Candidate molecular predictors of outcome after aneurysmal subarachnoid haemorrhage: a systematic review of haemoglobin metabolism, inflammation and oxidative injury pathways.

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Abstract

Aneurysmal subarachnoid haemorrhage (aSAH) is a devastating form of stroke associated with significant morbidity and mortality. Very little is known about the predictors of poor outcome and the pathophysiological mechanisms underlying neurological injury following aSAH. Three molecular pathways have been shown to be important: haemoglobin metabolism, inflammation and oxidative injury. The aim of this review is to use a systematic approach to identify a panel of key genes within these three pathways in order to focus future studies investigating predictors of poor outcome and the mechanisms of neurological injury following aSAH. Manual searching and bioinformatic mining tools were used. Studies of experimental or human SAH were included, and outcome was broadly defined to include all encountered readouts such as mortality, neurological scores, and neuropathological markers of tissue damage. If two or more molecules belonged to the same biochemical pathway, this pathway was examined in detail to identify all its components, which were then searched individually for any evidence of association with outcome using the same broad definition as before. This resulted in the identification of 58 candidate genes within the three pathways of interest (haemoglobin metabolism, inflammation and oxidative injury) potentially linked to outcome after aSAH.

Introduction

Aneurysmal subarachnoid haemorrhage (aSAH) results from the release of blood into the subarachnoid space as a consequence of a cerebral artery aneurysm rupture. aSAH is associated with significant morbidity and mortality. Twenty-eight day mortality rates of up to 42% have been reported[1] with the largest proportion of patients dying within two days of ictus[2]. Long term morbidity affecting survivors is significant with around half of survivors having cognitive impairments and only a third returning to the same work as prior to haemorrhage[3]. The socioeconomic burden of aSAH in the UK has been estimated as £510 million annually[4].

Neurological injury following aSAH can be broadly considered in two categories: firstly, an early brain injury (EBI) occurring within the first 72 hours of ictus, followed by a delayed brain injury occurring days to weeks after injury. EBI is thought to occur as a consequence of a spike in intracranial pressure and an associated fall in cerebral blood flow. This initiates toxic cascades causing global cerebral ischemia, blood-brain-barrier (BBB) disruption, cerebral oedema and ultimately cell death[5]. The toxic cascades initiated by EBI and the presence of blood and its breakdown products in the subarachnoid space are thought to cause a delayed brain injury characterised by spreading cortical depression, loss of autoregulation, microthrombi, inflammation, oxidative stress and vasospasm[6-8].

Blood within the subarachnoid space is broken down and cleared in a multi-step pathway involving a number of scavenging molecules[9]. This clearance system within the central nervous system (CNS) is not as well developed compared to the rest of the body meaning that blood break down products within the CNS persist for a relatively long time causing inflammation and oxidative stress. Blood breakdown

product scavenging molecules have been shown to play an important role in mitigating toxicity and limiting the neurological injury caused by blood breakdown products. Several authoritative reviews[9-15] have concluded that haemoglobin metabolism, inflammatory and oxidative mechanisms play a significant role in neurological injury following SAH (Figure 1).

The aim of this review is to identify a panel of molecules within the three themes of haemoglobin metabolism, inflammation and oxidative stress, which are of potential prognostic value after human aSAH. Candidate molecules were identified by an exhaustive search of the literature, using a combination of manual and text-mining methodology.

The candidate molecules identified here will be of use to any investigator who plans to perform a targeted study of the three pathways and outcome after SAH, using genetic, proteomic or metabolomic methods. As an example, in our case, we plan to perform a gene exome sequencing study, during which the exons and upstream regions of the candidates will be sequenced; the association of genetic variation in these regions with clinical outcome after aSAH will be investigated. Further study of these pathways will: (1) provide further evidence of the importance of haemoglobin breakdown product metabolism, inflammation and oxidative stress after human SAH; (2) improve prognostic tools; (3) give insight into the mechanisms of neurological injury following aSAH; (4) identify therapeutic targets for aSAH.

Methods

Rationale

A combination of manual and semi-automated methodology was used, rather than fully automated methods. In order to illustrate the need for this combined approach, we give an example. In mouse and rat models of SAH, two studies[16,17] compared RNA expression in cortical tissue from SAH versus control animals, and found that inflammation is a key theme. Blood breakdown products and oxidative stress are intricately linked to inflammation[18-23], yet these molecules are less likely to feature in automated pathway or network analyses of unbiased proteomic and RNA expression datasets since they are not well described in terms of KEGG pathways and GO ontology terms, and their networks are composed of a small number of molecules. For instance, when the 324 genes identified to be related to SAH by GLAD4U[24] (a PubMed gene retrieval and prioritization tool) are run through Over-Representation Analysis (ORA), Gene Set Enrichment Analysis (GSEA), and Network Topology-based Analysis (NTA) bioinformatic pipelines, oxidative stress and blood breakdown product metabolism are not identified. Hence enrichment of potential candidates within these pathways, based on most likely biologic targets reported in the literature, is needed to study components of these pathways which have been shown to be important in determining outcome after experimental or human SAH.

Overview

A literature search was performed for molecules shown to be important in influencing outcome after experimental or human aSAH within the three themes of haemoglobin metabolism, inflammation and oxidative stress. Outcome was broadly defined and included all encountered readouts such as mortality, neurological scores, and neuropathological markers of tissue damage.

The search strategy consisted of a manual Pubmed search specifically addressing the three themes of haemoglobin metabolism, inflammation and oxidative stress, supplemented by the full-text literature mining gene retrieval and functional analysis tool SciMiner[25] (http://hurlab.med.und.edu/SciMiner/). Publications retrieved were manually screened to ensure that the molecule was positively associated with outcome after SAH. For the reasons explained under “Rationale”, a combination of literature and pathway analysis was used in a sequential

multi-stage process, which is described next.

Description of stages

The stages of this search are depicted graphically in Figure 2. First, a primary literature search was performed in order to identify molecules (referred to as 'Group A' molecules) relevant to SAH from the three key themes: blood breakdown product metabolism, inflammation and oxidation. PubMed was searched for articles published in English prior to January 2019 using the following criteria: "oxidation", "anti-oxidant", "oxidative", "redox", "inflammation", "inflammatory", "h(a)emoglobin", "haem/heme" and "iron" in single combination with "subarachnoid h(a)emorrhage". The reference lists of published articles were manually searched for further articles. The results were manually curated to select those molecules related to SAH outcome.

If two or more molecules identified during the primary search belonged to the same biochemical pathway, this pathway was examined using different pathway analysis tools to identify all its components: the Reactome pathway database[26], KEGG pathway repository[27], textbooks and the literature. The genes identified by this search that were not present in 'Group A' are referred to as the 'Group B' molecules.

In order to ensure that none of these 'Group B' molecules were overlooked in the primary search, a secondary PubMed search was undertaken. In the secondary search the 'Group B' molecules were individually searched in combination with "subarachnoid h(a)emorrhage". Genes of this molecules were searched using official symbol provided by HGNC[28] and any common alternatives. 'Group B' molecules identified in the literature review to have evidence of a role in SAH outcome are referred to as 'Group C' molecules. 'Group D' molecules represent a more comprehensive set of molecules of interest in SAH outcome ('Group A' + 'Group C'). See Figure 2 for a summary of methodology and molecule groups A-D.

To ensure no molecules were missed, Pubmed IDs from both primary and secondary searches were text-mined using the full-text literature mining gene retrieval and functional analysis tool SciMiner[25] (http://hurlab.med.und.edu/SciMiner/). Genes which had not been identified manually were traced back to the original publication to identify whether they were associated with outcome after human or experimental SAH.

Results

As of 3rd August 2019, 1554 publications were retrieved by the primary Pubmed search. Automated text mining of these publications on SciMiner yielded 295 genes. Manual curation of the publications linked to these genes yielded 43 Group A molecules. A further 15 Group C molecules were identified during the secondary Pubmed search (Figure 2).

The final group of molecules (n=58) relevant to outcome after SAH (Group D = A + C) are shown in Table 1. Table 3 provides further detail on the function of each Group D molecule.

Conclusion

Blood breakdown product metabolism along with inflammatory and oxidative pathways play an important role after SAH. Experimental manipulation and/or genetic variation of molecules in these pathways have been linked to outcome after SAH and they therefore represent a very attractive target for future research studies. Further investigation of these molecules may help inform the mechanisms underlying poor outcome and help develop treatments to mitigate the devastating complications of SAH. It is important to note that these pathways are not independent but intimately linked. Both inflammation and oxidative stress can activate the other pathway, and transcription factors such as Nrf2, PPARγ and NFκB influence both pathways[23,29]. Blood breakdown products can stimulate both inflammation and oxidative stress and their scavengers play an important role to mitigate toxicity by clearing the breakdown products[18,22,30-32].

In summary, the panel of molecules presented in Table 3 have been identified using a systematic comprehensive search of the literature and will be useful to investigators planning to perform candidate or targeted molecular studies of outcome after SAH. This search will be updated at future intervals.

Figure 1 – Schematic summarising blood breakdown (1), inflammatory (2) and oxidative (3) pathways involved in SAH.

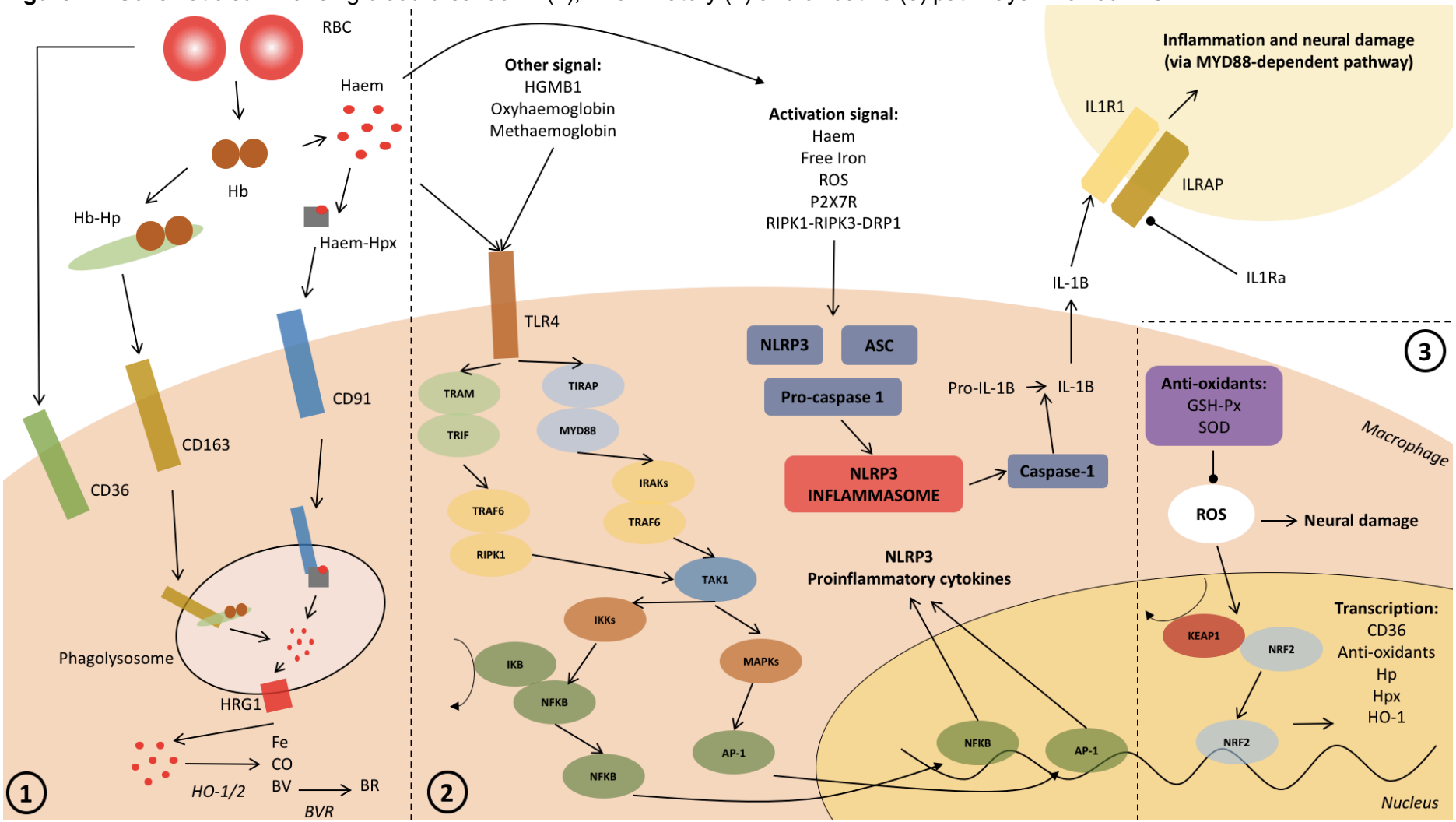

Figure 2 – Methods flowchart

STEP 1 ↓	Primary Pubmed search by theme (and outcome after human or experimental SAH), to identify **Group A** molecules (see Table 1)	**Theme 1:** Blood breakdown product metabolism	**Theme 2:** Inflammation	**Theme 3:** Oxidative response
STEP 2 ↓	Identification of distinct molecular pathways within themes 1-3, using multiple biochemical pathway analyses methodologies (see text)	Blood breakdown metabolism pathway	Group A molecules belonged to multiple pathways within the inflammation theme. However multiple molecules from TLR4, NLRP3, and IL1R1 pathways were identified.	All group A molecules belonged to one pathway – the NFE2L2 pathway
STEP 3 ↓	All known molecules within the pathways identified in Step 2 – **Group Z** molecules (see Table 2)	All known molecules within the blood breakdown metabolism pathway	All known molecules within the TLR4, NLRP3, and IL1R1 pathways	All known molecules within the NFE2L2 pathway
STEP 4 ↓	Identification of molecules needing secondary Pubmed search (**Group B**, where Group B = Group Z - Group A) (see Table 1)			
STEP 5 ↓	Secondary Pubmed search to identify 'Group B' molecules with evidence of effect on outcome after human or experimental SAH (**Group C**) (see Table 1)			
STEP 6	Molecules from primary and secondary Pubmed searches combined (**Group D**, where Group D = Group A + Group C) (see Table 1 & 3)			

Table 1. Molecules are denoted by their gene name

Molecule group	**Theme 1:** Blood breakdown product metabolism	**Theme 2:** Inflammation			**Theme 3:** Oxidative response		
A	HP HPX CD163 ADAM17 LRP1 HMOX1	TLR4 TLR2 TIRAP MYD88 TICAM1 RIPK1 NFKB1 RELA NFKBIA AGER	P2RX7 PANX1 NLRP3 PYCARD CASP1 RIPK3 DNM1L TNF IL6 IL17A	IL23A IL1B IL1A IL1R1 IL1RN MMP9 ICAM1 VCAM1 HMGB1	NFE2L2 KEAP1 PPARG BACH1 CYBB SOD1 SOD2 GPX1		
B	APOL1 HPR APOA1 AMBP CD36 BLVRA FTL FTH1 SLC48A1 HMOX2	TAB2 TAB1 TAB3 IL12A IL12B MAPK1 MAPK3 MAPK14 MAPK11 MAPK13 MAPK12 MAPK8 MAPK9 MAPK10 IRAK4 IRAK1	TRAF6 MAP3K7 JUN FOS CHUK IKBKB IKBKG TRAM1 TXN TXNIP HSP90AB1 SUGT1 RAK3 BTRC FBXW11	NKIRAS1 NKIRAS2 PELI1 TNIP2 PELI3 NFKBIB CUL1 SQSTM1 UBE2V1 MAP2K1 NFKB2 S100A12 SKP1 UBA52 RPS27A	CREB1 NQO1 GSTO2 GSTA5 GSTA1 GSTA2 GSTA3 GSTA4	GSTM1 GSTM2 GSTM3 GSTM4 GSTM5 GSTP1 GSTT1	GSTT2 MGST1 MGST2 MGST3 GSTT2B GSTO1 TXNRD1
C	FTL FTH1	TRAF6 TAB1 TAB3 MAP3K7	JUN FOS CHUK	IKBKB TXN TXNIP	CREB1 NQO1 GSTA1		
D	HP HPX CD163 ADAM17 LRP1 HMOX1 FTL FTH1	TLR4 TLR2 TIRAP MYD88 TICAM1 RIPK1 RIPK3 DNM1L NFKB1 RELA NFKBIA AGER P2RX7 PANX1	NLRP3 PYCARD CASP1 TNF IL6 IL17A IL23A IL1B IL1A IL1R1 IL1RN MMP9 ICAM1 VCAM1	HMGB1 TRAF6 TAB1 MAP3K7 JUN FOS CHUK IKBKB TXN TXNIP S100B	NFE2L2 KEAP1 PPARG BACH1 CYBB SOD1 SOD2 GPX1 CREB1 NQO1 GSTA1		

Table 2. Molecules are denoted by their gene name

Theme	1: Blood breakdown product metabolism		2: Inflammation					3: Oxidative response
Pathway of interest	Scavenging of heme		TLR4		IL1R1		NLRP3	NFE2L2
Source of pathway	Reactome		KEGG		KEGG & Reactome		Reactome	KEGG
Pathway Identifier	Scavenging of heme in plasma		N00186 N00188		N00435 N00438 IL-1 family signaling		NLR signalling pathways	N00243
Components	HPX LRP1 APOL1 HPR APOA1 HP CD163 AMBP		TLR4 TIRAP MYD88 IRAK4 IRAK1 TRAF6 MAP3K7 TAB2 CHUK IKBKB IKBKG NFKBIA NFKB1 RELA TNF	IL6 IL12A IL12B MAPK1 MAPK3 MAPK14 MAPK11 MAPK13 MAPK12 MAPK8 MAPK9 MAPK10 FOS JUN	IL1A IL1B IL1R1 IL1RAP MYD88 IRAK1 IRAK4 TRAF6 TAB1 TAB2 TAB3 MAP3K7 MAP2K3 MAP2K6 MAPK14 MAPKAPK2 ZFP36 MAP2K4 MAP2K7 MAPK8 MAPK9 MAPK10 FOS JUN TLR2	TLR4 TIRAP CHUK IKBKB IKBKG NFKBIA NFKB1 RELA TNF IL6 IL12A IL12B MAPK1 MAPK3 MAPK14 MAPK11 MAPK13 MAPK12 MAPK10 IRAK3 BTRC FBXW11 NKIRAS1 NKIRAS2 PELI1	TNIP2 PELI3 NFKBIB AGER CUL1 SQSTM1 UBE2V1 MAP2K1 NFKB2 S100A12 SKP1 UBA52 RPS27A RBX1 UBE2N MAP3K8 IL1R2 IL1RN SAA1 UBC UBB HMGB1 APP S100B MAP3K7	TXN TXNIP HSP90AB1 SUGT1 NLRP3 PYCARD CASP1	KEAP1 NFE2L2 HMOX1 NQO1 GSTO2 GSTA5 GSTA1 GSTA2 GSTA3 GSTA4 GSTM1 GSTM2 GSTM3 GSTM4 GSTM5 GSTP1 GSTT1 GSTT2 MGST1 MGST2 MGST3 GSTT2B GSTO1 TXNRD1
Molecules identified by additional literature review	CD36 BLVRA FTL	FTH1 SLC48A1 HMOX2	TRAM1					CREB1

Table 3. Molecules are denoted by their gene name

	Gene name	Symbol (HGNC)	Function
Inflammatory pathway molecules	Purinergic receptor P2X 7	P2RX7	ATP receptor
	Pannexin-1	PANX1	Gap junction protein
	Nuclear factor kappa B subunit 1	NFKB1	Component of NFkB transcription factor
	RELA proto-oncogene	RELA	Component of NFkB transcription factor
	NKKB inhibitor alpha	NFKBIA	IκB
	NLR family pyrin domain containing 3	NLRP3	Inflammasome complex component
	PYD and CARD domain containing	PYCARD	Inflammasome complex component
	Caspase 1	CASP1	Inflammasome complex component
	Receptor interacting serine/threonine kinase 1	RIPK1	Inflammasome complex activator
	Receptor interacting serine/threonine kinase 3	RIPK3	Inflammasome complex activator
	Dynamin 1 like	DNM1L	Inflammasome complex activator
	TNFα	TNF	Cytokine
	Interleukin 6	IL6	Cytokine
	Interleukin 17	IL17A	Cytokine
	Interleukin 23	IL23A	Cytokine
	Interleukin 1 β	IL1B	Cytokine
	Interleukin 1 α	IL1A	Cytokine
	Interleukin 1 receptor type 1	IL1R1	Interleukin 1 receptor
	Toll-like receptor 4	TLR4	Pattern recognition receptor
	Toll-like receptor 2	TLR2	Pattern recognition receptor
	TIR domain containing adaptor protein	TIRAP	Adaptor protein in IL-1 and TLR signalling pathways
	Mitogen-activated protein kinase kinase 7	MAP3K7	Adaptor protein in IL-1 and TLR signalling pathways
	TGF-beta activated kinase 1 (MAP3K7) binding protein 1	TAB1	MAP3K7 modifier
	TNF receptor associated factor 6	TRAF6	Adaptor protein in IL-1 and TLR signalling pathways
	Jun proto-oncogene	JUN	AP-1 component
	Fos proto-oncogene	FOS	AP-1 component
	Conserved helix-loop-helix ubiquitous kinase	CHUK	IKK complex component
	Inhibitor of nuclear factor kappa B kinase subunit beta	IKBKB	IKK complex component
	Myeloid differentiation primary response 88	MYD88	Adaptor protein in IL-1 and TLR signalling pathways
	Toll like receptor adaptor molecule 1	TICAM1	Adaptor protein in TLR signalling pathways
	High mobility group box 1	HMGB1	TLR4 activator
	Advanced glycosylation end-product specific receptor	AGER	HMGB1 receptor
	Interleukin 1 receptor antagonist	IL1RN	Inhibitor of IL1 at IL1R1
	Matrix metallopeptidase 9	MMP9	Matrix metalloproteinase
	Intercellular adhesion molecule 1	ICAM1	Cell surface glycoprotein
	Vascular cell adhesion molecule 1	VCAM1	Cell adhesion molecule
	Thioredoxin	TXN	Antioxidant
	Thioredoxin-interacting protein	TXNIP	TXN modifier
	S100 calcium binding protein B	S100B	Intracellular calcium binding protein

Oxidative pathway molecules	Nuclear factor erythroid 2 like 2	NFE2L2	Transcription factor promoting expression of anti-inflammatory genes
	Kelch like ECH associated protein 1	KEAP1	Regulator of NFE2L2 activity
	Peroxisome proliferator activated receptor gamma	PPARG	Nuclear receptor
	BTB domain and CNC homolog 1	BACH1	Regulator of NFE2L2 activity
	cAMP responsive element binding protein 1	CREB1	Regulator of NFE2L2 and NFκB activity
	Cytochrome b-245 beta chain	CYBB	NADPH oxidase
	Superoxide dismutase 1	SOD1	Antioxidant
	Superoxide dismutase 2	SOD2	Antioxidant
	Glutathione peroxidase 1	GPX1	Antioxidant
	NAD(P)H quinone oxidoreductase	NQO1	Antioxidant
	glutathione S-transferase-α1	GSTA1	Antioxidant
Blood breakdown product scavenger molecules	Haptoglobin	HP	Haemoglobin scavenger
	Hemepexin	HPX	Heme scavenger
	CD163	CD163	Facilitates endocytosis of haemoglobin-haptoglobin complexes into microglia/macrophages
	LDL receptor related protein 1	LRP1	Facilitates endocytosis of heme-hemopexin complexes
	ADAM metallopeptidase domain 17	ADAM17	Facilitates shedding of CD163 from cell surface
	Ferritin light chain	FTL	Intracellular iron storage protein
	Ferritin heavy chain 1	FTH1	Intracellular iron storage protein
	Heme oxygenase 1	HMOX1	Cleaves heme forming biliverdin

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