (*n*=1412)
Directly acquired mRS		
0	112 (8.1%)	107 (7.6%)
1	292 (21.1%)	254 (18.0%)
2	259 (18.7%)	266 (18.8%)
3	220 (15.9%)	234 (16.6%)
4	250 (18.1%)	268 (19.0%)
5	83 (6.0%)	113 (8.0%)
6	166 (12.0%)	170 (12.0%)
Primary outcome: mRS=3-6	719 (52.0%)	785 (55.6%)
Converted GOS		
1	166 (12.0%)	170 (12.0%)
2	-	-
3	553 (40.0%)	615 (43.6%)
4	259 (18.7%)	266 (18.8%)
5	404 (29.2%)	361 (25.6%)
Converted primary outcome: GOS=1-3	719 (52.0%)	785 (55.6%)

The mRS was specifically developed to assess outcome following stroke, although predominantly in ischaemic rather than haemorrhage stroke.[2] GOS, on the other hand, was designed in the context of head injury[3] and its use in stroke has been questioned.[9] The GOS, however, remains a commonly used outcome score, especially in haemorrhagic stroke, emphasising the importance of understanding its relationship to the mRS. While prospective studies should collect the same consistent outcome measure, retrospective individual patient level studies may require the combined use of existing mRS and GOS datasets, for which this study provides the appropriate evidence base and methodology. As observed in a single publication of ischaemic stroke[4] the mRS and GOS are also highly correlated in haemorrhagic stroke, both overall and within individual haemorrhage types.

No previous studies have assessed the optimum dichotomisation threshold for agreement between mRS and GOS. In this study the kappa statistic identified that the best agreement occurred using a dichotomisation threshold of GOS=4-5 and mRS=0-2 to define "good" outcome (κ=0.83, 95% confidence interval: 0.81-0.85, Table 2), supporting the use of this threshold when pooling dichotomised datasets. This dichotomisation includes GOS=4 (moderate disability, Fig. 1), which is not universally considered as a "good" outcome. If moderate disability is excluded from the definition of "good" outcome then the best agreement occurs when mRS=0-1 and GOS=5 are used to define "good" outcome (κ=0.81, 95% confidence interval: 0.79-0.84, Table 2); this threshold excludes mRS=2 (slight disability, Fig. 1) from the definition of "good" outcome. As the kappa statistic 95% confidence intervals overlap for these two dichotomisation thresholds, it is not immediately obvious that one is superior to the other and the decision should be guided by study context.

Defining "good" outcome as mRS=0-1 may be preferable when one considers Table 3, which provides the score-by-score interconversion between the mRS and GOS (with the probability that an individual's true score was the same as their converted score). When converting from mRS to GOS, a score of GOS=2 had a low probability (see Table 3 and legend); this was expected as there is no equivalent of GOS=2 (vegetative state) in the mRS. When converting from GOS to mRS, a score of mRS=3 also had a low probability (see Table 3 and legend). Although some incongruence is expected when converting a 5-point (GOS) to a 7-point scale (mRS), the low probability of conversion to GOS=2 and mRS=3 does limit the use of the conversion tables. It, therefore, follows that dichotomization into "good" and "poor" outcome is preferable to using the conversion tables. Moreover, dichotomisation of scores avoiding mRS=3 on the boundary between "good" outcome and "poor" outcome, i.e. defining "good" outcome as mRS=0-1, may offer the most reliable way of merging mRS and GOS data.

In pooled mRS and GOS datasets, when a decision is made to convert scores rather than dichotomize into "good" and "poor" outcome, and a choice of conversion direction is available, mRS to GOS conversion is superior to the reverse direction. This is likely because the GOS has fewer levels than the mRS reducing the spread of distribution. It should be noted that converting from the seven-point mRS to the five-point GOS may reduce the sensitivity of outcome assessment, for example the mRS has five levels covering what is conventionally considered to be good to moderate outcome whereas the GOS only has two such levels.

A major strength of this study is the large sample size drawn from multiple international cohorts, including different types of haemorrhagic strokes and study designs (observational and randomised controlled trials), enhancing generalizability. We also demonstrate in an external cohort that the proposed dichotomisation threshold and method of score interconversion does not alter the results of the INTERACT2 randomised controlled trial. This supports the application of these interconversion methods to external datasets.

Prior to this study there was no evidence base to support the interconversion of mRS and GOS in neurological disease. This study adds to the literature by providing practical methodological approaches to interconvert mRS and GOS. This will allow researchers to pool multiple studies using these different outcome measures to generate large sample sizes. Ultimately, analyses of large datasets will further our understanding of neurological disease and impact clinical practice, for example, by providing valuable prognostic information, improving outcome prediction and advancing our understanding of the pathophysiological mechanisms involved.

Limitations

A number of limitations merit discussion. This study focussed on the GOS and not its 8-point extended version (GOS-E)[10] but this was purposeful, since the GOS-E can be easily converted to the GOS, but not vice versa. Another limitation is that the dataset did not include other neurological conditions. While caution may be required when translating the results from haemorrhagic stroke to other conditions, one would expect the basic principles of disability assessment by the mRS and GOS, and the mathematical relationship between the scores, to be agnostic of the pathological process leading to neurological disability. When it is necessary to follow disability with time in the same patient, it is important to ensure when possible that outcome is measured with the same scale, and ideally by the same rater to minimize the effects of inter-rater reliability.[11,12]

Future directions

The results of this study are based on individuals with haemorrhagic stroke and tested in a randomised controlled trial in ICH. Although it is likely that the results

are translatable to other neurological conditions future studies are required to validate the findings in different cohorts. In addition to GOS and mRS other disease-specific outcome scales are being increasingly used in research, for example the Subarachnoid Haemorrhage Outcome Tool (SAHOT).[13] Future work is required to assess how these scales correlate with mRS and GOS to maximise the number of studies that can be included in pooled analyses.

Conclusion

In the context of haemorrhagic stroke, this study demonstrates that the mRS and GOS are highly correlated, establishes the optimum dichotomisation threshold for agreement, provides a method for interconversion and shows that mRS to GOS conversion is superior to the reverse direction if a choice is available.

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Author contribution

IG and DB conceived the study. All authors contributed to the study design and provided data. DR and BG performed statistical analyses. The first draft of the manuscript was written by BG, IG and DB, all authors revised the manuscript. All authors read and approved the final manuscript.

Declarations of Competing Interest

None.

Acknowledgments: National Institute of Neurological Disorders and Stroke (NINDS) for providing data.

References

1. Rankin J. Cerebral vascular accidents in patients over the age of 60. II. Prognosis. Scott Med J 1957;2(5):200-215.
2. Farrell B, Godwin J, Richards S, Warlow C. The United Kingdom transient ischaemic attack (UK-TIA) aspirin trial: final results. J Neurol Neurosurg Psychiatry 1991;54(12):1044-1054.
3. Jennett B, Bond M. Assessment of outcome after severe brain damage. Lancet 1975;1(7905):480-484.
4. Tilley BC, Marler J, Geller NL, Lu M, Legler J, Brott T, et al. Use of a global test for multiple outcomes in stroke trials with application to the national institute of neurological disorders and Stroke t-PA stroke trial. Stroke. 1996;27(11):2136-2142.
5. Gaastra B, Ren D, Alexander S, Bennett ER, Bielawski DM, Blackburn SL, et al. Haptoglobin genotype and aneurysmal subarachnoid hemorrhage: individual patient data analysis. Neurology 2019;92(18):e2150-e2e64.
6. Hanley DF, Lane K, McBee N, Ziai W, Tuhrim S, Lees KR, et al. Thrombolytic removal of intraventricular haemorrhage in treatment of severe stroke: results of the randomised, multicentre, multiregion, placebo-controlled CLEAR III trial. Lancet 2017;389(10069):603-611.
7. Hanley DF, Thompson RE, Rosenblum M, Yenokyan G, Lane K, McBee N, et al. Efficacy and safety of minimally invasive surgery with thrombolysis in intracerebral haemorrhage evacuation (MISTIE III): a randomised, controlled, open-label, blinded endpoint phase 3 trial. Lancet 2019;393(10175):1021-1032.
8. Anderson CS, Heeley E, Huang Y, Wang J, Stapf C, Delcourt C, et al. Rapid blood-pressure lowering in patients with acute intracerebral hemorrhage. N Engl J Med 2013;368(25):2355-2365.
9. Lees KR, Bath PM, Schellinger PD, Kerr DM, Fulton R, Hacke W, et al. Contemporary outcome measures in acute stroke research: choice of primary outcome measure. Stroke 2012;43(4):1163-1170.
10. Wilson JT, Pettigrew LE, Teasdale GM. Structured interviews for the Glasgow Outcome scale and the extended Glasgow Outcome scale: guidelines for their use. J Neurotrauma 1998;15(8):573-585.
11. Wilson JT, Hareendran A, Hendry A, Potter J, Bone I, Muir KW. Reliability of the modified Rankin Scale across multiple raters: benefits of a structured interview. Stroke 2005;36(4):777-781.
12. Anderson SI, Housley AM, Jones PA, Slattery J, Miller JD. Glasgow Outcome scale: an inter-rater reliability study. Brain Inj 1993;7(4):309-317.
13. Pace A, Mitchell S, Casselden E, Zolnourian A, Glazier J, Foulkes L, et al. A subarachnoid haemorrhage-specific outcome tool. Brain 2018;141(4):1111-1121.

Appendix B Co-author declarations

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Sheila Alexander

Publication: **Haemoglobin scavenging in intracranial bleeding: biology and clinical implications**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship alongside Ian Galea and Diederik Bulters.*

Publication: **Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Authored first draft of manuscript.*

Publication: **Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship.*

Publication: **Evidence-based interconversion of the Glasgow Outcome and modified Rankin scales: pitfalls and best practices**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Secondary role in data analysis. Authored first draft of manuscript.*

Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: Sheila A. Alexander Date: 11/10/22

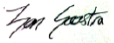
Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Varinder Alg

Publication: **Genetic variation in NFE2L2 is associated with outcome following aneurysmal subarachnoid haemorrhage**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 02/12/22

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Monica Ashokumar

Publication: **Auditory outcome following aneurysmal subarachnoid haemorrhage**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 10/11/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Issam Awad

Publication: **Evidence-based interconversion of the Glasgow Outcome and modified Rankin scales: pitfalls and best practices**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Secondary role in data analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: November 10, 2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Mark Bakker

Publication: **Genetic variation in NFE2L2 is associated with outcome following aneurysmal subarachnoid haemorrhage**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Publication: **Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship.*

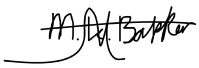
Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 14/11/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Peter Barron

Publication: **CRP (C-reactive protein) in outcome prediction after subarachnoid haemorrhage and the role of machine learning**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: Date: 12/11/2022.

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Ellen Bennett

Publication: **Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: Ellen Bennett Date: 11/29/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Hemant Bhagat

Publication: **Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship.*

Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: Hemant Bhagat Date: 11/11/2022

DR HEMANT BHAGAT MD, DM
Professor
Core Faculty of Neuroanaesthesia
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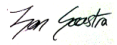
Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Dawn Bielawski

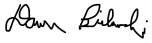
Publication: **Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: November 10, 2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Philippe Bijlenga

Publication: **Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship.*

Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: Philippe Bijlenga Date: 02/12/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Spiros Blackburn

Publication: **Haemoglobin scavenging in intracranial bleeding: biology and clinical implications**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship alongside Ian Galea and Diederik Bulters.*

Publication: **Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis**

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Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 11/17/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Mark Borsody

Publication: **Haemoglobin scavenging in intracranial bleeding: biology and clinical implications**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship alongside Ian Galea and Diederik Bulters.*

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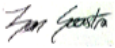
Signed:  Date: 09/11/2022

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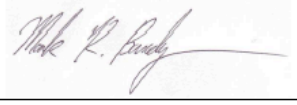
Signed:  Date: 11/10/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Diederik Bulters

Publication: **Long-term cognitive outcome following aneurysmal subarachnoid haemorrhage**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Publication: **Auditory outcome following aneurysmal subarachnoid haemorrhage**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Publication: **Duration and characteristics of persistent headache following aneurysmal subarachnoid hemorrhage**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript with Harry Carmichael.*

Publication: **Long-term fatigue following aneurysmal subarachnoid haemorrhage and the impact on employment**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

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Publication: **Haptoglobin genotype and outcome after subarachnoid haemorrhage: new insights from a meta-analysis**

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The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Authored first draft of manuscript.*

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The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

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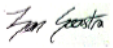
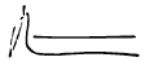
Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 05/12/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 06/12/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Nicci Campbell

Publication: **Auditory outcome following aneurysmal subarachnoid haemorrhage**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 09/11/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Harry Carmichael

Publication: **Duration and characteristics of persistent headache following aneurysmal subarachnoid hemorrhage**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript with Harry Carmichael.*

Publication: **Long-term fatigue following aneurysmal subarachnoid haemorrhage and the impact on employment**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: Date: 10/11/22

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Simran Chhugani

Publication: **CRP (C-reactive protein) in outcome prediction after subarachnoid haemorrhage and the role of machine learning**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: S. Chhugani Date: 25/11/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Malie Collins

Publication: **Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship.*

Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 11/25/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Sylvain Doré

Publication: Haemoglobin scavenging in intracranial bleeding: biology and clinical implications

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship alongside Ian Galea and Diederik Bulters.*

Publication: Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Authored first draft of manuscript.*

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Publication: A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: S. Doré Date: 11/28/2022

Sylvain Doré

Digitally signed by Sylvain Doré
DN: cn=Sylvain Doré, o=University of Florida, ou, email=sdore@ufl.edu, c=US
Date: 2022.11.28 07:06:03 -05'00'

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Poppy Duncan

Publication: **Genetic variation in NFE2L2 is associated with outcome following aneurysmal subarachnoid haemorrhage**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 15/11/2022

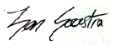
Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Frederick Ewbank

Publication: **Long-term cognitive outcome following aneurysmal subarachnoid haemorrhage**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 12/11/22

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Ian Galea

Publication: **Long-term cognitive outcome following aneurysmal subarachnoid haemorrhage**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Publication: **Auditory outcome following aneurysmal subarachnoid haemorrhage**

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The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 05/12/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 5/12/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: James Galea

Publication: **Haemoglobin scavenging in intracranial bleeding: biology and clinical implications**

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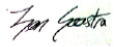
Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 17 NOV 2022

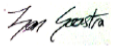
Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Patrick Garland

Publication: **Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 10 NOV 2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: James Glazier

Publication: **Haptoglobin genotype and outcome after subarachnoid haemorrhage: new insights from a meta-analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation, analysis and manuscript preparation alongside James Glazier.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 10/11/22

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Christoph Griessenauer

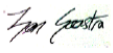
Publication: **Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship.*

Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: Christoph J. Griessenauer Date: 11/28/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Dan Hanley

Publication: **Evidence-based interconversion of the Glasgow Outcome and modified Rankin scales: pitfalls and best practices**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Secondary role in data analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: Dan Hanley Date: 12/9/22

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Tian He

Publication: **Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 10/11/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Philipp Hendrix

Publication: **Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship.*

Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: Date: November 11, 2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Eunpyo Hong

Publication: **Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship.*

Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 11/10/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Isabel Hostettler

Publication: **Genetic variation in NFE2L2 is associated with outcome following aneurysmal subarachnoid haemorrhage**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

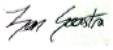
Publication: **Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship.*

Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date:10/11/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Henry Houlden

Publication: **Genetic variation in NFE2L2 is associated with outcome following aneurysmal subarachnoid haemorrhage**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

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Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 10/11/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Koji Iihara

Publication: **Haemoglobin scavenging in intracranial bleeding: biology and clinical implications**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship alongside Ian Galea and Diederik Bulters.*

Publication: **Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Authored first draft of manuscript.*

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Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: K Iihara Date: 14/11/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Jin Pyeong Jeon

Publication: **Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship.*

Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: Date: 9/11/2022.

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Yoichiro Kawamura

Publication: **Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: Yoichiro Kawamura Date: 10/11/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Bong Jun Kim

Publication: **Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship.*

Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: Bong jun Kim Date: 23 / 11 / 2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Peter Kirkpatrick

Publication: **CRP (C-reactive protein) in outcome prediction after subarachnoid haemorrhage and the role of machine learning**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 02 Dec 2022

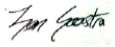
Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Munish Kumar

Publication: **Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 11/11/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Jenna Leclerc

Publication: **Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: Jenna Leclerc Date: 12/3/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Jiang Li

Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: Date: 11/28/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Ben Macarthur

Publication: **CRP (C-reactive protein) in outcome prediction after subarachnoid haemorrhage and the role of machine learning**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: Ben MacArthur Date: 10/11/2022

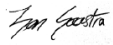
Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: James Meschia

Publication: **Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: December 6, 2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Sandrine Morel

Publication: **Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship.*

Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 10.11.2022

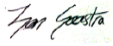
Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Laura Newitt

Publication: **CRP (C-reactive protein) in outcome prediction after subarachnoid haemorrhage and the role of machine learning**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 28.11.22

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Paul Nyquist

Publication: **Haemoglobin scavenging in intracranial bleeding: biology and clinical implications**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship alongside Ian Galea and Diederik Bulters.*

Publication: **Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Authored first draft of manuscript.*

Publication: **Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship.*

Publication: **Evidence-based interconversion of the Glasgow Outcome and modified Rankin scales: pitfalls and best practices**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Secondary role in data analysis. Authored first draft of manuscript.*

Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: Paul Nyquist Date: 11/09/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Michael Pizzi

Publication: **Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 11/28/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Dianxu Ren

Publication: **Haemoglobin scavenging in intracranial bleeding: biology and clinical implications**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship alongside Ian Galea and Diederik Bulters.*

Publication: **Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis**

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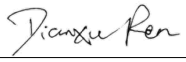
Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 11/10/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Ynte Ruigrok

Publication: **Genetic variation in NFE2L2 is associated with outcome following aneurysmal subarachnoid haemorrhage**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Publication: **Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship.*

Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: YR Date: 10/11/2022

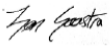
Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Rafael Tamargo

Publication: **Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: December 6, 2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Will Tapper

Publication: **Long-term cognitive outcome following aneurysmal subarachnoid haemorrhage**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Publication: **Genetic variation in NFE2L2 is associated with outcome following aneurysmal subarachnoid haemorrhage**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Publication: **Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship.*

Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed: Ben Gaastra Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed: Will Tapper Date: 10/11/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Carole Turner

Publication: **CRP (C-reactive protein) in outcome prediction after subarachnoid haemorrhage and the role of machine learning**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 11/11/22

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: David Werring

Publication: **Genetic variation in NFE2L2 is associated with outcome following aneurysmal subarachnoid haemorrhage**

The candidate's specific contribution to the publication was: *Primary role in study design, data curation and analysis. Authored first draft of manuscript.*

Publication: **Genome-wide association study of clinical outcome after aneurysmal subarachnoid haemorrhage: protocol**

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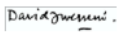
Publication: **A genome wide association study of outcome after aneurysmal subarachnoid haemorrhage: discovery analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection, curation and analysis. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 28/11/2022

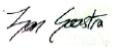
Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Wuyang Yang

Publication: **Haptoglobin genotype and aneurysmal subarachnoid haemorrhage: individual patient data analysis**

The candidate's specific contribution to the publication was: *Primary role in study design, data collection and curation. Authored first draft of manuscript.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 11/28/2022

Declaration of co-authorship of published work

Name of candidate: Ben Gaastra
Name of co-author: Ardalan Zolnourian

Publication: **Haemoglobin scavenging in intracranial bleeding: biology and clinical implications**

The candidate's specific contribution to the publication was: *Primary role in manuscript authorship alongside Ian Galea and Diederik Bulters.*

Statement by candidate: The above contribution statement(s) are accurate.

Signed:  Date: 09/11/2022

Statement by co-author: I agree with the above declaration(s) of the candidate.

Signed:  Date: 11/11/2022

References

1. Feigin VL, Lawes CM, Bennett DA, Barker-Collo SL, Parag V. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. *Lancet Neurol*. Apr 2009;8(4):355-69. doi:10.1016/S1474-4422(09)70025-0

2. de Rooij NK, Linn FH, van der Plas JA, Algra A, Rinkel GJ. Incidence of subarachnoid haemorrhage: a systematic review with emphasis on region, age, gender and time trends. *J Neurol Neurosurg Psychiatry*. Dec 2007;78(12):1365-72. doi:10.1136/jnnp.2007.117655

3. Macdonald RL, Schweizer TA. Spontaneous subarachnoid haemorrhage. *Lancet*. 02 2017;389(10069):655-666. doi:10.1016/S0140-6736(16)30668-7

4. Molyneux AJ, Birks J, Clarke A, Sneade M, Kerr RS. The durability of endovascular coiling versus neurosurgical clipping of ruptured cerebral aneurysms: 18 year follow-up of the UK cohort of the International Subarachnoid Aneurysm Trial (ISAT). *Lancet*. Feb 21 2015;385(9969):691-7. doi:10.1016/S0140-6736(14)60975-2

5. Larsen CC, Astrup J. Rebleeding after aneurysmal subarachnoid hemorrhage: a literature review. *World Neurosurg*. Feb 2013;79(2):307-12. doi:10.1016/j.wneu.2012.06.023

6. Connolly ES, Rabinstein AA, Carhuapoma JR, et al. Guidelines for the management of aneurysmal subarachnoid hemorrhage: a guideline for healthcare professionals from the American Heart Association/american Stroke Association. *Stroke*. Jun 2012;43(6):1711-37. doi:10.1161/STR.0b013e3182587839

7. Steiner T, Juvela S, Unterberg A, et al. European Stroke Organization guidelines for the management of intracranial aneurysms and subarachnoid haemorrhage. *Cerebrovasc Dis*. 2013;35(2):93-112. doi:10.1159/000346087

8. Roos YB, de Haan RJ, Beenen LF, Groen RJ, Albrecht KW, Vermeulen M. Complications and outcome in patients with aneurysmal subarachnoid haemorrhage: a prospective hospital based cohort study in the Netherlands. *J Neurol Neurosurg Psychiatry*. Mar 2000;68(3):337-41. doi:10.1136/jnnp.68.3.337

9. Vergouwen MD, Vermeulen M, van Gijn J, et al. Definition of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage as an outcome event in clinical trials and observational studies: proposal of a multidisciplinary research group. *Stroke*. Oct 2010;41(10):2391-5. doi:10.1161/STROKEAHA.110.589275

10. Dorhout Mees SM, Rinkel GJ, Feigin VL, et al. Calcium antagonists for aneurysmal subarachnoid haemorrhage. *Cochrane Database Syst Rev*. Jul 2007;(3):CD000277. doi:10.1002/14651858.CD000277.pub3

11. Pickard JD, Murray GD, Illingworth R, et al. Effect of oral nimodipine on cerebral infarction and outcome after subarachnoid haemorrhage: British aneurysm nimodipine trial. *BMJ*. Mar 1989;298(6674):636-42.

12. Macdonald RL. Delayed neurological deterioration after subarachnoid haemorrhage. *Nat Rev Neurol*. Jan 2014;10(1):44-58. doi:10.1038/nrneurol.2013.246

13. Johnston SC, Selvin S, Gress DR. The burden, trends, and demographics of mortality from subarachnoid hemorrhage. *Neurology*. May 1998;50(5):1413-8. doi:10.1212/wnl.50.5.1413

14. Lovelock CE, Rinkel GJ, Rothwell PM. Time trends in outcome of subarachnoid hemorrhage: Population-based study and systematic review. *Neurology*. May 11 2010;74(19):1494-501. doi:10.1212/WNL.0b013e3181dd42b3

15. Schatlo B, Fung C, Stienen MN, et al. Incidence and Outcome of Aneurysmal Subarachnoid Hemorrhage: The Swiss Study on Subarachnoid Hemorrhage (Swiss SOS). *Stroke*. Jan 2021;52(1):344-347. doi:10.1161/STROKEAHA.120.029538

16. Wallmark S, Ronne-Engström E, Lundström E. Predicting return to work after subarachnoid hemorrhage using the Montreal Cognitive Assessment (MoCA). *Acta Neurochir (Wien)*. Feb 2016;158(2):233-9. doi:10.1007/s00701-015-2665-4

17. Taylor TN, Davis PH, Torner JC, Holmes J, Meyer JW, Jacobson MF. Lifetime cost of stroke in the United States. *Stroke*. Sep 1996;27(9):1459-66.

18. Rivero-Arias O, Gray A, Wolstenholme J. Burden of disease and costs of aneurysmal subarachnoid haemorrhage (aSAH) in the United Kingdom. *Cost Eff Resour Alloc*. Apr 2010;8:6. doi:10.1186/1478-7547-8-6

19. Rankin J. Cerebral vascular accidents in patients over the age of 60. II. Prognosis. *Scott Med J*. May 1957;2(5):200-15. doi:10.1177/003693305700200504

20. Farrell B, Godwin J, Richards S, Warlow C. The United Kingdom transient ischaemic attack (UK-TIA) aspirin trial: final results. *J Neurol Neurosurg Psychiatry*. Dec 1991;54(12):1044-54.

21. Jennett B, Bond M. Assessment of outcome after severe brain damage. *Lancet*. Mar 1 1975;1(7905):480-4.

22. Nieuwkamp DJ, Setz LE, Algra A, Linn FH, de Rooij NK, Rinkel GJ. Changes in case fatality of aneurysmal subarachnoid haemorrhage over time, according to age, sex, and region: a meta-analysis. *Lancet Neurol*. Jul 2009;8(7):635-42. doi:10.1016/S1474-4422(09)70126-7

23. Al-Khindi T, Macdonald RL, Schweizer TA. Cognitive and functional outcome after aneurysmal subarachnoid hemorrhage. *Stroke*. Aug 2010;41(8):e519-36. doi:10.1161/STROKEAHA.110.581975

24. Nussbaum ES, Mikoff N, Paranjape GS. Cognitive deficits among patients surviving aneurysmal subarachnoid hemorrhage. A contemporary systematic review. *Br J Neurosurg*. Dec 21 2020:1-18. doi:10.1080/02688697.2020.1859462

25. Campbell N, Verschuur C, Mitchell S, et al. Hearing impairment after subarachnoid hemorrhage. *Ann Clin Transl Neurol*. 03 2019;6(3):420-430. doi:10.1002/acn3.714

26. Galea JP, Dulhanty L, Patel HC, Collaborators UaISHD. Predictors of Outcome in Aneurysmal Subarachnoid Hemorrhage Patients: Observations From a Multicenter Data Set. *Stroke*. 11 2017;48(11):2958-2963. doi:10.1161/STROKEAHA.117.017777

27. Cahill J, Cahill WJ, Calvert JW, Calvert JH, Zhang JH. Mechanisms of early brain injury after subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. Nov 2006;26(11):1341-53. doi:10.1038/sj.jcbfm.9600283

28. Dodd WS, Laurent D, Dumont AS, et al. Pathophysiology of Delayed Cerebral Ischemia After Subarachnoid Hemorrhage: A Review. *J Am Heart Assoc*. 08 03 2021;10(15):e021845. doi:10.1161/JAHA.121.021845

29. Zolnourian A, Galea I, Bulters D. Neuroprotective Role of the Nrf2 Pathway in Subarachnoid Haemorrhage and Its Therapeutic Potential. *Oxid Med Cell Longev*. 2019;2019:6218239. doi:10.1155/2019/6218239

30. D'Souza S. Aneurysmal Subarachnoid Hemorrhage. *J Neurosurg Anesthesiol*. Jul 2015;27(3):222-40. doi:10.1097/ANA.0000000000000130

31. Macdonald RL, Pluta RM, Zhang JH. Cerebral vasospasm after subarachnoid hemorrhage: the emerging revolution. *Nat Clin Pract Neurol*. May 2007;3(5):256-63. doi:10.1038/ncpneuro0490

32. Garland P, Morton MJ, Haskins W, et al. Haemoglobin causes neuronal damage. *Brain Commun*. Jan 2020;2(1):fcz053. doi:10.1093/braincomms/fcz053

33. Macdonald RL, Katan M. Haptoglobin and hemoglobin in subarachnoid hemorrhage: A tale of 2 globins. *Neurology*. Apr 30 2019;92(18):831-832. doi:10.1212/WNL.0000000000007399

34. Dutra FF, Bozza MT. Heme on innate immunity and inflammation. *Front Pharmacol*. 2014;5:115. doi:10.3389/fphar.2014.00115

35. Dutra FF, Alves LS, Rodrigues D, et al. Hemolysis-induced lethality involves inflammasome activation by heme. *Proc Natl Acad Sci U S A*. Sep 30 2014;111(39):E4110-8. doi:10.1073/pnas.1405023111

36. Brough D, Rothwell NJ, Allan SM. Interleukin-1 as a pharmacological target in acute brain injury. *Exp Physiol*. Dec 2015;100(12):1488-94. doi:10.1113/EP085135

37. Ayer RE, Zhang JH. Oxidative stress in subarachnoid haemorrhage: significance in acute brain injury and vasospasm. *Acta Neurochir Suppl*. 2008;104:33-41.

38. Reeder BJ, Sharpe MA, Kay AD, Kerr M, Moore K, Wilson MT. Toxicity of myoglobin and haemoglobin: oxidative stress in patients with rhabdomyolysis and subarachnoid haemorrhage. *Biochem Soc Trans*. Aug 2002;30(4):745-8. doi:10.1042/

39. Hugelshofer M, Buzzi RM, Schaer CA, et al. Haptoglobin administration into the subarachnoid space prevents hemoglobin-induced cerebral vasospasm. *J Clin Invest*. 12 02 2019;129(12):5219-5235. doi:10.1172/JCI130630

40. Lin G, Macdonald RL, Marton LS, Kowalczuk A, Solenski NJ, Weir BK. Hemoglobin increases endothelin-1 in endothelial cells by decreasing nitric oxide. *Biochem Biophys Res Commun*. Jan 26 2001;280(3):824-30. doi:10.1006/bbrc.2000.4167

41. Voetsch B, Jin RC, Loscalzo J. Nitric oxide insufficiency and atherothrombosis. *Histochem Cell Biol*. Oct 2004;122(4):353-67. doi:10.1007/s00418-004-0675-z

42. Dreier JP, Korner K, Ebert N, et al. Nitric oxide scavenging by hemoglobin or nitric oxide synthase inhibition by N-nitro-L-arginine induces cortical spreading ischemia when K+ is increased in the subarachnoid space. *J Cereb Blood Flow Metab*. Sep 1998;18(9):978-90. doi:10.1097/00004647-199809000-00007

43. Macdonald RL, Higashida RT, Keller E, et al. Clazosentan, an endothelin receptor antagonist, in patients with aneurysmal subarachnoid haemorrhage undergoing surgical clipping: a randomised, double-blind, placebo-controlled phase 3 trial (CONSCIOUS-2). *Lancet Neurol*. Jul 2011;10(7):618-25. doi:10.1016/S1474-4422(11)70108-9

44. Dorhout Mees SM, Algra A, Vandertop WP, et al. Magnesium for aneurysmal subarachnoid haemorrhage (MASH-2): a randomised placebo-controlled trial. *Lancet*. Jul 07 2012;380(9836):44-9. doi:10.1016/S0140-6736(12)60724-7

45. Kirkpatrick PJ, Turner CL, Smith C, Hutchinson PJ, Murray GD, Collaborators S. Simvastatin in aneurysmal subarachnoid haemorrhage (STASH): a multicentre randomised phase 3 trial. *Lancet Neurol*. Jul 2014;13(7):666-75. doi:10.1016/S1474-4422(14)70084-5

46. Zhang S, Wang L, Liu M, Wu B. Tirilazad for aneurysmal subarachnoid haemorrhage. *Cochrane Database Syst Rev*. Feb 2010;(2):CD006778. doi:10.1002/14651858.CD006778.pub2

47. Hillman J, Fridriksson S, Nilsson O, Yu Z, Saveland H, Jakobsson KE. Immediate administration of tranexamic acid and reduced incidence of early rebleeding after aneurysmal subarachnoid hemorrhage: a prospective randomized study. *J Neurosurg*. Oct 2002;97(4):771-8. doi:10.3171/jns.2002.97.4.0771

48. Saber H, Desai A, Palla M, Mohamed W, Seraji-Bozorgzad N, Ibrahim M. Efficacy of Cilostazol in Prevention of Delayed Cerebral Ischemia after Aneurysmal Subarachnoid Hemorrhage: A Meta-Analysis. *J Stroke Cerebrovasc Dis*. Nov 2018;27(11):2979-2985. doi:10.1016/j.jstrokecerebrovasdis.2018.06.027

49. Pace A, Mitchell S, Casselden E, et al. A subarachnoid haemorrhage-specific outcome tool. *Brain*. Feb 2018;doi:10.1093/brain/awy003

50. Visser-Meily JM, Rhebergen ML, Rinkel GJ, van Zandvoort MJ, Post MW. Long-term health-related quality of life after aneurysmal subarachnoid hemorrhage: relationship with psychological symptoms and personality characteristics. *Stroke*. Apr 2009;40(4):1526-9. doi:10.1161/STROKEAHA.108.531277

51. Rinkel GJ, Algra A. Long-term outcomes of patients with aneurysmal subarachnoid haemorrhage. *Lancet Neurol*. Apr 2011;10(4):349-56. doi:10.1016/S1474-4422(11)70017-5

52. Sudlow C, Gallacher J, Allen N, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*. Mar 2015;12(3):e1001779. doi:10.1371/journal.pmed.1001779

53. Vos EM, Greebe P, Visser-Meily JMA, Rinkel GJE, Vergouwen MDI. Subjective hearing impairment after subarachnoid haemorrhage: Prevalence and risk factors. *J Neurol Sci*. Jan 2017;372:184-186. doi:10.1016/j.jns.2016.11.062

54. Audiology BSo. Position Statement and Practice Guidance

Auditory Processing Disorder (APD). 2018.

55. Chia EM, Wang JJ, Rochtchina E, Cumming RR, Newall P, Mitchell P. Hearing impairment and health-related quality of life: the Blue Mountains Hearing Study. *Ear Hear*. Apr 2007;28(2):187-95. doi:10.1097/AUD.0b013e31803126b6

56. Kutlubaev MA, Barugh AJ, Mead GE. Fatigue after subarachnoid haemorrhage: a systematic review. *J Psychosom Res*. Apr 2012;72(4):305-10. doi:10.1016/j.jpsychores.2011.12.008

57. van Donkelaar CE, Bakker NA, Birks J, et al. Prediction of Outcome After Aneurysmal Subarachnoid Hemorrhage. *Stroke*. 04 2019;50(4):837-844. doi:10.1161/STROKEAHA.118.023902

58. Jaja BN, Cusimano MD, Etminan N, et al. Clinical prediction models for aneurysmal subarachnoid hemorrhage: a systematic review. *Neurocrit Care*. Feb 2013;18(1):143-53. doi:10.1007/s12028-012-9792-z

59. Jaja BNR, Saposnik G, Lingsma HF, et al. Development and validation of outcome prediction models for aneurysmal subarachnoid haemorrhage: the SAHIT multinational cohort study. *BMJ*. Jan 18 2018;360:j5745. doi:10.1136/bmj.j5745

60. Dengler NF, Madai VI, Unteroberdörster M, et al. Outcome prediction in aneurysmal subarachnoid hemorrhage: a comparison of machine learning methods and established clinico-radiological scores. *Neurosurg Rev*. Oct 2021;44(5):2837-2846. doi:10.1007/s10143-020-01453-6

61. Booker J, Barron P, Newitt L, et al. Changes in Outcome Prediction During the First Week After Subarachnoid Hemorrhage. *Neurosurgery Open*. 2022;3(4)

62. Jeon YT, Lee JH, Lee H, et al. The postoperative C-reactive protein level can be a useful prognostic factor for poor outcome and symptomatic vasospasm in patients with aneurysmal subarachnoid hemorrhage. *J Neurosurg Anesthesiol*. Oct 2012;24(4):317-24. doi:10.1097/ANA.0b013e31826047a2

63. Rothoerl RD, Axmann C, Pina AL, Woertgen C, Brawanski A. Possible role of the C-reactive protein and white blood cell count in the pathogenesis of cerebral vasospasm following aneurysmal subarachnoid hemorrhage. *J Neurosurg Anesthesiol*. Jan 2006;18(1):68-72. doi:10.1097/01.ana.0000181693.30750.af

64. Turner CL, Budohoski K, Smith C, et al. Elevated Baseline C-Reactive Protein as a Predictor of Outcome After Aneurysmal Subarachnoid Hemorrhage: Data From the Simvastatin in Aneurysmal Subarachnoid Hemorrhage (STASH) Trial. *Neurosurgery*. Nov 2015;77(5):786-92; discussion 792-3. doi:10.1227/NEU.0000000000000963

65. Habehh H, Gohel S. Machine Learning in Healthcare. *Curr Genomics*. Dec 16 2021;22(4):291-300. doi:10.2174/1389202922666210705124359

66. Bzdok D, Krzywinski M, Altman N. Points of Significance: Machine learning: a primer. *Nat Methods*. Nov 2017;14(12):1119-1120. doi:10.1038/nmeth.4526

67. Bzdok D, Altman N, Krzywinski M. Statistics versus machine learning. *Nat Methods*. Apr 2018;15(4):233-234. doi:10.1038/nmeth.4642

68. Germanson TP, Lanzino G, Kongable GL, Torner JC, Kassell NF. Risk classification after aneurysmal subarachnoid hemorrhage. *Surg Neurol*. Feb 1998;49(2):155-63. doi:10.1016/s0090-3019(97)00337-6

69. de Toledo P, Rios PM, Ledezma A, Sanchis A, Alen JF, Lagares A. Predicting the outcome of patients with subarachnoid hemorrhage using machine learning techniques. *IEEE Trans Inf Technol Biomed*. Sep 2009;13(5):794-801. doi:10.1109/TITB.2009.2020434

70. Hostettler IC, Muroi C, Richter JK, et al. Decision tree analysis in subarachnoid hemorrhage: prediction of outcome parameters during the course of aneurysmal subarachnoid hemorrhage using decision tree analysis. *J Neurosurg*. 12 2018;129(6):1499-1510. doi:10.3171/2017.7.JNS17677

71. Liu J, Xiong Y, Zhong M, et al. Predicting Long-Term Outcomes After Poor-Grade Aneurysmal Subarachnoid Hemorrhage Using Decision Tree Modeling. *Neurosurgery*. Mar 2020;doi:10.1093/neuros/nyaa052

72. Savarraj JPJ, Hergenroeder GW, Zhu L, et al. Machine Learning to Predict Delayed Cerebral Ischemia and Outcomes in Subarachnoid Hemorrhage. *Neurology*. Jan 2021;96(4):e553-e562. doi:10.1212/WNL.0000000000011211

73. de Jong G, Aquarius R, Sanaan B, et al. Prediction Models in Aneurysmal Subarachnoid Hemorrhage: Forecasting Clinical Outcome With Artificial Intelligence. *Neurosurgery*. 04 15 2021;88(5):E427-E434. doi:10.1093/neuros/nyaa581

74. Sidey-Gibbons JAM, Sidey-Gibbons CJ. Machine learning in medicine: a practical introduction. *BMC Med Res Methodol*. 03 19 2019;19(1):64. doi:10.1186/s12874-019-0681-4

75. Yu D, Williams GW, Aguilar D, et al. Machine learning prediction of the adverse outcome for nontraumatic subarachnoid hemorrhage patients. *Ann Clin Transl Neurol*. 11 2020;7(11):2178-2185. doi:10.1002/acn3.51208

76. Haug T, Sorteberg A, Finset A, Lindegaard KF, Lundar T, Sorteberg W. Cognitive functioning and health-related quality of life 1 year after aneurysmal subarachnoid hemorrhage in preoperative comatose patients (Hunt and Hess Grade V patients). *Neurosurgery*. Mar 2010;66(3):475-84; discussion 484-5. doi:10.1227/01.NEU.0000365364.87303.AC

77. Kristiansen M, Graversen JH, Jacobsen C, et al. Identification of the haemoglobin scavenger receptor. *Nature*. 2001;409(6817):198-201.

78. Guetta J, Strauss M, Levy NS, Fahoum L, Levy AP. Haptoglobin genotype modulates the balance of Th1/Th2 cytokines produced by macrophages exposed to free hemoglobin. *Atherosclerosis*. Mar 2007;191(1):48-53. doi:10.1016/j.atherosclerosis.2006.04.032

79. Banerjee S, Jia Y, Siburt CJ, et al. Haptoglobin alters oxygenation and oxidation of hemoglobin and decreases propagation of peroxide-induced oxidative reactions. Research Support, Non-U.S. Gov't

Research Support, U.S. Gov't, Non-P.H.S. *Free Radic Biol Med*. Sep 15 2012;53(6):1317-26. doi:10.1016/j.freeradbiomed.2012.07.023

80. Chaichana KL, Levy AP, Miller-Lotan R, Shakur S, Tamargo RJ. Haptoglobin 2-2 genotype determines chronic vasospasm after experimental subarachnoid hemorrhage. *Stroke*. Dec 2007;38(12):3266-71. doi:10.1161/STROKEAHA.107.490003

81. Leclerc JL, Blackburn S, Neal D, et al. Haptoglobin phenotype predicts the development of focal and global cerebral vasospasm and may influence outcomes after aneurysmal subarachnoid hemorrhage. *Proceedings of the National Academy of Sciences of the United States of America*. 2015;112(4):1155-60.

82. Kantor E, Bayır H, Ren D, et al. Haptoglobin genotype and functional outcome after aneurysmal subarachnoid hemorrhage. *Journal of neurosurgery*. 2014;120(2):386-90.

83. Ohnishi H, Iihara K, Kaku Y, et al. Haptoglobin phenotype predicts cerebral vasospasm and clinical deterioration after aneurysmal subarachnoid hemorrhage. *Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association*. 2013;22(4):520-6.

84. Murthy SB, Caplan J, Levy AP, et al. Haptoglobin 2-2 Genotype Is Associated With Cerebral Salt Wasting Syndrome in Aneurysmal Subarachnoid Hemorrhage. *Neurosurgery*. 2016;78(1):71-6.

85. Galea J, Cruickshank G, Teeling JL, et al. The intrathecal CD163-haptoglobin-hemoglobin scavenging system in subarachnoid hemorrhage. *Journal of neurochemistry*. 2012;121(5):785-92.

86. Borsody M, Burke A, Coplin W, Miller-Lotan R, Levy A. Haptoglobin and the development of cerebral artery vasospasm after subarachnoid hemorrhage. *Neurology*. 2006;66(5):634-40.

87. Langlois MR, Delanghe JR. Biological and clinical significance of haptoglobin polymorphism in humans. *Clin Chem*. Oct 1996;42(10):1589-600.

88. Bamm VV, Tsemakhovich VA, Shaklai M, Shaklai N. Haptoglobin phenotypes differ in their ability to inhibit heme transfer from hemoglobin to LDL. *Biochemistry*. Apr 2004;43(13):3899-906. doi:10.1021/bi0362626

89. Philippidis P, Mason JC, Evans BJ, et al. Hemoglobin scavenger receptor CD163 mediates interleukin-10 release and heme oxygenase-1 synthesis: antiinflammatory monocyte-macrophage responses in vitro, in resolving skin blisters in vivo, and after cardiopulmonary bypass surgery. *Circ Res*. Jan 09 2004;94(1):119-26. doi:10.1161/01.RES.0000109414.78907.F9

90. Iliff JJ, Lee H, Yu M, et al. Brain-wide pathway for waste clearance captured by contrast-enhanced MRI. *J Clin Invest*. Mar 2013;123(3):1299-309. doi:10.1172/JCI67677

91. Morton MJ, Hostettler IC, Kazmi N, et al. Haptoglobin genotype and outcome after aneurysmal subarachnoid haemorrhage. *J Neurol Neurosurg Psychiatry*. 03 2020;91(3):305-313. doi:10.1136/jnnp-2019-321697

92. Ateia AM, Elbassiouny A, El-Nabi SH, et al. Predictive value of haptoglobin genotype as a risk of cerebral vasospasm after aneurysmal subarachnoid hemorrhage. *Clin Neurol Neurosurg*. 12 2020;199:106296. doi:10.1016/j.clineuro.2020.106296

93. Wilson DA, Nakaji P, Albuquerque FC, McDougall CG, Zabramski JM, Spetzler RF. Time course of recovery following poor-grade SAH: the incidence of delayed improvement and implications for SAH outcome study design. *J Neurosurg*. Sep 2013;119(3):606-12. doi:10.3171/2013.4.JNS121287

94. Han SW, Kim BJ, Kim TY, et al. Association of Haptoglobin Phenotype With Neurological and Cognitive Outcomes in Patients With Subarachnoid Hemorrhage. *Front Aging Neurosci*. 2022;14:819628. doi:10.3389/fnagi.2022.819628

95. Bulters D, Gaastra B, Zolnourian A, et al. Haemoglobin scavenging in intracranial bleeding: biology and clinical implications. *Nat Rev Neurol*. Jul 2018;14(7):416-432. doi:10.1038/s41582-018-0020-0

96. Baird L, Dinkova-Kostova AT. The cytoprotective role of the Keap1-Nrf2 pathway. *Arch Toxicol*. Apr 2011;85(4):241-72. doi:10.1007/s00204-011-0674-5

97. Zhang M, An C, Gao Y, Leak RK, Chen J, Zhang F. Emerging roles of Nrf2 and phase II antioxidant enzymes in neuroprotection. *Prog Neurobiol*. Jan 2013;100:30-47. doi:10.1016/j.pneurobio.2012.09.003

98. Zhao X, Song S, Sun G, et al. Neuroprotective role of haptoglobin after intracerebral hemorrhage. *J Neurosci*. Dec 2009;29(50):15819-27. doi:10.1523/JNEUROSCI.3776-09.2009

99. Chen G, Fang Q, Zhang J, Zhou D, Wang Z. Role of the Nrf2-ARE pathway in early brain injury after experimental subarachnoid hemorrhage. *J Neurosci Res*. Apr 2011;89(4):515-23. doi:10.1002/jnr.22577

100. Li T, Wang H, Ding Y, et al. Genetic elimination of Nrf2 aggravates secondary complications except for vasospasm after experimental subarachnoid hemorrhage in mice. *Brain Res*. Apr 2014;1558:90-9. doi:10.1016/j.brainres.2014.02.036

101. Starke RM, Kim GH, Komotar RJ, et al. Endothelial nitric oxide synthase gene single-nucleotide polymorphism predicts cerebral vasospasm after aneurysmal subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. Jun 2008;28(6):1204-11. doi:10.1038/jcbfm.2008.11

102. Gallek MJ, Conley YP, Sherwood PR, Horowitz MB, Kassam A, Alexander SA. APOE genotype and functional outcome following aneurysmal subarachnoid hemorrhage. *Biol Res Nurs*. Jan 2009;10(3):205-12. doi:10.1177/1099800408323221

103. Vilkki J, Lappalainen J, Juvela S, Kanarek K, Hernesniemi JA, Siironen J. Relationship of the Met allele of the brain-derived neurotrophic factor Val66Met polymorphism to memory after aneurysmal subarachnoid hemorrhage. *Neurosurgery*. Aug 2008;63(2):198-203; discussion 203. doi:10.1227/01.NEU.0000320382.21577.8E

104. Ruigrok YM, Slooter AJ, Bardoel A, Frijns CJ, Rinkel GJ, Wijmenga C. Genes and outcome after aneurysmal subarachnoid haemorrhage. *J Neurol*. Apr 2005;252(4):417-22. doi:10.1007/s00415-005-0661-y

105. Vergouwen MD, Frijns CJ, Roos YB, Rinkel GJ, Baas F, Vermeulen M. Plasminogen activator inhibitor-1 4G allele in the 4G/5G promoter polymorphism increases the occurrence of cerebral ischemia after aneurysmal subarachnoid hemorrhage. *Stroke*. Jun 2004;35(6):1280-3. doi:10.1161/01.STR.0000128707.48644.7e

106. Bakker MK, van der Spek RAA, van Rheenen W, et al. Genome-wide association study of intracranial aneurysms identifies 17 risk loci and genetic overlap with clinical risk factors. *Nat Genet*. 12 2020;52(12):1303-1313. doi:10.1038/s41588-020-00725-7

107. Hanley DF, Lane K, McBee N, et al. Thrombolytic removal of intraventricular haemorrhage in treatment of severe stroke: results of the randomised, multicentre, multiregion, placebo-controlled CLEAR III trial. *Lancet*. 02 11 2017;389(10069):603-611. doi:10.1016/S0140-6736(16)32410-2

108. Hanley DF, Thompson RE, Rosenblum M, et al. Efficacy and safety of minimally invasive surgery with thrombolysis in intracerebral haemorrhage evacuation (MISTIE III): a randomised, controlled, open-label, blinded endpoint phase 3 trial. *Lancet*. Mar 09 2019;393(10175):1021-1032. doi:10.1016/S0140-6736(19)30195-3

109. Mayer SA, Kreiter KT, Copeland D, et al. Global and domain-specific cognitive impairment and outcome after subarachnoid hemorrhage. *Neurology*. Dec 2002;59(11):1750-8. doi:10.1212/01.wnl.0000035748.91128.c2

110. Pillich RT, Chen J, Churas C, et al. NDEx: Accessing Network Models and Streamlining Network Biology Workflows. *Curr Protoc*. Sep 2021;1(9):e258. doi:10.1002/cpz1.258

111. Stark C, Breitkreutz BJ, Reguly T, Boucher L, Breitkreutz A, Tyers M. BioGRID: a general repository for interaction datasets. *Nucleic Acids Res*. Jan 01 2006;34(Database issue):D535-9. doi:10.1093/nar/gkj109

112. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. Nov 2003;13(11):2498-504. doi:10.1101/gr.1239303

113. Ritz A, Poirel CL, Tegge AN, et al. Pathways on demand: automated reconstruction of human signaling networks. *NPJ Syst Biol Appl*. 2016;2:16002. doi:10.1038/npjsba.2016.2

114. Gil DaL, JN and Murali, TM. The PathLinker app: Connect the dots in protein interaction networks [version 1; peer review: 1 approved, 2 approved with reservations]. *F1000Research*. 2017;6(58)doi:10.12688/f1000research.9909.1

115. Dobrosotskaya IY, Seegmiller AC, Brown MS, Goldstein JL, Rawson RB. Regulation of SREBP processing and membrane lipid production by phospholipids in Drosophila. *Science*. May 03 2002;296(5569):879-83. doi:10.1126/science.1071124

116. Kawahara A, Nishi T, Hisano Y, Fukui H, Yamaguchi A, Mochizuki N. The sphingolipid transporter spns2 functions in migration of zebrafish myocardial precursors. *Science*. Jan 23 2009;323(5913):524-7. doi:10.1126/science.1167449

117. Spiegel S, Milstien S. The outs and the ins of sphingosine-1-phosphate in immunity. *Nat Rev Immunol*. Jun 2011;11(6):403-15. doi:10.1038/nri2974

118. Gaire BP, Choi JW. Sphingosine 1-Phosphate Receptors in Cerebral Ischemia. *Neuromolecular Med*. 03 2021;23(1):211-223. doi:10.1007/s12017-020-08614-2

119. Sun N, Keep RF, Hua Y, Xi G. Critical Role of the Sphingolipid Pathway in Stroke: a Review of Current Utility and Potential Therapeutic Targets. *Transl Stroke Res*. 10 2016;7(5):420-38. doi:10.1007/s12975-016-0477-3

120. Männer A, Thomas D, Wagner M, et al. Sphingosine 1-phosphate levels in cerebrospinal fluid after subarachnoid hemorrhage. *Neurol Res Pract*. 2020;2:49. doi:10.1186/s42466-020-00093-x

121. Tosaka M, Okajima F, Hashiba Y, et al. Sphingosine 1-phosphate contracts canine basilar arteries in vitro and in vivo: possible role in pathogenesis of cerebral vasospasm. *Stroke*. Dec 01 2001;32(12):2913-9. doi:10.1161/hs1201.099525

122. Salomone S, Potts EM, Tyndall S, et al. Analysis of sphingosine 1-phosphate receptors involved in constriction of isolated cerebral arteries with receptor null mice and pharmacological tools. *Br J Pharmacol*. Jan 2008;153(1):140-7. doi:10.1038/sj.bjp.0707581

123. Moore JE, Purcaro MJ, Pratt HE, et al. Expanded encyclopaedias of DNA elements in the human and mouse genomes. *Nature*. 07 2020;583(7818):699-710. doi:10.1038/s41586-020-2493-4

124. Meuleman W, Muratov A, Rynes E, et al. Index and biological spectrum of human DNase I hypersensitive sites. *Nature*. 08 2020;584(7820):244-251. doi:10.1038/s41586-020-2559-3

125. Kundaje A, Meuleman W, Ernst J, et al. Integrative analysis of 111 reference human epigenomes. *Nature*. Feb 19 2015;518(7539):317-30. doi:10.1038/nature14248

126. Ward LD, Kellis M. HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. *Nucleic Acids Res*. Jan 2016;44(D1):D877-81. doi:10.1093/nar/gkv1340

127. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res*. 01 2019;47(D1):D886-D894. doi:10.1093/nar/gky1016

128. Tsai RY, Reed RR. Identification of DNA recognition sequences and protein interaction domains of the multiple-Zn-finger protein Roaz. *Mol Cell Biol*. Nov 1998;18(11):6447-56. doi:10.1128/MCB.18.11.6447

129. Roquer J, Cuadrado-Godia E, Guimaraens L, et al. Short- and long-term outcome of patients with aneurysmal subarachnoid hemorrhage. *Neurology*. 09 29 2020;95(13):e1819-e1829. doi:10.1212/WNL.0000000000010618

130. Passier PE, Visser-Meily JM, Rinkel GJ, Lindeman E, Post MW. Life satisfaction and return to work after aneurysmal subarachnoid hemorrhage. *J Stroke Cerebrovasc Dis*. 2011 Jul-Aug 2011;20(4):324-9. doi:10.1016/j.jstrokecerebrovasdis.2010.02.001

131. Wu S, Kutlubaev MA, Chun HY, et al. Interventions for post-stroke fatigue. *Cochrane Database Syst Rev*. Jul 02 2015;(7):CD007030. doi:10.1002/14651858.CD007030.pub3

132. Su Y, Yuki M, Otsuki M. Non-Pharmacological Interventions for Post-Stroke Fatigue: Systematic Review and Network Meta-Analysis. *J Clin Med*. Feb 25 2020;9(3)doi:10.3390/jcm9030621

133. Westerberg H, Jacobaeus H, Hirvikoski T, et al. Computerized working memory training after stroke--a pilot study. *Brain Inj*. Jan 2007;21(1):21-9. doi:10.1080/02699050601148726

134. Sun JH, Tan L, Yu JT. Post-stroke cognitive impairment: epidemiology, mechanisms and management. *Ann Transl Med*. Aug 2014;2(8):80. doi:10.3978/j.issn.2305-5839.2014.08.05

135. Sorrentino ZA, Laurent D, Hernandez J, et al. Headache persisting after aneurysmal subarachnoid hemorrhage: A narrative review of pathophysiology and therapeutic strategies. *Headache*. Oct 2022;62(9):1120-1132. doi:10.1111/head.14394

136. Morad AH, Tamargo RJ, Gottschalk A. The Longitudinal Course of Pain and Analgesic Therapy Following Aneurysmal Subarachnoid Hemorrhage: A Cohort Study. *Headache*. Nov 2016;56(10):1617-1625. doi:10.1111/head.12908

137. Hile GB, Cook AM. Treatment of headache in aneurysmal subarachnoid hemorrhage: Multimodal approach. *Interdisciplinary Neurosurgery*. 2020/12/01/ 2020;22:100857. doi:https://doi.org/10.1016/j.inat.2020.100857

138. Dorhout Mees SM, Bertens D, van der Worp HB, Rinkel GJ, van den Bergh WM. Magnesium and headache after aneurysmal subarachnoid haemorrhage. *J Neurol Neurosurg Psychiatry*. May 2010;81(5):490-3. doi:10.1136/jnnp.2009.181404

139. O'Brien JL, Lister JJ, Fausto BA, Clifton GK, Edwards JD. Cognitive Training Enhances Auditory Attention Efficiency in Older Adults. *Front Aging Neurosci*. 2017;9:322. doi:10.3389/fnagi.2017.00322

140. Goldberg J, Schoeni D, Mordasini P, et al. Survival and Outcome After Poor-Grade Aneurysmal Subarachnoid Hemorrhage in Elderly Patients. *Stroke*. 12 2018;49(12):2883-2889. doi:10.1161/STROKEAHA.118.022869

141. Gaastra B, Ren D, Alexander S, et al. Haptoglobin genotype and aneurysmal subarachnoid hemorrhage: Individual patient data analysis. *Neurology*. Apr 2019;92(18):e2150-e2164. doi:10.1212/WNL.0000000000007397

142. Kubota K, Egi M, Mizobuchi S. Haptoglobin Administration in Cardiovascular Surgery Patients: Its Association With the Risk of Postoperative Acute Kidney Injury. *Anesth Analg*. Jun 2017;124(6):1771-1776. doi:10.1213/ANE.0000000000002093

143. Nonaka T, Watanabe S, Chigasaki H, Miyaoka M, Ishii S. Etiology and treatment of vasospasm following subarachnoid hemorrhage. *Neurol Med Chir (Tokyo)*. Jan 1979;19(1):53-60.

144. Pezzini A. Long-term consequences of subarachnoid haemorrhage: Is it time for new strategies? *Eur J Neurol*. Sep 28 2022;doi:10.1111/ene.15580

145. Zhao X, Wen L, Dong M, Lu X. Sulforaphane activates the cerebral vascular Nrf2-ARE pathway and suppresses inflammation to attenuate cerebral vasospasm in rat with subarachnoid hemorrhage. *Brain Res*. Dec 2016;1653:1-7. doi:10.1016/j.brainres.2016.09.035

146. Zolnourian AH, Franklin S, Galea I, Bulters DO. Study protocol for SFX-01 after subarachnoid haemorrhage (SAS): a multicentre randomised double-blinded, placebo controlled trial. *BMJ Open*. 03 2020;10(3):e028514. doi:10.1136/bmjopen-2018-028514

147. McGinley MP, Cohen JA. Sphingosine 1-phosphate receptor modulators in multiple sclerosis and other conditions. *Lancet*. 09 25 2021;398(10306):1184-1194. doi:10.1016/S0140-6736(21)00244-0

148. Fu Y, Zhang N, Ren L, et al. Impact of an immune modulator fingolimod on acute ischemic stroke. *Proc Natl Acad Sci U S A*. Dec 23 2014;111(51):18315-20. doi:10.1073/pnas.1416166111

149. Fu Y, Hao J, Zhang N, et al. Fingolimod for the treatment of intracerebral hemorrhage: a 2-arm proof-of-concept study. *JAMA Neurol*. Sep 2014;71(9):1092-101. doi:10.1001/jamaneurol.2014.1065

150. Wang Y, Zhou S, Han Z, et al. Fingolimod administration improves neurological functions of mice with subarachnoid hemorrhage. *Neurosci Lett*. 09 25 2020;736:135250. doi:10.1016/j.neulet.2020.135250

151. Xu HL, Pelligrino DA, Paisansathan C, Testai FD. Protective role of fingolimod (FTY720) in rats subjected to subarachnoid hemorrhage. *J Neuroinflammation*. Jan 27 2015;12:16. doi:10.1186/s12974-015-0234-7

152. Altay O, Suzuki H, Hasegawa Y, et al. Isoflurane attenuates blood-brain barrier disruption in ipsilateral hemisphere after subarachnoid hemorrhage in mice. *Stroke*. Sep 2012;43(9):2513-6. doi:10.1161/STROKEAHA.112.661728

153. Altay O, Hasegawa Y, Sherchan P, et al. Isoflurane delays the development of early brain injury after subarachnoid hemorrhage through sphingosine-related pathway activation in mice. *Crit Care Med*. Jun 2012;40(6):1908-13. doi:10.1097/CCM.0b013e3182474bc1

154. Zhang L, Guo K, Zhou J, et al. Ponesimod protects against neuronal death by suppressing the activation of A1 astrocytes in early brain injury after experimental subarachnoid hemorrhage. *J Neurochem*. 08 2021;158(4):880-897. doi:10.1111/jnc.15457