UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE HEALTH AND BIOLOGICAL SCIENCES

SCHOOL OF MEDICINE

Doctor of Philosophy

Comparison of two screening strategies for haemochromatosis: A pilot study investigating uptake and acceptability, feasibility and cost.

by

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The work presented in this thesis was done wholly while registered in postgraduate candidature in the University of Southampton under the supervision of Drs Rosenberg and Roderick. It is my own original work.

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ABSTRACT

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Haemochromatosis, a treatable adult-onset condition of progressive iron overload is amenable to population screening. Initial enthusiasm for screening to increase early diagnosis has been modified since the identification of the HFE gene. It is possible to screen for this condition using a genetic or a biochemical testing strategy. The performance of these strategies is different and it has been considered that there is something different about genetic testing that makes it less acceptable than other types of medical tests. There is a consensus, that evaluation of screening programmes requires an assessment of the balance between the benefit of the screening programme and the harm that might be caused by screening. This should include evaluation of all the components of the programme such as uptake of screening and characteristics of the population being offered and accepting screening.

Design: Randomised controlled equivalence/non-inferiority trial of two screening strategies.

a) Biochemical screening for iron overload on a blood sample taken at the General Practitioner's surgery, followed by genetic analysis and clinical assessment in those screened positive.

b) Genetic screening for the at risk genotype on a saliva sample performed at home, followed by biochemical testing for iron overload and clinical assessment in those screened positive.

Results: Approximately 3000 individuals from a general practice population aged 30-70 were offered screening. There was no difference in the feasibility and acceptability of screening as assessed by uptake and psychological assessments. Overall uptake was low, 34%, the factors affecting the probability of accepting screening were age, gender and social deprivation. Both strategies detected cases requiring further management or treatment. The biochemical strategy had the lowest cost per case detected.

Discussion: This is the first reported study to systematically evaluate screening for haemochromatosis in primary care, specifically comparing a genetic and biochemical screening strategy. The genetic strategy was no less acceptable than the biochemical strategy and both strategies were feasible and detected cases. It is suggested that in the future genetic factors will be used to predict disease and may be used for screening. Evaluation of these technologies should apply existing methodologies from epidemiology and health services research. Evaluation of screening should be focused on the predictive value of the screening strategy, the effectiveness of diagnosis and treatment and the balance between the costs and benefits of the programme.

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Abbreviations

ALT	alanine aminotransferase
DNA	deoxyribonucleic acid
HAD	Hospital Anxiety and Depression Scale
HHC	Haemochromatosis
IQR	interquartile range
IRE	iron regulatory element
IRP	iron regulatory protein
LFT	liver function tests
NSC	National Screening Committee
PKU	Phenylketonuria
PSA	prostate specific antigen
RNA	ribonucleic acid
SD	standard deviation
STAI	State-Trait Anxiety Inventory
TS	transferrin saturation
WHO	World Health Organisation
YD	compound heterozygote C282Y H63D
YN	heterozygous (carrier) C282Y
YY	homozygous C282Y
DN	heterozygous (carrier) H63D
NN	no mutation

Introduction

Hereditary haemochromatosis (HHC) is a common autosomal recessive inherited disorder of iron metabolism. The carrier frequency is estimated at 1 in 10 of Northern Europeans with a prevalence of the at risk genotype of approximately 1 in 300 (Robson et al., 2000). Excessive iron absorption causes iron overload and deposition in the liver, pancreas, anterior pituitary gland and heart leading to liver cirrhosis, liver cancer, endocrine problems including diabetes and cardiomyopathy resulting in morbidity and mortality in middle life. Early symptoms are non-specific or absent and may often be ignored or misdiagnosed. Treatment by removing excess iron with phlebotomy is effective and if started before irreversible end organ damage restores normal life expectancy (Niederau et al., 1996).

The discovery of the HFE gene in 1996 (Feder et al., 1996) has led to increasing interest in haemochromatosis and has introduced DNA based predisposition testing as a possible tool for diagnosis and screening. The purpose of testing is case identification, since early identification of iron overload and initiation of treatment is considered to be effective. The prevalence of haemochromatosis from studies using biochemical assessments of iron overload and from population studies of the genotype frequencies is estimated to be 1 in 300 in Northern European populations (Hanson et al., 2001). These are overlapping but different groups since not all cases of haemochromatosis are accounted for by the at-risk genotype and not all individuals at genetic risk develop iron overload. This high prevalence makes haemochromatosis a common single gene disorder of Northern Europeans.

Studies examining the presence of symptoms relating to undiagnosed haemochromatosis in family members of diagnosed index cases have indicated that up to 50% of males and 16% of females who have the genetic predisposition may have such symptoms (Bulaj et al., 2000). Although the frequency of the genetic predisposition is the same in males and females, women have a lower incidence of the clinical phenotype. The explanation is probably that women lose iron through physiological blood loss (menstruation, childbirth) until they are post menopausal. The high prevalence of the genetic predisposition, the preventable serious

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consequences of progressive iron overload and the availability of effective treatment have been put forward as arguments for population screening in line with the World Health Organisation criteria (Wilson and Jungner, 1968).

Prior to the identification of the gene it had been suggested that population screening programmes based on transferrin saturation (a marker of iron status) be initiated (Adams et al., 1995; Phatak et al., 1994). Screening by biochemical means will detect iron overload (not necessarily progressive or due to haemochromatosis), and iron deficiency that require further investigation with the attendant cost implications but also potential benefits. If done at one time point and not repeated it misses those individuals with the genetic predisposition who may go on to develop iron overload in the future. Testing for the at-risk genotype is specific, can be done at any age but has the social and ethical implications of a genetic test. Since the identified mutations do not account for disease in all diagnosed patients, testing for the at risk genotype will miss the 10% of cases who do not have the common mutations and the prognostic value of the at-risk genotype in relation to clinically important disease is not known.

The identification of a genetic predisposition has happened alongside developments in the theory and principles of screening. The use of genetic technology is possibly scrutinised more closely than other medical tests and this together with the more stringent criteria that are now applied to the evaluation of screening, has led to a re-evaluation of screening for haemochromatosis.

The 'gold standard' for the evaluation of the effectiveness of any screening programme requires a randomised controlled trial allocating individuals, general practitioners, health care organisations or residents of a geographic area to a screening or non-screening arm. The decision about whether to implement a screening programme should include an assessment of all the costs and benefits associated with the programme. In screening for disease the outcome measure is the risk of complications of the disease that is being screened for i.e. the effect of screening on the natural history of the condition. In antenatal screening the outcome should be informed choice.

Evaluation of screening programmes firstly requires evaluation of all the components of the programme, and before screening trials are planned pilot studies are necessary to inform their design. In considering screening for haemochromatosis, there are many questions that need evaluating before planning a screening trial. Haemochromatosis is a condition that will be unfamiliar to the general population and this will impact on whether individuals accept the offer of screening. In addition there are now two possible methods of screening for haemochromatosis to consider, genotypic and phenotypic (iron overload).These may differ in their uptake, acceptability, feasibility and in the performance of the tests.

There are major questions to answer before a decision about screening for haemochromatosis can be made. There is limited information relating to the natural history and prognosis of the at-risk genotype and/or mild iron overload and the clinical effectiveness of early case identification strategies and treatment. These questions can only be answered with well designed, large scale follow-up studies which identify individuals at an early stage of disease who either have the genetic predisposition or evidence of early iron overload, to answer questions relating to the natural history and prognosis of the condition. This cohort of patients would ideally be identified through screening. In order to design these studies carefully to answer specific research questions, data are needed on the characteristics of the population accepting screening, the uptake of screening, the performance of the different screening and diagnostic tests and the acceptability and feasibility of offering screening.

The following study is designed to compare these outcomes in two strategies of testing for haemochromatosis: genetic testing for the at-risk genotype and biochemical testing for the phenotype (iron overload).

Chapter 1. Haemochromatosis

1.1 Clinical Aspects

1.1.1 History

Trousseau (cited in Sheldon (Sheldon, 1935)) is credited with the first description of a patient with haemochromatosis in 1865, but no specific name was attached to the condition. The next case was described by Troisier in 1871 and his description delineated the classic clinical triad of diabetes, liver cirrhosis and darkening of the skin. The term haemochromatosis was first used by Von Recklinghausen (1889) to describe the post mortem findings in patients who had died from 'bronze diabetes', thus suggesting that iron may be a contributing factor in the development of the condition.

Sheldon reviewed the world literature in 1935 and correctly identified haemochromatosis as an inborn error of iron metabolism (Sheldon, 1934; Sheldon, 1935). He also suggested that there may be a familial component to the disease. The exact pattern of inheritance was debated over the next 40 years, with some family studies suggesting recessive inheritance and some dominant. The observation of a sex difference in the manifestation of haemochromatosis with most cases being observed in males, also added to the debate on the nature of the familial association in the disease.

The demonstration of an excess of HLA A3 alleles in individuals with haemochromatosis as compared to the normal population in 1976 led to the conclusion that haemochromatosis was inherited as an autosomal recessive trait caused by an undefined gene that was tightly linked to the HLA locus on chromosome 6 (Simon et al., 1976). Pseudo dominant inheritance was shown to be caused by inheritance of a disease allele from an unaffected carrier parent in addition to one from an affected parent. Despite this relatively early success for linkage methods for suggesting the chromosomal location of a disease gene it took a further 20 years before the causative gene was identified through classical positional cloning techniques (Feder et al., 1996; Rosenberg et al., 2002).

It has been suggested that there are three distinct periods in the management of haemochromatosis. From 1865 until 1920 prior to the development of insulin it was considered to be an invariably fatal disorder with death occurring through the complications of diabetes soon after diagnosis. Insulin therapy increased life expectancy to an average of three years after diagnosis with principal causes of death being due to liver or cardiac complications. Even at this time however it was noted that there were several documented cases of survival up to twenty years after diagnosis (Finch and Finch, 1955). The third phase of development was when treatment by venesection was initiated in the 1960's and it was realised that early diagnosis and initiation of treatment improved outcome. It could be said that with the advances in understanding of the molecular genetics of haemochromatosis we are now in a fourth stage of development. The focus is currently on more careful consideration of the case definition, diagnostic and management strategies and further research relating to the understanding of iron metabolism and the role of the HFE protein in its absorption and regulation.

1.1.2 Case definition

Central to any discussion of the merits of early case identification and the benefits or otherwise of treatment in haemochromatosis is how a case is defined. Previously a diagnosis of haemochromatosis was made on the clinical and pathological demonstration of a characteristic pattern of iron overload in the absence of other known causes. Diagnosis was established on the basis of a liver biopsy, or quantitative phlebotomy. Quantitative phlebotomy is a direct measurement of the iron that is available for haemoglobin synthesis and patients with haemochromatosis will require more units of blood to be removed to induce anaemia. Liver biopsy and assessment of the hepatic iron index was the 'gold standard' for diagnosis of haemochromatosis. Liver biopsies have a mortality rate ranging between 1:1000 to 1:10000 (Shah et al., 1999).

Since the discovery of the gene there has been much discussion as to whether the case definition should be genotypic i.e. solely based on having the genetic predisposition or phenotypic i.e. showing evidence of iron overload. In recognition that a minority of cases previously regarded as being typical of haemochromatosis lack the mutations

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commonly associated with the disease an expert group has issued a statement suggesting that haemochromatosis be defined phenotypically. Thus the presence of the genetic predisposition is not sufficient for the diagnosis of HHC but it will suggest a susceptibility to developing the phenotype. The definition is as follows; 'hereditary haemochromatosis is an inherited disorder resulting from an inborn error of metabolism which leads to progressive iron loading of the parenchymal cells in the liver, pancreas and heart. In its fully developed stage organ structure and functions are impaired'(Adams et al., 2000a).

1.1.3 Prevalence

The question of the prevalence of haemochromatosis has been addressed by routine mortality data, autopsy studies, screening studies for iron overload and screening studies for the at-risk genotypes.

Mortality data will in theory reflect the frequency of clinically severe complications of haemochromatosis. However this is likely to be an underestimate given that end organ damage leads to other causes of death e.g. coronary heart disease, liver failure etc. which may be unrecognised as being due to haemochromatosis. Without a detailed post-mortem the contribution of haemochromatosis as a cause of death may be missed. Moreover mortality data only reflects fatal cases and provides no evidence of clinical morbidity.

Studies based on post-mortem records may give an indication of the prevalence of significant iron overload in the population having post mortems. This iron overload may or may not have been diagnosed and may or may not have caused morbidity during life. In addition the population having post-mortem examination is not representative of all deaths. Differences in post-mortem rates, the nature of the pathological investigation, true variation in prevalence due to ethnic differences and different populations and changes in diagnostic classification and strategies over time will also affect the prevalence estimates.

If earlier diagnosis and treatment does affect morbidity due to iron overload, deaths attributable to haemochromatosis may fall as most diagnosed cases are treated and

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this too will change prevalence rates estimated from these types of study, although it may be balanced by increased acquisition and reporting of cases.

Table 1.1 summarises three prevalence studies based on routine mortality data or autopsy studies and the prevalence ranges from 3 per 10,000 to 19 per 10,000. There is a wide variation in this prevalence which may be explained by different populations studied, the USA population would be expected to have more people in it from non-Caucasian populations where the prevalence of haemochromatosis is lower.

Table 1.1 Prevalence of haemochromatosis in autopsy studies.

Study		Prevalence
(MacSween and Scott, 1973)	21,565 autopsies in Scotland Haemochromatosis as the diagnosis on death certificate.	19/10,000
(Lindmark and Eriksson, 1985)	8,834 male autopsies in Sweden. Haemochromatosis as diagnosis at P.M.	9/10,000
(Yang et al., 1998)	Analysis of all cause mortality data in the USA Reported cause of death among persons with autopsy.	3/10,000

Routine mortality data from England and Wales by ICD code from the UK Office of National Statistics report 22 deaths from haemochromatosis in males and 8 in females in 2001. These figures have been fairly constant since 1968 (data provided by Dr J Goddard). Haemochromatosis does have an ICD9 and 10 code however although these codes exclude anaemia they are likely to include other disorders of iron metabolism. These numbers are very small; they are based on the underlying cause of

death as reported on death certificates and will be subject to the caveats discussed previously. The rates per million are presented in figure 1.1.

Figure 1.1 Rates per million for deaths from iron overload disorder by year.
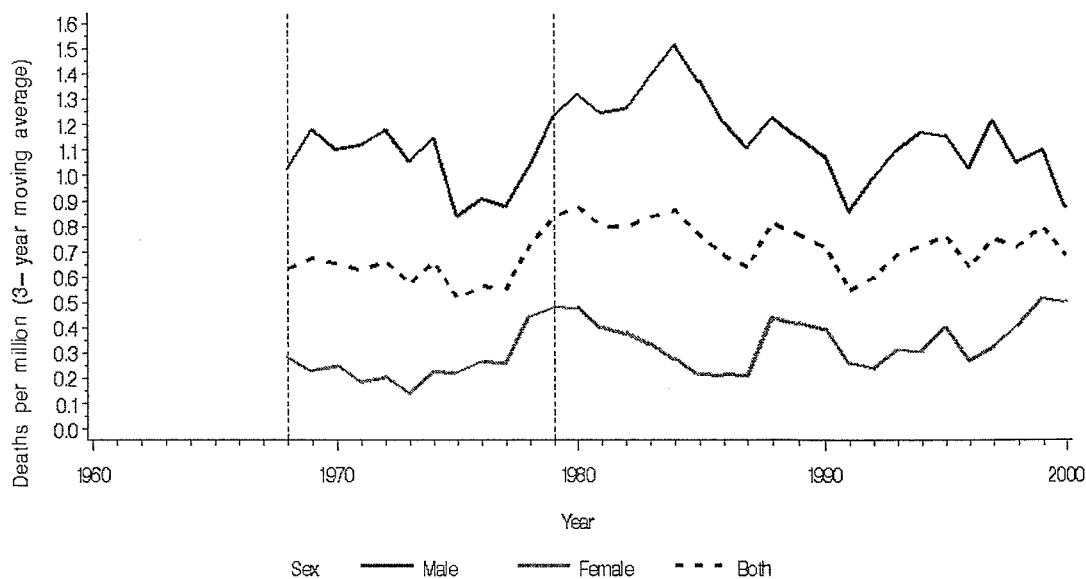
In the biochemical prevalence studies the unadjusted prevalence of iron overload assumed to be due to genetic haemochromatosis in screened populations ranges from 15 to 66 per 10,000 (Table 1.2). Reviews of these studies are complicated by the different populations used e.g. healthy blood donors, patients attending hospital, employees and population samples. The prevalence in these studies is also affected by the age and sex distribution of the population and by the use of different case definitions such as raised biochemical measurements of iron indices or evidence of iron overload on liver biopsy which vary between the studies.

A review of prevalence studies that included studies from a general population, adequate follow up and consistent case definitions estimated an unadjusted prevalence of iron overload of about 25 per 10,000. The prevalence adjusted for compliance and false negatives was estimated to be 50 per 10,000 (Bradley et al., 1996a). This review did not address questions relating to the evaluation of screening programmes. Estimates of prevalence will be complicated by lack of consensus relating to the case definition and the lack of knowledge about the natural history of the genotypes or early iron overload.

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Table 1.2 Prevalence of haemochromatosis in biochemical screening studies.

Study	Setting and population	Number screened	Case definition	Unadjusted prevalence in those tested.
(Edwards et al., 1988)	Blood donors	11065	quantitative phlebotomy or liver biopsy	19/10,000
(Velati et al., 1990)	Blood donors	1301	quantitative phlebotomy or liver biopsy	15/10,000
(Wiggers et al., 1991)	Blood donors	4302	quantitative phlebotomy or liver biopsy	23/10,000
(Bell et al., 1997)	Blood donors	NA	quantitative phlebotomy or liver biopsy	18/10,000
(Jonsson et al., 1991)	Cardiovascular risk factor research clinics	2592	quantitative phlebotomy or liver biopsy	15/10,000
(Baer et al., 1995)	Males presenting for health checkups	3977	quantitative phlebotomy or liver biopsy	20/10,000
(McDonnell et al., 1998)	Persons having health appraisal. Hospital in-patients	18001	raised ferritin and transferrin saturation	42/10,000
(Balan et al., 1994)	Patients having blood tests	12258	quantitative phlebotomy or liver biopsy	30/10,000
(Phatak et al., 1998)	Primary care patients attending clinic	16031	quantitative phlebotomy or liver biopsy	16/10,000
(Stave et al., 1999)	Employees having blood taken for other reasons	1968	raised ferritin and transferrin saturation	15/10,000
(Leggett et al., 1990)	Employees	1967	quantitative phlebotomy or liver biopsy	36/10,000
(Smith et al., 1997)	Employee screening programme stored serum samples	2294	quantitative phlebotomy or liver biopsy	22/10,000
(Niederau et al., 1998)	Employees and primary care patients attending clinic	6039	quantitative phlebotomy or liver biopsy	46/10,000
(McDonnell et al., 1999a)	Employees	1653	raised ferritin and transferrin saturation	40/10,000
(Burt et al., 1998)	Random sample from electoral rolls	1064	raised liver iron stores	28/10,000
(Olynyk et al., 1999)	Randomly selected sample from cohort study	3011	raised ferritin and transferrin saturation	66/10,000
(Asberg et al., 2001)	General population	65,238	raised ferritin and transferrin saturation	41/10,000

Since the identification of the gene several studies have assessed the gene frequencies in varying populations. There are two common mutations which will be discussed in more detail in section 1.2.8. Homozygosity for the C282Y mutation has a prevalence in diagnosed patients of greater than 75% (Hanson et al., 2001) and up to 90% in a UK population (The UK haemochromatosis consortium, 1997). Compound heterozygosity for the C282Y mutation in conjunction with the H63D mutations is thought to account for a further 5% of patients. The prevalence of these two genotypes from a pooled analysis is presented in table 1.3.

Table 1.3 HFE frequencies in general population by geographic area (Hanson et al., 2001)

	Genotype	
Area	Homozygous C282Y	Compound heterozygote C282Y/H63D
Europe N=6203	0.4%	1.8%
North America N=3752	0.5%	2.1%
Global N=11668	0.4%	1.6%

Since the H63D mutation frequency is relatively high in the Northern European population, compound heterozygosity for the two mutations occurs more frequently than homozygosity for the C282Y mutation. This is in contrast to the genotype frequencies in diagnosed cases where C282Y predominates. The predictive value of the two at risk genotypes is therefore very different. Although the H63D mutation does increase transferrin saturation and ferritin levels in population samples, there is a suggestion that compound heterozygosity is not sufficient for the development of clinically diagnosed haemochromatosis in isolation, but does confer excess risk in the presence of other contributory factors such as alcoholic liver disease (Britton and Bacon, 2002; Fletcher et al., 2002; Gochee et al., 2002)

The prevalence of iron overload in adults assumed to be due to genetic haemochromatosis is estimated and widely accepted to be approximately 30 per

10,000 or 1 in 300 individuals from a Northern European population. The prevalence of the at-risk genotype is also about 1 in 300. As stated before these are not completely overlapping populations, since despite the majority of individuals with haemochromatosis possessing the at-risk genotype, the proportion of people with the at-risk genotype who will develop haemochromatosis is not known, but penetrance is unlikely to be 100%.

This prevalence is much higher than the prevalence of diagnosed haemochromatosis ascertained by autopsy studies or routine death certification data, leaving unanswered questions about the precise risk of severe disease in this condition.

1.1.4 Pathophysiology

A consensus expert document (Adams et al., 2000a) outlines the main signs, symptoms and clinical presentation of haemochromatosis as follows:

Liver

In reports of case series the liver is one of the most common organs to be affected and hepatomegaly is one of the most frequent findings at clinical presentation (Adams and Valberg, 1996). It is assumed that progressive iron overload leads to liver fibrosis and ultimately cirrhosis. The percentage of patients who are reported to have cirrhosis at the time of presentation varies but there is a suggestion that it is reducing over time, possibly because of earlier referral and diagnosis. In one case series this was from 95% to 70% over 40 years (Milman et al., 2001), in another from 80% to 40 % (Niederau et al., 1996). The Niederau study reports experience from one referral centre and the Milman study is a retrospective cohort study in a defined geographical area. It may be that the reduction in cirrhosis in referred cases reflects the referral of a group of patients who may not have gone on to develop cirrhosis over time so any conclusions must be tentative. Using comparisons with population mortality data both of these studies indicate that diagnosis and the initiation of phlebotomy therapy before liver disease is established, significantly increases life expectancy. The presence of cirrhosis at diagnosis was predictive of poorer survival in these studies and this was also found in a more recent case series (Adams et al., 2000c).

One explanation is that cirrhosis contributes to the development of hepatocellular carcinoma. In Niederau's case series 19 out of a total of 69 deaths were from primary liver cancer (28% 95% confidence interval 18-39). The ratio of observed to expected deaths (expected deaths derived from standardised rates for the population in the same region of Germany) was 118. In addition two patients who were still alive at the end of the study had biopsy proven hepatocellular carcinoma. This huge ratio is partly explained by the very low prior probability of hepatocellular carcinoma in the general population. In Milman's study standardised mortality rates are not reported separately for hepatocellular carcinoma, however the observed number of deaths from all cancer was 48, 32 of which were from hepatocellular carcinoma (23% of the patient sample). The standardised mortality ratio for cancer was 4.96 (95% CI 3.66 -6.58). These two studies appear to show a disparity in the standardised mortality rates. This may in part be explained by the fact that the Niederau case series is a series of patients who have been referred to a specialist hepatology centre and a smaller number detected through family testing. This referral pattern would tend to bias the population sample towards those individuals who present with signs of liver disease. The Milman study was a nationwide survey of the population of Denmark and included a matched control population. Hepatocellular carcinoma was still a significant cause of death in the patient sample and the standardised mortality ratio in this study is probably an underestimate since it includes deaths from all cancers not solely from liver cancer. Both studies indicate that hepatocellular carcinoma usually occurred in the presence of cirrhosis.

A case note review of patients with hepatocellular carcinoma presenting to a single referral centre in America indicated that 33 out of 314 (11%) of patients had a diagnosis of haemochromatosis. It was noted that the sample included 18 Asian patients, who would be unlikely to have haemochromatosis and more likely to have hepatitis B as the underlying causative pathology (Cogswell et al., 1998). A second study cited in the same review of the evidence for screening for haemochromatosis found 15% of 60 patients having surgical resection for hepatocellular carcinoma in a centre in France had haemochromatosis. Studies of patients having liver transplants suggest that

undiagnosed haemochromatosis is not infrequent, that the occurrence of unsuspected hepatocellular carcinoma in this group is increased, and that life expectancy post transplant for patients with undiagnosed haemochromatosis is significantly reduced (Kowdley et al., 1995; Kilpe et al., 1993).

In addition to hepatocellular carcinoma the fibrosis and cirrhosis caused by progressive iron overload will be a cause of progressive liver disease with the attendant morbidity and mortality.

Joints

Characteristically the second and third metacarpal joints are affected but all joints may be involved, particularly the wrists ankles and knees. The arthropathy presents with bony swellings of the joints and may resemble osteoarthritis. The pathophysiology of the arthropathy of haemochromatosis is not characterised (von Kempis, 2001), although there is evidence of iron deposition in the articular cartilage of patients with haemochromatosis (Schumacher et al., 1998). Arthropathies are found in 40 to 75 percent of patients (Niederau et al., 1994; Milman et al., 2001) but the occurrence may be overestimated since arthritis is a common symptom, estimates are usually based on patient information and the actual site and severity of the arthropathy is often not characterised. In a survey of 2851 patients self reported doctor diagnosed arthritis was present in 10 percent of patients although 43% reported joint pain. Comparison with population norms showed that diagnosed arthritis was more frequent in patients with haemochromatosis under 60 than in the general population of the same age (McDonnell et al., 1999b). Arthritis as a symptom of haemochromatosis appears to be associated with a reduced quality of life (Adams and Speechley, 1996) and unfortunately is one of the symptoms that is probably not improved by venesection therapy and may in fact deteriorate in some patients (McDonnell et al., 1999b; Niederau et al., 1996).

Endocrine

Diabetes mellitus is the major endocrine disorder associated with HHC. The mechanisms responsible are still obscure, but it is thought to be caused by destruction of pancreatic β cells through iron promoted formation of free

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radicals, which leads to insulin resistance. In Niederau's case series 27% of patients had insulin dependent diabetes at diagnosis and 20% were non insulin dependent. The occurrence of diabetes was significantly associated with the presence of liver cirrhosis. In another retrospective case series the percentage of patients with insulin dependent diabetes was between 24% and 50% with a higher percentage of patients diagnosed during the period 1948 to 1968 as compared to 1980 to 1985 (Milman et al., 2001). The same issues regarding the prevalence of cirrhosis at diagnosis apply to the changing prevalence of diabetes over time. Namely, that changing testing and referral patterns may result in the diagnosis and treatment of a group of patients who would not develop serious disease, rather than reducing the incidence of serious disease by early treatment. In a postal survey of patients physician diagnosed diabetes was present in 7.2% of patients but also in 7.9% of a general population sample (in whom there was no information regarding age or gender or if there was a diagnosis of haemochromatosis) with the diabetes being more commonly diagnosed in patients with haemochromatosis under the age of 60 compared to the general population, suggesting that haemochromatosis may lead to an earlier onset of diabetes (McDonnell et al., 1999b).

Hypogonadism also occurs and is caused primarily by gonadotropin deficiency resulting from iron deposition in the pituitary or hypothalamus. Other endocrine disorders including impairment of the thyroid, parathyroid, or adrenal glands have been reported but are rarely seen.

Heart

Cardiac manifestations of haemochromatosis are thought to be associated with iron deposition in the myocardium. Congestive heart failure has been seen in 2 to 35 percent and arrhythmias are present in 7 to 36 percent of HHC patients in various case series (Witte et al., 1996). ECG abnormalities have been reported to be more common in patients than in controls and it is suggested that abnormalities of cardiac conduction precede the development of cardiomyopathy and may be reversible by treatment (Cecchetti et al., 1991). It is not clear from the reported studies how thoroughly patients have been

investigated in terms of cardiac function and this may account for the variability in reporting of these signs and symptoms and cardiac findings.

In summary: discussion of the prevalence of the clinical presentation and pathophysiology in haemochromatosis is complicated by the lack of studies including control groups. The non-specific nature of the early signs and symptoms of haemochromatosis leads to problems in diagnosis and since many of these early signs and symptoms are common it may be unwise to attribute all the symptoms that are complained of to a diagnosis of haemochromatosis.

Early reports of case series such as a report of a series of 30 patients in the 1930's (prior to the initiation of phlebotomy therapy but after insulin therapy had been introduced) suggested that the chief symptoms on diagnosis in 50% of patients were fatigue and symptoms of diabetes. The primary physical finding was skin pigmentation and enlarged liver (Butt and Wilder, 1938).

The authors comment on the increased survival in patients with the initiation of insulin therapy. Prior to the development of insulin haemochromatosis was considered to be a fatal disease often undiagnosed before death (Sheldon, 1935). The predominance of fatigue as an early symptom is confirmed in other case series together with joint pain, abdominal pain or sexual dysfunction (Niederau et al., 1994; Haddow and Ledue, 1994). Clinical findings include abnormal liver function tests, diabetes and ECG abnormalities.

As was noted in the original description of the disease the presence of symptoms is more common in men than women, the assumption being that women lose iron through physiological blood loss (menstruation, childbirth etc.). The onset of symptoms also appears to be later in women than in men. A comparison of male and female patients with diagnosed haemochromatosis however suggests that in women with diagnosed haemochromatosis full phenotypic expression can be seen (Moirand et al., 2000). Asymptomatic persons identified through family screening have been identified with possible life threatening complications such as cirrhosis, indicating that clinically important complications may also be present in the absence of significant symptoms (Bulaj et al., 2000).

Phenotypic expression of haemochromatosis, which is variable, appears to depend on a complex interplay of the status of the HFE gene, other genetic factors, age, sex, and such environmental influences as dietary iron, the extent of iron losses from other processes, and the presence of other diseases or toxins (e.g. alcohol).

1.1.5 Natural history and impact of treatment

Some information on the burden of disease may be gained from routine hospitalisation data. Routine data from the UK is presented in table 1.4.

Table 1.4 Hospital episode statistics for haemochromatosis in the U.K. (Department of Health, 2003b).

Year	Finished Consultant Episodes	Male	Mean Age	Day case
2001/ 2002	10,627	74%	55	92%
2000/ 2001	8,839	76%	55	89%
1999/ 2000	7627	76%	54	89%
1998/ 1999	6,890	75%	54	87%

These data would suggest that the majority of hospitalisations relating to haemochromatosis are waiting list day case admissions which may relate to venesection. However these data will overestimate the number of patients since one patient having weekly venesections will have a number of day case admissions or finished consultant episodes. These data are therefore of little use in estimating the burden of disease for a patient with haemochromatosis. The age/sex profile is as would be suggested by what is known about the condition i.e. that it affects men more than women and the age of onset is in middle life. There appears to be an increasing trend of admissions and finished consultant episodes, which may reflect increasing diagnosis and treatment or a change in treatment strategy to more frequent treatment.

A review of the national discharge survey and census in America estimated that the rate of haemochromatosis associated hospitalisations in the period 1979 to 1997 was

2.3 per 100,000 individuals (Brown et al., 2001). Haemochromatosis will be predominantly treated on an outpatient basis and may be treated through blood donation services; both will contribute to an underestimation of the hospitalisation rates in the condition.

As in the discussion relating to symptoms it is difficult to assess the natural history in haemochromatosis since there is a lack of studies with control groups. In addition since there is an accepted therapy for haemochromatosis it would be unethical to conduct a standard randomised controlled trial of treatment or follow up an untreated cohort. Reports of case series from referral centres and studies of relatives of affected patients will overestimate morbidity whereas screening studies carried out in selected healthy populations such as blood donors will tend to underestimate morbidity. Screening studies carried out in a general population would give an estimate of morbidity in previously undiagnosed individuals.

In 1996 Bradley et al. reported their analysis of prevalence studies that screened general populations and included information on the presence of clinical symptoms in persons identified with iron overload who complied with further investigations (Bradley et al., 1996a). Fifty percent of the 28 men and 44% of nine women identified as cases of haemochromatosis had at least one clinical manifestation, with asymptomatic fibrosis of the liver accounting for this in 21% of men and 11% of women. However the numbers were small and the confidence intervals of these estimates must be wide. In a pooled analysis of homozygous (based on HLA typing) family members, 73% (95% CI 52-58) of males over 40 and 44% (95% CI 22-69) of females over the age of 40 had one or more of the following: liver fibrosis, cirrhosis or hepatomegaly, cardiomyopathy, arthropathy, diabetes, abdominal pain (Bradley et al., 1996b). These pooled analyses were again complicated by the fact that there were no control groups. However it would not be ethical to test an age and sex matched control group for the presence of asymptomatic fibrosis since this would necessitate a liver biopsy which would pose unacceptable risks in an asymptomatic population.

The evidence regarding the predictive value of the at-risk genotypes in an asymptomatic population is also unclear. A study which genotyped autopsy specimens from patients diagnosed with liver cancer or cirrhosis and compared the frequency of

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genotypes with a population sample derived from the same geographic population found that haemochromatosis alleles were over-represented in the both diagnostic groups. In the hepatocellular carcinoma group this was a statistically significant association despite having a small sample size (thirty four liver cancer specimens). The frequency of C282Y homozygosity in the liver cancer specimens was 10%, whereas in the normal population it was estimated at 0.4%. The authors were attempting to answer questions relating to the risk of developing hepatocellular carcinoma in a person with the homozygous genotype and conclude that it is a rare event. (Willis et al., 2000). However it is known that there is non-penetrance and homozygosity for the C282Y mutation is not a diagnostic criterion for haemochromatosis. In addition 50% of the samples were not genotyped due to technical problems. This added to problems of ascertainment of cases of hepatocellular carcinoma and cirrhosis over the time period studied. This study does not provide data to answer the question of the risk of hepatocellular carcinoma in a person with haemochromatosis, but in common with the other studies discussed in section 1.1.4 supports the finding that haemochromatosis is a significant risk factor for the development of hepatocellular carcinoma.

A recent report of a prospective genetic screening study comparing symptoms in people who were subsequently found to be homozygous with those who were not, suggests a low penetrance of 1% for the severe manifestation of haemochromatosis (liver disease, diabetes and skin pigmentation) (Beutler et al., 2002a). The conclusions of this study have been extensively criticised and robustly defended (Poullis et al., 2002; Cox et al., 2002; Allen et al., 2002; Beutler et al., 2002b). The low penetrance suggested in the study was of the severe phenotype, however greater than 50% of the genotype positive patients did have a raised ferritin and were more likely to report liver disorders or have raised aspartate aminotransferase (a marker for liver fibrosis) compared to those without the at-risk genotype. Criticisms have also been levelled at the nature of the population studied. This was patients of a health maintenance organisation in California and care has to be taken in making assumptions about the representativeness of such a population since it may include a greater proportion of healthy individuals in employment. In addition previously diagnosed HHC patients who were identified through a biochemical screening programme were not included.

A community based study in Australia identified 16 homozygotes and compared their iron parameters to historical samples collected four years previously. Four of the sixteen had already been diagnosed clinically with the condition. Of the remaining 12 patients, 7 had raised ferritin levels (5 males and 2 females) of those, ferritin levels increased over the four years in 6 suggesting that 50% of previously undiagnosed homozygotes had increasing body iron stores. The numbers are small, however there is a suggestion that there was an excess of males in the group with rising ferritin levels as would be expected (Olynyk et al., 1999).

The classic triad of liver disease, diabetes and skin bronzing occurs in a minority of patients at presentation: 38 out of 142 cirrhotic patients and 5 out of 109 non-cirrhotic patients in one case series (Niederau et al., 1996). However this presentation represents the severe complications of HHC. It also represents a small subset of patients with advanced haemochromatosis and could be said to be a failure of early diagnosis

In summary haemochromatosis presenting clinically undoubtedly has significant morbidity and mortality with the patient's quality of life being adversely affected. However the progress of disease in persons diagnosed prior to the development of signs and symptoms is less clear. In addition there is a lack of evidence concerning the contribution of untreated iron overload or clinically diagnosed haemochromatosis to disease in the population i.e. what the public health implications are.

1.1.6 Treatment
Venesection as a therapy for haemochromatosis is considered to be safe, inexpensive and effective. Practice guidelines suggest removal of iron by weekly or twice weekly phlebotomy until the patient is marginally iron deficient. Subsequently the frequency of phlebotomy is adjusted according to serum transferrin saturation and serum ferritin (Adams et al., 2000a).

Evidence for the benefits of treatment comes from observational studies. These include an early report comparing a small series of treated persons with patients who were untreated either because they refused treatment or because they were diagnosed prior to it being available (Bomford and Williams, 1976a). This series using current

and historical controls indicated a benefit of treatment with prolonged survival and reduction in signs and symptoms in the treated group. Since there was no random allocation of treatment this may be confounded by systematic differences between the treated and untreated groups.

The most widely reported evidence for the benefits of treatment come from a cohort of patients followed in a German centre (Niederau et al., 1996). As mentioned previously this series includes patients who are referred to this specialist centre together with family members identified through family screening. Analysis of the outcome in these patients suggests that if treatment is initiated prior to the development of irreversible cirrhosis, diabetes or cardiomyopathy then mortality in the treated group is no different from mortality in the population from which they are derived making comparisons using population mortality data. However, although this study uses age and sex matched data as a control, there is no concurrent control group and it cannot be assumed that 100% of the group who were diagnosed without symptoms would have developed serious complications of the condition. Similar findings of the benefit of treatment have been reported from other studies (Adams et al., 2000c; Milman et al., 2001). It would now be unethical to conduct a randomised controlled trial of treatment versus non-treatment since the evidence is strongly suggestive of the benefit of treatment in individuals diagnosed clinically and patients report symptomatic improvement.

In a postal survey of diagnosed patients 86% reported that some or all of their symptoms improved with therapy (McDonnell et al., 1999b). In patients with established iron overload and symptomatic disease, liver function, weakness, fatigue, loss of libido, cardiomyopathy and skin pigmentation usually improve (Barton et al., 1998). As discussed previously response to treatment for arthritis is variable. Removal of excess iron does not reverse cirrhosis or diabetes but it can be stabilised and insulin requirements reduced (Bomford and Williams, 1976b; Niederau et al., 1996).

Although venesection as a therapy for preventing the complications of haemochromatosis is considered to be simple the patient perspective has not been evaluated. One anecdotal report suggests that the adverse effects of treatment may not be as trivial as is usually assumed (Seamark and Hutchinson, 2000a; Seamark and

Hutchinson, 2000b). In a postal survey 12% of patients expressed a negative attitude towards phlebotomy citing problems with venous access, the time involved and also dissatisfaction that the blood was discarded (McDonnell et al., 1999b).

Although the evidence supports the benefit of treatment by venesection in haemochromatosis there are still unanswered questions. These include questions about who benefits from treatment-do all people with iron overload require venesection, are current treatment protocols being adequately followed, when to start treatment and how to identify those that need treatment before progression to advanced disease is irreversible.

1.2 Iron Metabolism

In order to understand the pathophysiology of haemochromatosis it is necessary to provide an overview of what is understood about iron metabolism generally.

Iron is vital for all living organisms as it is an essential component of a wide variety of metabolic reactions including transport of oxygen, DNA synthesis and electron transport. However iron concentrations in the tissue need to be tightly regulated since excessive iron is toxic as a result of the formation of free radicals. The control of iron uptake and storage is therefore complex.

1.2.1 Intracellular regulation of iron uptake and storage

The majority of total body iron (60-70%) is present in haemoglobin in the erythrocyte pool, another 10% is present in the form of myoglobins, cytochromes and iron-containing enzymes and in a healthy individual, the remaining storage iron is sequestered by ferritin and haemosiderin in the liver, spleen and bone marrow. There is a constant turnover of iron for haemoglobin synthesis by erythroid precursor cells in the bone marrow; the majority of iron for this is recovered from the destruction of red blood cells. Iron is transported in the blood tightly bound to transferrin and although this is less than 1% of the total body iron store, because of its high turnover it is the most significant body iron pool (Brock et al., 1994; Lieu et al., 2001; Worwood, 1999; Andrews, 1999).

In addition to its role in cellular processes such as DNA and RNA synthesis, electron transport, cellular respiration, proliferation and differentiation and as a key component of many cellular enzymes, iron also maintains cellular iron homeostasis. It does this through the regulation of gene expression at the posttranscriptional level.

A constant balance between uptake, transport, utilisation and storage of iron is needed to maintain cellular iron homeostasis. This is mediated through iron regulatory proteins (IRP) 1 and 2 and their interaction with iron responsive elements (IRE) encoded in the transcripts of genes regulating iron metabolism. In conditions of iron deficiency iron regulatory proteins bind to the iron-responsive element of ferritin messenger RNA inhibiting translation. In addition, IRP's bind to the IRE on

transferrin receptor 1 messenger RNA, increasing its stability and up regulating translation. The net result being increased expression of the transferrin receptor at the cell surface with a concomitant increased uptake of iron transferrin complexes. The decreased expression of ferritin down regulates the uptake of iron into the storage pool at the cellular level, further enhancing the circulating iron pool. The reverse happens when iron is plentiful with down regulation of transferrin receptor 1 and up regulation of ferritin (Harford et al., 1994).

The iron regulatory proteins act at the level of the individual cell. Iron homeostasis is also maintained at the level of the whole organism. This tight regulation depends upon the constant movement of iron bound to transferrin in the plasma, between the functional iron pool and the storage iron pool. Because of its low solubility iron is not excreted, although iron is lost through menstruation, other blood loss and desquamation of epithelial cells from the gastrointestinal and urogenital systems, and the skin. The primary level at which body iron content is controlled is by variation in the amount of iron absorbed from the diet at the level of the small intestine.

1.2.3 Absorption and regulation of iron.

Sources of iron

Iron in food exists in two main forms

- heme iron from meat as part of haemoglobin and myoglobin
- non-heme iron from cereals vegetables and other foods

These two forms are absorbed by different pathways and with different degrees of efficiency depending upon chemical form, other components of the diet and the level of iron stores (Hallberg, 1981). Twenty to 30% of available heme iron is absorbed and this is relatively unaffected by other factors.

However the absorption of non-heme iron can be affected by other dietary factors which can enhance or inhibit absorption (British Nutrition Foundation Task Force, 1995). These other factors include citric and ascorbic acid which increase iron

absorption and chelating substance such as polyphenols which are present in tea and some cereals can decrease absorption.

The amount of iron in food varies considerably and the most important sources are those sources which are not only rich in iron but are eaten frequently and from which iron is easily absorbed. For example liver and shellfish are rich sources of iron but may form a small part of most individual's diets in the UK where the major sources of iron are fortified cereals, meats and meat products and vegetables.

Absorption of iron occurs mainly in the small intestine, at the villous tips of the duodenum and jejunum. Uptake of iron occurs across the brush border of the small intestine and release of iron into the circulation occurs across the basolateral membrane of gut epithelial cells. It is at these sites that iron absorption is regulated. Recent work has started to elucidate the mechanisms involved (Reviewed in (Andrews, 1999; Andrews, 2000; Lieu et al., 2001)).

The luminal surface of the enterocytes in the small intestine contains no transferrin receptors and therefore the transferrin-transferrin receptor pathway is not involved in the absorption of iron across the apical membrane. Absorption of non-heme iron after being reduced to its ferrous state is mediated by a transporter called DMT1 or Nramp2. Heme iron is processed in the gut lumen and enters the enterocytes intact. Intracellular iron is either stored within the cell as ferritin or transported across the basolateral membrane by ferroportin1 into the plasma. Ferroportin1 is thought to function together with the proteins hephaestin and ceruloplasmin, the exact mechanism of action is not yet understood (Figure1.1).

A recently discovered protein, hepcidin has been shown to play a role in regulating the absorption of iron in the enterocytes being up regulated and inhibiting absorption in conditions of iron overload, and down regulated in conditions of iron deficiency facilitating absorption. Again the mechanism of action is not known (Nicolas et al., 2002)

Figure 1.2 Absorption of iron in the gut
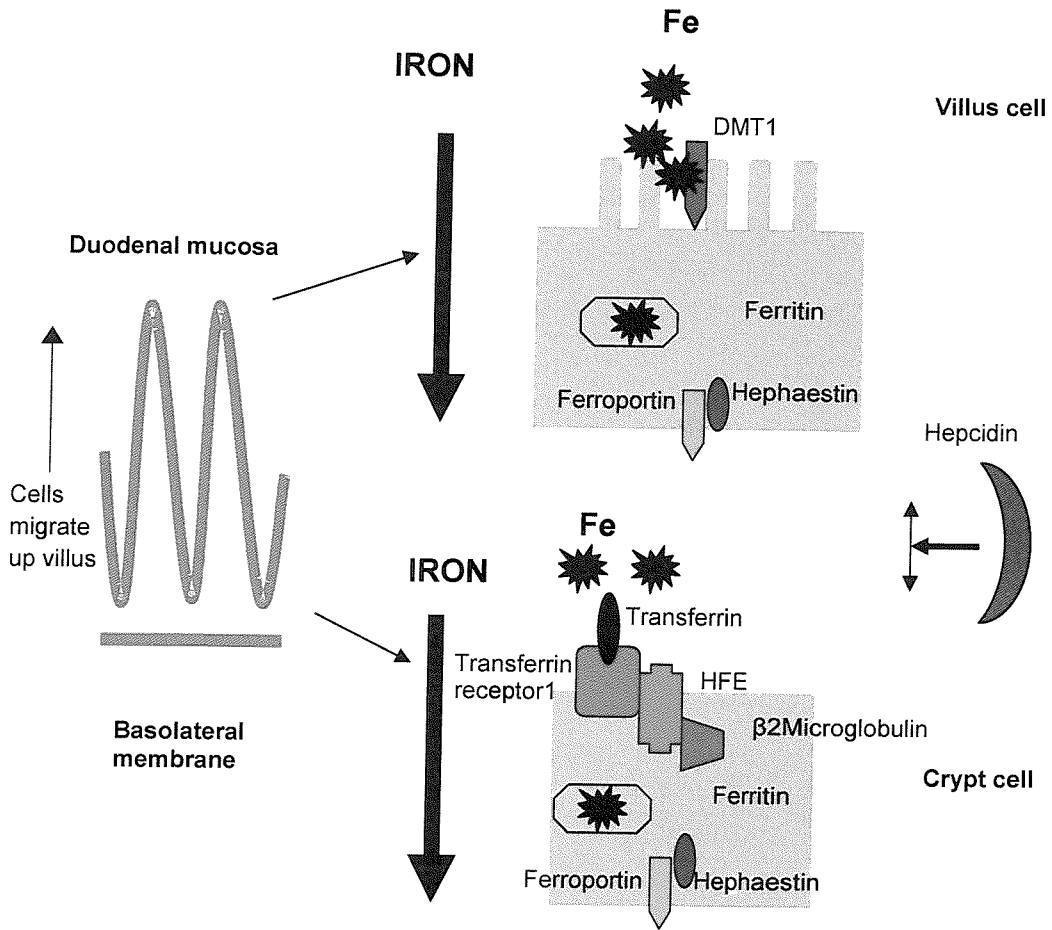
Intestinal iron absorption is regulated in at least three ways.

1. The concept of mucosal block was first described in the 1940's (Hahn et al., 1943). The hypothesis is that ferritin in the intestinal mucosa blocks the transfer of unwanted iron. Administration of iron orally results in absorption of iron into the gut epithelial cells, which is sequestered by ferritin. Until this iron is transported out of the epithelial cells, the absorption of more iron is temporarily blocked. The mucosal block down regulates absorption of iron even in the presence of iron deficiency (Andrews, 1999). Although mechanisms regulating absorption of iron have not been adequately tested in longitudinal studies an inverse relationship between ferritin and absorption of iron has been demonstrated after iron supplementation (Hunt and Roughead, 2000; Roughead and Hunt, 2000).

2. A second regulatory mechanism is termed the 'stores regulator' (Finch, 1994). It senses total body iron stores and regulates absorption by a small factor of two to three in iron deficient states compared to iron replete states. The mechanism responsible for this is unknown. The primary defect in hereditary haemochromatosis is an inability to control the absorption of iron that occurs at the level of the crypt cells of the duodenum. The role of the HFE protein and putative mechanisms will be discussed in section (1.2.8).

It is possible that a recently identified protein, hepcidin, may have a function (Bridle et al., 2003; Gehrke et al., 2003; Nicolas et al., 2002; Roetto et al., 2003). Hepcidin is produced in the liver and levels appear to be responsive to inflammation, anaemia, hypoxia and levels of iron. When hepcidin levels increase iron absorption in macrophages and intestinal enterocytes decreases and in the opposite situation iron absorption is increased.

3. The third regulatory mechanism does not respond to cellular iron levels but is a response to the requirements for erythropoiesis. The erythropoietic regulator has a greater capacity to increase iron absorption than the stores regulator (Finch, 1994). The mechanism for this is not yet fully understood but hepcidin may also play a role.

1.2.4 Anaemia

Iron absorption is up regulated in several anaemic states including iron deficiency anaemia. These include the thalassaemias, but not sickle cell anaemia. Although haemochromatosis is a disorder of iron overload, any screening programme utilising biochemical assessments of iron status will detect cases of low iron indices in addition to cases of raised iron.

Iron deficiency anaemia is an important public health problem (Beard, 2001; British Nutrition Foundation Task Force, 1995) particularly in children and women of reproductive age. Iron deficiency will result from any condition where dietary intake does not meet the body's demand. Therefore anaemia can result from insufficient dietary availability of iron, defects of absorption, increased blood loss or increased requirements of the body for example during periods of rapid growth. Anaemia of chronic disease is not related to an imbalance between availability and utilisation of iron and the pathophysiology is not yet understood. It may have evolved as a mechanism of defence against microbial infection since withholding of iron from pathogens may inhibit replication and therefore attenuate infections. Again it is possible that hepcidin plays a role.

Elucidation of the biochemical pathways and genes disrupted in rare congenital defects of iron metabolism causing various types of anaemia and iron overload have contributed greatly to the understanding of normal iron metabolism (Lieu et al., 2001).

1.2.5 Iron overload

Iron overload usually presents in one of two characteristic patterns. In situations where the excess iron results from increased turn over of erythrocytes, for example acquired transfusional iron overload, iron is deposited in the reticuloendothelial macrophages first before spilling over into the parenchymal cells. In cases where erythropoiesis is normal but the plasma iron content exceeds the binding capacity of transferrin, iron is deposited in the parenchymal cells of the liver, heart and a subgroup of endocrine tissues. Parenchymal iron deposition leads to fibrosis of the liver and subsequent organ damage. Hereditary haemochromatosis is a cause of primary iron overload.

Iron overload can result as a secondary consequence of other conditions such as chronic hepatitis C, alcohol abuse and as mentioned above from blood transfusions. The liver is the major organ for the storage of iron therefore any disorder that disturbs the liver's ability to function may also affect the ability of the liver to appropriately sequester and store iron.

1.2.6 Laboratory testing for iron

Serum iron, total iron binding capacity and transferrin saturation

Measurement of serum iron alone is of little clinical use since there is considerable variation from hour to hour in normal individuals. More information is obtained by measuring serum iron concentration and total iron binding capacity (serum iron plus unbound iron binding capacity) as a surrogate for percentage saturation of transferrin. Problems with standardisation of these assays are recognised (McCullen et al., 2000; Worwood, 1997).Transferrin may also be measured by immunological methods and transferrin saturation calculated directly. Transferrin saturation (TS) is considered the 'gold standard' for assessment of iron overload; however there remain issues relating to test standards and quality control whichever technique is used. TS can also be decreased in inflammatory states, can be increased by alcohol consumption and can be artefactually changed by recent ingestion of iron or vitamins.

Serum ferritin

Serum ferritin is considered to correlate with the total amount of storage iron in normal individuals. Iron overload is correlated with a high serum ferritin however serum ferritin may be high in other forms of liver disease, cancer, infection, inflammation and chronic disease (Worwood, 1997).

All the above biochemical measures are non-specific markers of iron overload.

Quantitative phlebotomy

This is used to measure iron stores. It provides a direct measurement of the amount of iron available for haemoglobin synthesis. Blood is removed weekly and after a number of venesections the patient is unable to maintain his or her normal haemoglobin level. At this point it is assumed that the available iron

stores have been used and the amount of iron removed can be calculated. Although there is no 'gold standard' against which this can be evaluated. Individuals with normal iron stores become iron deficient after the removal of approximately 1.5-2g of iron i.e. four 500ml units of blood (Worwood, 1997). Individuals with iron overload will require more venesections to deplete their storage iron (Powell et al., 1994).

Liver biopsy
Liver biopsy is the definitive test for a diagnosis of haemochromatosis and allows histochemical estimation of tissue iron, assessment of the extent of fibrosis or cirrhosis and chemical measurement of hepatic iron concentration. The degree of stainable liver iron is usually graded and the consensus is that grades 0-1 are normal; grades 2-4 represent increased parenchymal iron stores. Iron deposition in the liver may be increased in various forms of liver disease including alcoholic cirrhosis.

It has been accepted that a hepatic iron index (hepatic iron concentration divided by age) greater than 1.9 discriminates between hepatic iron overload caused by hereditary haemochromatosis and that caused by other liver diseases. This is based on the concept that iron overload in haemochromatosis increases with age, but is stable in other chronic liver diseases. A hepatic iron index greater than 1.9 was considered to be the 'gold standard' test for haemochromatosis (Powell et al., 1994; Kowdley et al., 1997). Careful evaluation suggests that while it is a useful test particularly in patients without cirrhosis it should be evaluated in conjunction with other clinical information and genetic analysis (Chalasani and Gitlin, 1998; Adams et al., 1997).

1.2.7 Iron metabolism in Haemochromatosis
The discovery of the gene associated with hereditary haemochromatosis in 1996 (Feder et al., 1996) has facilitated understanding of the basic mechanisms underpinning the development of haemochromatosis as well as pushing forward the understanding of iron metabolism in general. Since the discovery of the HFE gene a number of other genes have been discovered which also cause haemochromatosis. For completeness sake these are summarised in table 1.5, together with a summary of the differences in the phenotype from classical haemochromatosis

Table 1.5 Non HFE related haemochromatosis.

		Chromosomal location	Inheritance	Gene	Protein	Differences in phenotype from classical haemochromatosis	Reference
Classical haemochromatosis	HFE	6p21.3	Autosomal recessive	HFE	hfe		(Feder et al., 1996)
Juvenile haemochromatosis	HFE2A	1q21	Autosomal recessive	?	?	more severe earlier onset greater cardiac and endocrine manifestations equal prevalence in males and females	(Roetto et al., 1999)
Juvenile haemochromatosis	HFE2B	19q13	Autosomal recessive	HAMP	hepcidin antimicrobial peptide		(Roetto et al., 2003)
	HFE3	7q22	Autosomal recessive	TFR2	transferrin receptor 2		(Camaschella et al., 2000)
	HFE4	2q22	Autosomal dominant	SLC11A3 (IREG1)	ferroportin (iron regulated transporter1)	early accumulation of iron in reticuloendothelial cells marked increase in ferritin prior to raised transferrin saturation .	(Njajou et al., 2001)

1.2.8 HFE gene

In 1996 Feder et al identified a gene in which two missense mutations accounted for 88% of the affected probands in their study .This gene previously called HLA-H is now called HFE. Further prevalence studies have confirmed the relationship between the two common mutations and haemochromatosis. These mutations are a G to A transition at nucleotide 845 on the HFE gene causing aspartate to substitute for histidine at position 282 on the HFE protein (C282Y) and a G to C transition at nucleotide 187 causing an aspartate to histidine substitution at position 63 in the HFE protein (H63D) (Figure 1.2).

The majority of patients are homozygous for the C282Y mutation, with a smaller minority compound heterozygotes for both mutations (Beutler et al., 1996; The UK haemochromatosis consortium, 1997; Feder et al., 1996; Jazwinska et al., 1996). In a recent review of genotyping studies in clinically affected probands the frequency of homozygosity for C282Y ranged from 52 per cent to 100 percent with approximately 5% of cases being compound heterozygotes for the two mutations (Hanson et al., 2001). This review included studies that were from ethnically diverse populations some of whom would not be expected to have a high frequency of the C282Y mutation.

Figure 1.3 Chromosome, HFE gene and HFE protein (figure provided by M Gomez)
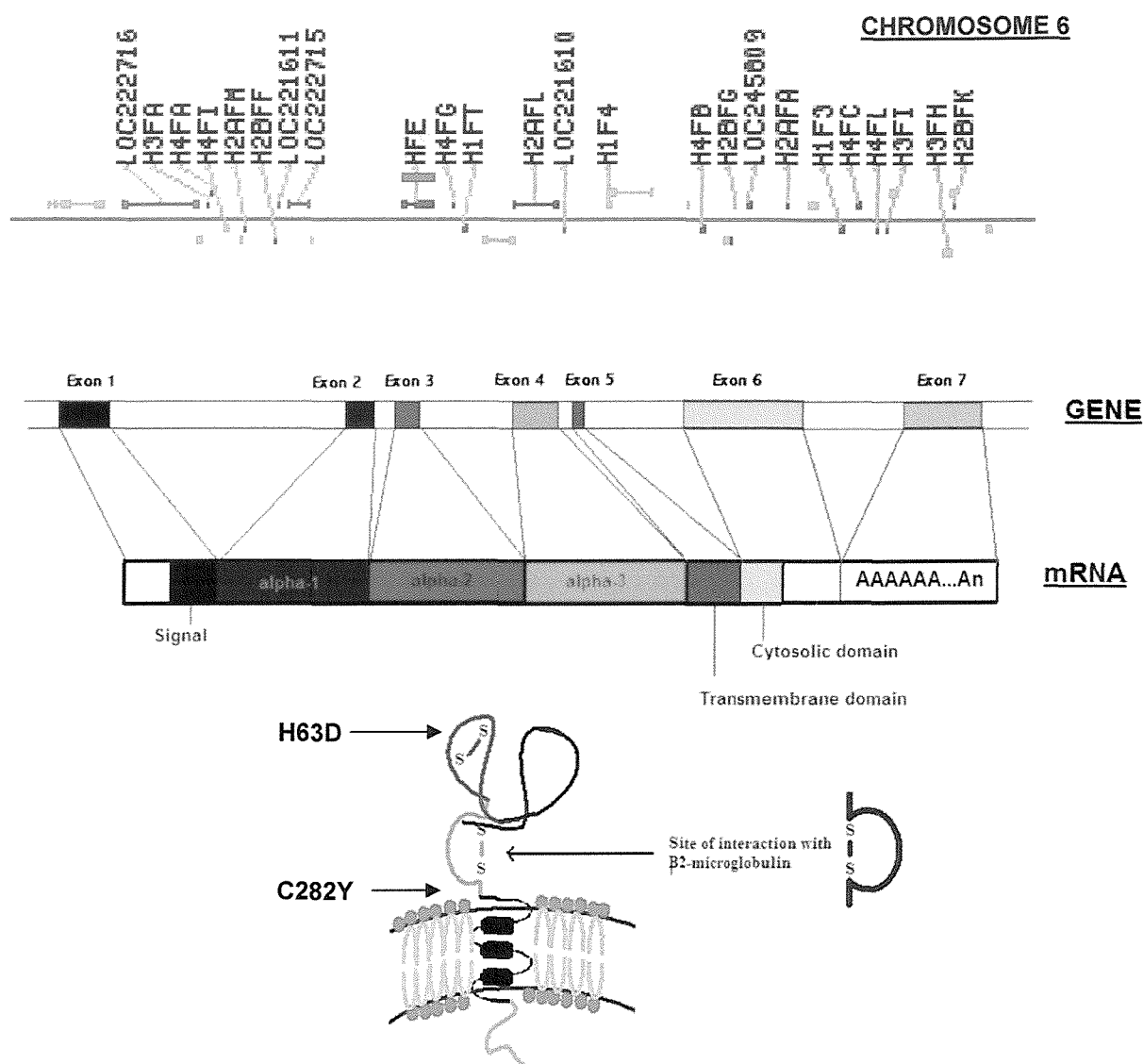
As discussed throughout this chapter there are also difficulties with the case definition of haemochromatosis and as many as 10 % of cases of haemochromatosis are not accounted for by these common mutations. Other mutations within the HFE gene have been identified but their clinical significance is unclear (Pointon et al., 2000). In addition rare families have been reported in whom iron overload disorders have been shown to be caused by mutations in genes for other proteins involved in iron metabolism pathways (Table 1.5)

Haemochromatosis is generally considered to be a disease of Northern Europeans and prevalence studies have indicated that the common mutations are found primarily in people of Northern European descent with particularly high prevalence in 'Celtic' populations (Merryweather-Clarke et al., 1997; Merryweather-Clarke et al., 2000; Smith et al., 1997). There have also been recent claims that the C282Y mutation mutations arose in a Scandinavian population rather than a Celtic one and spread to areas of Viking settlement (Milman and Pedersen, 2003).

There is considerable variability in the expression of the HFE phenotype with some individuals who are homozygous for the common defective alleles showing little or no evidence of progressive iron overload. Some of this variability may be due to the effects of modifier genes and there is evidence from animal models and experiments of the existence of naturally occurring autosomal and sex linked variants that influence the severity of iron loading (Fleming et al., 2001; Sproule et al., 2001).

1.2.9 HFE protein and its role in intestinal absorption of iron

The HFE protein is a type 1 trans-membrane protein that associates with class 1 light chain beta2 microglobulin (Feder et al., 1996). The wild type protein binds to the transferrin receptor and reduces its affinity for transferrin (Feder et al., 1998). The C282Y mutation alters the HFE protein structure and disrupts its transport and presentation to the cell surface (Lebron et al., 1998). Since the luminal cells in the small intestine do not contain transferrin receptors this mechanism of action of the HFE protein is not implicated in the increased absorption of dietary iron in haemochromatosis. The H63D mutation does not appear to prevent cell surface expression of HFE, however studies on cell associated transferrin expression suggest that in contrast to over expressed wild type protein the H63D mutant protein does not decrease the affinity of transferrin receptor for transferrin, providing evidence of a functional role of the H63D mutation in the genesis of haemochromatosis (Feder et al., 1998).

The HFE protein, which is defective in hereditary haemochromatosis, is normally expressed in crypt enterocytes of the duodenum where it has a unique, predominantly intracellular localisation (Parkkila et al., 1997). One hypothesis is that the HFE

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protein modulates the uptake of transferrin-bound iron from plasma by the crypt enterocytes and participates in the mechanism by which the crypt enterocytes sense the level of body iron stores (Waheed et al., 1997). It is thought that this mechanism may be mediated by the divalent metal transporter DMT1. The suggestion is that HFE mutations lead to low iron levels within the duodenal crypt cells. The production of DMT1 is up regulated and stabilised leading to increased absorption of dietary iron by the epithelial cells as they migrate and mature into intestinal villi enterocytes. Expression of DMT1 has been shown to be increased in duodenal biopsies from patients with haemochromatosis or iron deficiency anaemia compared to controls (Zoller et al., 2001; Zoller et al., 1999). HFE protein is also expressed in the human placenta at the site of maternal fetal transport of transferrin bound iron suggesting a role in iron homeostasis at this site.

A further hypothesis suggests a possible mechanism for the role of the HFE protein in iron homeostasis, the hypothesis being that the HFE protein inhibits the release of iron from cells by interacting with ferroportin and also competes with iron bound transferrin for transferrin receptor. In conditions of high transferrin saturation HFE dissociates from transferrin receptor 1, binds with ferroportin and inhibits iron release from the cell. In the crypt cell this would programme the cell to be iron loaded and thus as it matures it will absorb less iron. In conditions of low transferrin saturation the opposite occurs and more iron is absorbed and exported to the blood stream. Mutations in the HFE gene disrupt this negative feedback allowing the crypt cell to behave as if it were iron deficient even in the presence of high transferrin saturation (Townsend and Drakesmith, 2002).

Although the mechanism by which mutations in the HFE gene cause haemochromatosis is not yet understood, the discovery of the HFE gene has led to a number of advances in the understanding of iron biology. It has also opened the debate on the exact phenotype of haemochromatosis and allowed for re-examination of the desirability for screening for this condition.

1.3 Screening for haemochromatosis

Haemochromatosis is a condition that may be amenable to population screening in that it has significant morbidity and mortality, there is an early pre-symptomatic phase during which intervention may be possible, there is effective treatment and screening tests are available. However there remain many unanswered questions relating to the natural history of the condition when it is diagnosed early, which screening test to use and their utility, the acceptability and feasibility of screening programmes and an assessment of the benefits and dis-benefits of screening as a strategy for early case identification.

A randomised controlled trial of screening in haemochromatosis would require a very large sample size to have enough power to detect an effect. Therefore pilot studies are needed to assess such questions as the feasibility and acceptability of screening in this condition, the performance of the screening tests used and the characteristics of the population accepting screening. Information from such pilot studies would then be used to inform the design of large screening trials or to recruit for studies of the natural history or early treatment. In chapter two population screening and screening for haemochromatosis will be discussed in more detail.

Chapter 2 Screening for haemochromatosis

2.1 Population Screening

'Screening is a public health service in which members of a defined population, who do not necessarily perceive they are at risk of, or are already affected by a disease or its complications, are asked a question or offered a test, to identify those individuals who are more likely to be helped than harmed by further tests or treatment to reduce the risk of a disease or its complications'. First report of the UK National Screening Committee (NSC, 1988).

It is suggested that screening programmes be defined as risk reduction programmes (NSC, 2000). The primary question in evaluating screening programmes should be balancing the benefits and harms of the programme, the benefit being whether the programme reduces the risk of morbidity or mortality of the disease that is being screened for. The evaluation should include both the benefit produced in terms of risk reduction balanced against the potential harms caused by screening. Therefore the purpose of a screening programme for haemochromatosis should be to reduce the risk of developing the complications of having the genetic predisposition for haemochromatosis. This definition has to be modified somewhat when considering antenatal screening programmes e.g. for Down syndrome, the purpose of screening in those programmes is to allow pregnant couples informed choice. However evaluation of most antenatal screening programmes still does not focus on this as the primary outcome. By conceptualising a screening programme as a risk reduction programme, the evaluation of its effectiveness requires comparison of screened versus not screened to compare relative risk of morbidity and mortality.

Since screening involves approaching a large number of individuals to detect a much smaller number of individuals who might benefit from the intervention, the assessment of potential harm caused to those who screen negative is particularly relevant when screening programmes are being evaluated. The classic criteria for evaluation of screening programmes suggested by Wilson and Jungner establish the importance of considering a number of factors prior to recommending the implementation of a screening programme. These include: the seriousness of the

condition that is to be screened for, the effectiveness of early diagnosis and subsequent management, the sensitivity and specificity of the screening tests and careful assessment of the whole screening programmes before recommending implementation (Wilson and Jungner, 1968). These have been expanded by the National Screening Committee in the UK to take account of the more rigorous standards that are now needed to demonstrate effectiveness and greater concern about possible harm caused by screening. In particular the committee ask that there be 'evidence from high quality Randomised Controlled Trials that the screening programme is effective at reducing mortality or morbidity', that there be evidence that the *complete* programme is clinically, socially and ethically acceptable, that the benefit outweighs the harm and that all other options for managing the condition should have been considered. All these conditions should be met before pilot screening studies are conducted to evaluate the delivery and effectiveness of the screening programme outside of a research environment. The full criteria are in appendix 1.

Evaluating population screening as a strategy for early case detection in haemochromatosis against these criteria suggests that introduction of screening for haemochromatosis is premature (Table 2.1). However the caution relates to the absence of evidence rather than evidence of ineffectiveness. There is a lack of evidence relating to the natural history of cases diagnosed early, the precise burden of disease in the general population, effectiveness of screening strategies and evaluation of other strategies for early diagnosis. All these require further research including piloting screening programmes to asses their acceptability and feasibility.

Table 2.1 Evaluation of screening for haemochromatosis against National Screening Committee criteria (NSC, 1988) (Not all the criteria are included)

The condition	Important health problem	The genetic predisposition for haemochromatosis and the prevalence of iron overload is 1 in 200 to 1 in 500. These may not be the same populations. Untreated haemochromatosis has significant morbidity and mortality. The true disease burden is unknown
	The natural history and epidemiology of the condition should be understood	There is considerable uncertainty about the natural history and epidemiology. There is a lack of prospective cohort and case control studies. Progression to end organ damage does not appear to be an inevitable consequence of either the genetic predisposition or early abnormal iron indices.
	There should be a recognised latent period or pre-symptomatic stage	Early symptoms are non-specific. Raised transferrin saturation appears to be a sensitive although not specific screening tests in asymptomatic individuals. Genetic testing will detect those at risk of the condition at any age. The true risk is undetermined.
The test	a) There should be a simple, safe, precise and validated screening test which is acceptable to the target population. b) The cut-off value level should be defined and agreed	a) Providing standardisation and quality control issues are attended to both biochemical and genetic tests are simple and safe. The positive predictive value in an asymptomatic population is unclear. There is concern about the acceptability of genetic tests. b) Cut-off levels for transferrin saturation and ferritin are known. The risk of disease associated with genetic predisposition is not yet known
The treatment	Effective and acceptable treatment should be available	Phlebotomy therapy appears to be effective for symptomatic individuals. The effectiveness in asymptomatic individuals diagnosed through screening programmes is unclear.
	There should be agreed evidence based management and treatment protocols	Treatment and management guidelines are currently based on expert opinion rather than clear evidence
	Clinical management of the condition and patient outcomes should be optimised	It is not clear that guidelines for management are adhered to. Treatment is fragmented and there are undoubtedly ways in which the clinical care of patients with HHC could be optimised.
The screening programme	All other options for managing the condition should have been considered	The impact on early diagnosis of increased physician awareness and extended family testing has not yet been evaluated

2.1.1 Current adult screening programmes

In this context adult screening programmes refers to programmes that offer a screening test to an adult in order to reduce the risk of disease in that adult. Antenatal screening programmes although offered to adults, are screening programmes designed to offer pregnant couples reproductive choice and to facilitate informed decision making regarding further diagnostic tests for disease or disability in the unborn child and will not be discussed in detail. Screening programmes and guidelines for screening vary between countries both in whether a particular programme is offered at all and if it is offered what the eligibility criteria and the screening intervals should be.

These variations will affect the performance of the screening tests, acceptability, uptake and cost effectiveness of screening. In addition programmes will vary according to the organisation of health care systems. For example in the UK all national screening programmes are free of charge and provision of screening has to be shown to be cost effective since it diverts resources from other health care provision. In the United States charges may be made and will be reimbursed in various ways such as through Medicare, different states and different managed care organisations will have different policies regarding screening and possible further diagnostic tests.

Guidelines for current screening programmes or pilots in the United States, Canada, Australia and the UK are summarised in table 2.2. The only screening programmes that are recommended widely are for breast cancer and cervical cancer, which clearly are targeted at women. Screening for colorectal cancer is being evaluated in a number of countries. There has been a debate concerning PSA (prostate specific antigen) testing for prostate cancer. Although in the UK this is available at the patient's request it is not recommended as a national programme and this was the same for all countries' guidelines that were examined.

Table 2.2 Adult screening programmes

Country	Recommended programmes	Pilot/Planned programmes
U.K. (National Screening Committee, 2002).	Breast cancer Females 50-64 Mammogram 3 yrly	Chlamydia Females age 16-24 Urine test
	Cervical cancer Females 20-64 PAP smear 3-5 yrly	Colorectal cancer Males and females 50-69 Faecal occult blood
U.S.A. (U.S.Preventative Services Task Force, 2002).	Breast cancer Females >40 Mammography 1-2 yrly	
	Cervical Cancer Females 18-67 PAP smear 3 yrly	
	Colorectal cancer Males and females > 50 Faecal occult blood yrly	
	Lipid disorders Males>35 Females>45 Cholesterol 5 yrly	
Canada (Canadian task force on preventative health care, 2002).	Breast cancer Females 50-69 Mammography yrly	
	Cervical Cancer Females 18-69 PAP smear 3 yrly	
	Colorectal cancer Males and females>50 Faecal occult blood 1-2 yrly	
Australia (National health and medical research council of Australia, 2002).	Breast Screening Females 50-69 Mammography 2 yrly	Colorectal Cancer Males and females>50 Faecal occult blood
	Cervical Cancer Females 18-69 2 yrly	

2.2.1 Harm caused by screening

Interpretation of the benefit of screening is complicated by the fact that screening test may detect people early in he course of their disease, which would appear to increase survival. However unless the screening prevents mortality the person has not had their life extended they have simply known they had the disease for longer. This is known as lead time bias as shown in figure 2.1

Figure 2.1 Lead time bias

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                        ┌──────────┐
                        │ Symptoms │
                        └──────────┘
         5 years             │          5 years
                             ▼
    ━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━▶
    ▲                                           ▲
    │                                           │
    │                                           │
┌──────────┐                              ┌──────────┐
│ Start of │                              │  Death   │
│ disease  │   ┌──────────────┐           └──────────┘
└──────────┘   │ Early diagnosis│              │
    │          │ with screening │              │
    │          └──────────────┘               │
    │              │ Lead time │               │
    ▼   3 years    ▼ 2 years   ▼   7 years     ▼
    ━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━▶

                    10 years
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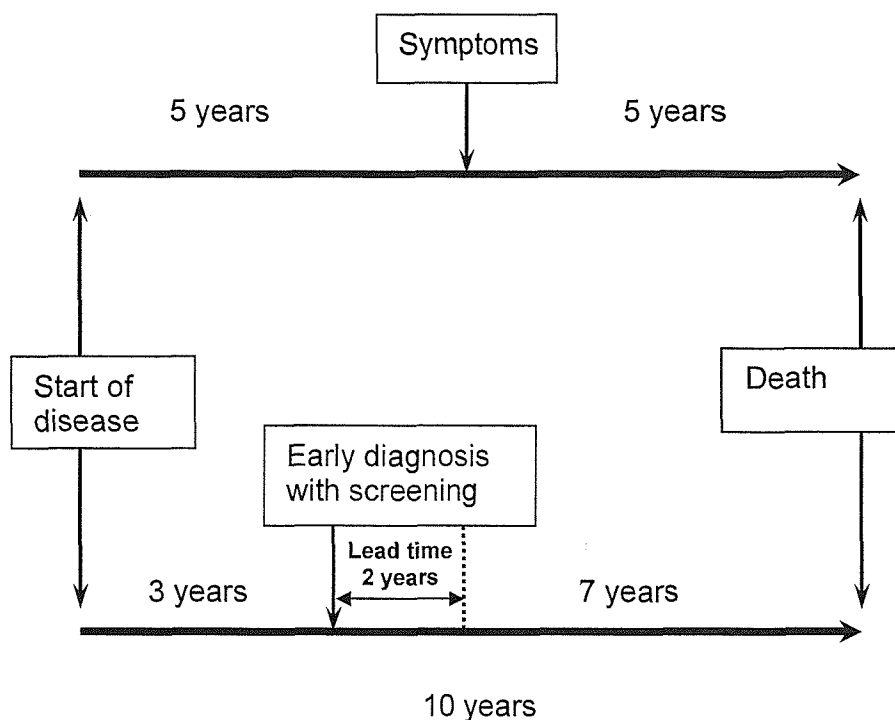
Consideration of the negative as well as the positive effects of screening underpins the recent decision by the UK National Screening Committee not to recommend PSA testing for the detection of prostate cancer. The decision was made on the basis that the costs in terms of complications of treatment-impotence, incontinence, postoperative mortality and psychological disturbance was not balanced by evidence of a reduction in deaths in those diagnosed early (Stewart-Brown and Farmer, 1997).

The screening and diagnostic tests and subsequent treatments may have the potential to cause harm. One concern raised in relation to colorectal cancer screening is the potential complications of the further diagnostic test-colonoscopy. Since all screening tests have a false positive rate then a number of people will have colonoscopies that have no disease. The risk of the procedure for them has no benefit (Robinson et al., 1999). Screening for aortic aneurysms by ultrasound has been piloted in a research setting, however the treatment is surgery which has its own mortality and morbidity (Scott, 2002). The critical question is balancing the benefit of intervening early with the risk associated with the intervention.

There may also be harmful consequences in screening programmes arising from false negative results. That is the consequences when individuals are screened negative and reassured but later go on to have the condition that is being screened for. A review of false negative results in screening programmes found little evidence that would allow their consequences to be evaluated. They did find evidence of legal consequences and suggest that another outcome may to provide false reassurance with the possibility of delaying detection of, for example, breast or cervical cancer (Petticrew et al., 2000).

The psychological consequences of false positive and false negative results have been investigated. Screening tests usually require further diagnostic tests to demonstrate the presence of disease, however there is the risk that a positive screening test itself becomes a surrogate for disease. Screening for hypertension in workplace settings has been shown to have adverse psychological consequences in those who are diagnosed as hypertensive: increased sickness absence, reduced perceived health status, increased anxiety irrespective of whether their hypertension merited treatment (Haynes et al., 1978). A randomised controlled trial of cardiovascular risk factor screening found little evidence for harmful effects of anxiety or lower self-rated health in the screened group. There was a suggestion however that the screen negative group were falsely reassured in the presence of continuing albeit lower cardiovascular risk (Marteau et al., 1996). Investigations of the outcome of false negative results in antenatal screening found that parents of the Down syndrome child born after screening were more likely to blame others for the outcome and to have higher parenting stress scores than parents of a Down syndrome child born after screening was declined (Hall et al., 2000b).

Population screening programmes have the potential to cause harm to many more individuals than they benefit, therefore careful evaluation of all the costs and benefits needs to be made before their introduction. In addition the whole programme should be evaluated, not simply the performance of the screening test itself. The application of a screening test outside of carefully controlled research environments means that attention has to be paid to the quality of the programme as it is delivered in the real world (NSC, 2000).

In summary, the potential risks of screening programmes include:

1. Complications arising from the screening test, further diagnostic tests and treatment.
2. Treatment of individuals who although they have the disease may never have developed complications from it.
3. Adverse consequences of being labelled with a condition
4. Costs and inconvenience of investigation and treatment
5. Anxiety generated by the process of screening
6. The consequences of a false positive result and the false reassurance of a false negative result (NSC, 1988; Stewart-Brown and Farmer, 1997; Peckham and Dezateux, 1998)

2.1.2 Uptake of screening programmes

Population screening programmes are developed as public health initiatives and as such they need a high uptake in order to have a significant impact on reducing morbidity and mortality in the population. The importance of this is confirmed by the fact that uptake of cervical cancer screening and breast cancer screening are included as performance indicators for general practitioners and health authorities in the UK (Department of Health, 2002a). The current figures for uptake in England are 83% for cervical cancer screening and 69% for breast cancer screening.

There have been many debates, about the desirability of attaining high uptake rates without consideration of issues surrounding informed choice (Austoker, 1999; Bekker

et al., 1993; McQueen, 2002). The policy shift in the UK towards informed choice is outlined in the guidelines produced by the National Screening Committee (NSC, 2000). In addition the General Medical Council has produced guidelines on the information that should be provided when offering screening, which should include information on the condition as well as the likelihood of any test result and it's meaning (General Medical Council, 1999).

Examples of programmes where informed choice is seen to be paramount are those programmes where, the consequences of uninformed choice are seen to be unacceptably high, for example in antenatal screening programmes.

There is a tension between maximising the public health benefit of screening by minimising information about the possible harm at an individual level and promoting informed uptake. Evidence from prostate cancer screening trials suggest that providing information about the possible consequences of early diagnosis and the side effects of treatment decreases patients interest in screening (Flood et al., 1996). Although population screening is intended to benefit the whole community this cannot be at the expense of the individual, the consensus remains as to the importance of informed uptake rather than maximising uptake at all costs.

A review of the factors that might affect the uptake of screening found little primary evidence that would allow an assessment to be made and the authors comment that the determinants that were possibly significant varied across programmes. The review included international studies. The three factors that were shown to be significant across a number of screening programmes were age, insurance status and previous screening behaviour i.e. attendance at one screening programme was predictive of acceptance of another (Jepson et al., 2000).These reviewers were not able to assess whether the effect of age was to increase attendance in younger or older patients and also comment that insurance status is irrelevant within the UK setting.

Although genetic testing based on family history cannot be categorised as screening it is currently used to predict the risk of disease and uptake appears to be higher if treatment is available. In Huntingtons disease where no treatment is available uptake

of genetic testing has been reported to be about 10% (Crauford et al., 1989), in familial cancer syndromes it was 77% in men and 93% in women (Evans et al., 1997).

Psychological theories of health behaviour have been applied to screening decisions (Cameron, 1997). In Leventhal's self regulatory model, perception of health threats can be described by variations along five dimensions; personal characteristics, perceptions of the cause of the condition, whether the condition is curable or can be controlled, the consequences of the health related decision and the time line of the decision and possible consequences (Leventhal et al., 1997). The way in which the threat is perceived will vary according to personal characteristics and the way information is presented.

An experimental study approached a community sample of people, gave information about haemochromatosis and asked hypothetically about whether the person would want screening. One in 5 of the individuals approached agreed to take part. All were given standard information including the statement that treatment was most effective before age 30. The mean likelihood of accepting a test was 46%. After an intervention of either focusing on the positive statements about the condition or focusing on negative statements, the mean likelihood of accepting a test increased for both groups in people under the age of 30. However in those over thirty there was a significant reduction in the likelihood of accepting the test in the group who had focused on negative statements, demonstrating an effect on the decision by the framing of information and the personal characteristics of the participant (Salkovskis et al., 1999). This interaction with age was not seen in a similar study offering a hypothetical test for heart disease. In this study, the likelihood of accepting a test decreased in all age groups in those who focused on negative statements and increased in the group focusing on the positive aspects. However the perceived severity and anxiety decreased in the group that focused on the negative aspects, and the authors' comment that this may be due to an effect of threat minimisation (Wroe and Salkovskis, 2000).

There is a lack of rigorous evidence relating to the determinants of uptake in current screening programmes and there is a lack of consensus as to how to evaluate informed decision making in the context of screening programmes.

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It is considered that genetic screening is a special case that merits extra appraisal of the possible harmful consequences and this is discussed more fully in the following section.

2.1.3 Genetic Screening

Concern about the special nature of genetic screening has led to a number of reports and guidelines being produced. The argument that is made is that genetic screening is distinguished from other types of medical screening by the genetic nature of the disorder which may result in risk implications to family members of the person screened, even though family members may not be, nor perhaps wish to be, included in the screening programme. Another difference that is claimed for genetic screening is that the aim is not necessarily to prevent or treat diseases in the person screened; it may be used for health related reproductive or lifestyle choices (Ayme et al., 2000). An early UK report on the ethical aspects of genetic screening offers the following definition of population genetic screening (Nuffield Council on Bioethics, 1993).

" a search in a population to identify individuals who may have, or be susceptible to, a serious genetic disease, or who, though not at risk themselves, as gene carriers may be at risk of having children with that genetic disease. While it is individuals who are screened, the results will normally have wider implications. Depending on the nature of the genetic defect that is identified and its pattern of inheritance, siblings and other blood relations, as well as existing and future offspring, may be affected." Nuffield Council on Bioethics

Because of the wider implications genetic information may have for family members this report focuses on issues of confidentiality and consent. The above definition would include biochemical tests for genetic disease such as phenylalanine used in the screening programme for PKU. This clearly is a widely accepted screening programme which, since it was established in the 1960's has, at least in it's history, not involved the level of consent or information that is now seen to be best practice. The Nuffield Council's report was published in 1993 and has no reference to what may be the major advance in genetic science in the medium term, which is the identification of genetic polymorphisms that confer susceptibility to common diseases. However the

report does make recommendations regarding the possibility of discrimination by insurance companies and employers.

A more recent report on behalf of the European Society for Human Genetics does focus more on the predictive possibilities of genetic screening and concludes that: the benefits of genetic screening include pre-symptomatic detection of diseases or susceptibility to diseases for prevention, early diagnosis, care and treatment, the detection of genetic variation that may confer predisposition to harm caused by environmental factors and the detection of carrier status in order to facilitate reproductive choice. The potential harm identified by these authors includes: anxiety raised by information which cannot be used for therapy, preventative measures or lifestyle choices, the provision of information which is difficult to understand or interpret, concerns about possible stigmatisation of individuals at increased genetic risk or those who choose to refuse an offer of screening, concerns about disclosure of information to family members and possible misuse of information by insurers or employers (Ayme et al., 2000).

A report by the World Health Organisation also focuses on the need for informed consent and adequate information in relation to genetic screening and recommends that screening programmes should be voluntary, that there should be no disclosure of results without consent and that test results should be followed by genetic counselling (World Health Organisation, 1998).

The above reports make an assumption that population genetic screening has additional implications, which require extra consideration over and above that which is necessary for other population screening programmes. These additional implications are said to arise because of:

- the inescapable involvement of families
- the involvement of genetic screening in reproductive choice
- the predictive nature of genetic screening for future disease
- The possibility for stigmatisation or discrimination particularly by employers or insurance companies.

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Implications of genetic testing for families

Clinical genetic services have focused on the implications of genetic disease for families and some would claim that this is part of their specialism that is unique in medicine (Bradbury et al., 1999). It should be emphasised that current practice of identifying an index affected case and offering family testing based on the inheritance pattern is not genetic screening. It is genetic testing since the family is presenting for medical care having already been identified as being at risk. Diagnosis of an autosomally inherited genetic disease undoubtedly does have implications for the extended family but this identification does not depend on the application of a genetic test but on a clinical diagnosis being made in the index case. A neurological diagnosis of Huntington's disease clearly has familial implications as does a diagnosis of familial hypercholesterolaemia made after a myocardial infarction.

Undoubtedly any proposed screening programme that is being considered should include full information about possible consequences including possible implications for family members. In practice the majority of single gene disorders would be too rare or have insufficient public health implications for the initiation of a population screening programme. The most effective strategy will probably remain targeted family testing after the identification of an index case. The exceptions to this include PKU screening, which although a rare disease has serious consequences which can be prevented if the diagnosis is made early enough. Universal screening programmes for the haemoglobinopathies and cystic fibrosis are also being considered. The primary aim is to identify affected children neonatally, although antenatal screening for carriers of haemoglobinopathy genes is being proposed in order to give couples reproductive choice.

The gene frequency for haemochromatosis is high and there is the possibility of effective treatment, which makes it a condition where screening could be considered. The concern about the effects on the extended family apply to both population screening programmes utilising biochemical tests for iron overload

and to those using mutation analysis. The same concerns regarding fully informed consent apply to both methods.

Genetic testing and reproductive choice

The use of genetic diagnosis in prenatal diagnosis with the subsequent termination of affected pregnancies has raised concerns about the abuse of reproductive genetic technology. Population screening programmes for Down syndrome or for carriers of recessive diseases such as the haemoglobinopathies and cystic fibrosis awaken concerns relating to the past history of clinical genetics and the eugenics movement. For a historical review see Kevles (Kevles, 1995; Butler and Barton, 1999). There is a desire to formalise such screening programmes in terms of individual reproductive choice rather than designing them to reduce the incidence of children born with Down syndrome or cystic fibrosis. Issues surrounding informed consent in such programmes therefore become paramount and are actively being addressed in the UK (NSC, 2000). The issue of reproductive choice is not relevant when considering screening or testing for haemochromatosis. In addition to it being an adult onset disease with effective treatment, the predictive value of the genotype is not known.

Genetic testing and prediction

The promise that is held out for the 'New Genetics' is a change in clinical practice from diagnosis and treatment to prediction and prevention (Collins and McKusick, 2001). This implies that there would be the possibility for effective prevention strategies once a genetic predisposition was identified.

The debate about the predictive power of genetics in the past has focused on diseases such as Huntington's disease where there is no effective therapy and the gene mutation confers a one hundred percent risk of developing a devastating disease. Models of care involving counselling protocols prior to predictive testing have been developed (Crauford and Tyler, 1992; International Huntington's Disease Association, 2002). These protocols have been adapted for use in other single gene disorders where predictive testing is possible for example in families with mutations in the BRCA1 breast cancer

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gene. The development of these protocols was driven by a concern about the possible harmful effects on an individual of such testing. However evidence to date shows little evidence of such testing causing more than a temporary disruption and although adverse events have been reported in Huntington's disease predictive testing programmes they have been very few and associated with negative results (i.e. the individual does not have the gene) as well as positive ones (Almqvist et al., 1999; Huggins et al., 1992) A review of reports of the psychological consequences of predictive testing programmes that included Huntington's disease in addition to polyposis coli, BRCA1 breast cancer genes, HIV and hypercholesterolaemia also found little evidence of long term psychological effects (Shaw et al., 1999).

The protocols that have been developed in response to predictive genetic testing for Huntington's disease are not appropriate when considering utilising genetic technology for population screening. As discussed previously in this section population screening programmes should only be considered when there is therapy or treatment that is effective, safe and acceptable which is not the case in Huntington's disease.

Although current population screening programmes such as those for breast and cervical cancer are designed to detect early disease in order to initiate treatment, other proposed programmes such as cholesterol testing or cardiovascular risk factor screening are designed to detect persons at risk in order to initiate treatment or behaviour change. It may be that genetic polymorphisms that produce an individualised risk assessment may be introduced into clinical practice. However the principles used in evaluating the introduction of these tests should be same as those used in the evaluation of the utility of any risk factor in preventing disease. There is an increasing recognition particularly in the area of cardiovascular disease that genetic risk assessment should be evaluated alongside other risk assessment tools in terms of its clinical utility. There is no difference between a genotypic and phenotypic test in this situation since evaluation of the clinical utility requires knowledge of the predictive value of the test.

However identification of those at risk is only the first step to a reduction in mortality and morbidity. The second step is acceptance of treatment and/or behavioural change. It may be that assessing a person at high genetic risk will influence the way in which they respond to medical advice regarding future treatment and health promotion strategies. A genetic risk factor may be perceived as being uncontrollable which may change individual's motivation for engaging in health promoting behaviour (Marteau and Lerman, 2001; Marteau and Croyle, 1998).

This has been investigated using Leventhal's self regulatory model as a theoretical framework. A qualitative study was conducted interviewing parents of children participating in a neonatal screening study for hypercholesterolaemia. When the test was perceived as detecting raised cholesterol, it was not threatening and was perceived as dietary in origin. When it was perceived as genetic it was seen as uncontrollable and therefore threatening (Senior et al., 1999). This was further investigated in an experimental study using hypothetical scenarios and similar conclusions were drawn (Senior et al., 2000).

If the use of a DNA based test did lead to a lower uptake of a screening test or non- adherence to treatment and management strategies this would change the effectiveness of any intervention based on a genetic test. This would require evaluation when assessing the effectiveness of using genetic tests to identify at-risk individuals in order to initiate treatment or prevention.

Genetic testing and discrimination

The other well publicised issue that has been raised in regard to genetic screening is stigmatisation by insurance companies or employers. There has been considerable debate in the literature and media about the potential for discrimination, both in employment and insurance if predictive genetic testing is carried out. The concern is that if an individual has a genetic test that gives a risk of specific illnesses developing that individual will be unable to purchase insurance (Chadwick et al., 2001). However, it should be borne in mind that if an individual discloses a family history of a genetic condition, this would itself

have an impact on obtaining insurance and the premiums paid. It is not only genetic testing that affects the situation, but family history. In addition actuarial decisions are currently made based on non-medical information such as smoking behaviour or weight.

The potential impact of genetic discrimination will be very different according to public policy in individual countries. For example in the USA where much of the concern is expressed, many of the population depend on private insurance for their health care provision, and consequently an individual alteration in risk will have implications for their insurability. In the UK where health care is universally funded (at the present time) and contribution is universal, compulsory and based on ability to pay, the issues of genetic testing in relation to healthcare provision are different. Although, this may become more relevant if genetic testing for predisposition to elderly onset diseases such as dementia becomes possible, since funding for nursing home care for the elderly is constantly under review. In the UK discussions between the government, the insurance industry, the clinical genetics community, consumers and other stakeholders have led to a five year moratorium (from October 2001) on the use of genetic tests in assessing applications for life insurance, critical illness, long term care and income protection policies up to specific financial limits.

The same principles that are used in evaluating any screening programme should be applied when evaluating genetic screening. The concern that has been raised about informed consent and choice in relation to population genetic screening has now been accepted as an appropriate outcome for any population screening programme as discussed earlier and this provides a challenge for those designing such programmes (Marteau and Kinmonth, 2002).

There may be need for extra caution when considering genetic screening for the reasons outlined above. However there is some confusion as to the exact definition of genetic screening. There is confusion as to what constitutes 'genetic' and as to what constitutes 'screening'.

As discussed previously genetic testing within the context of a known family history is not screening. The use of the word screening should be in relation to the definition outlined at the beginning of this section.

A test for a genetic disease does not only involve DNA or chromosome analysis: asking about family history at an antenatal booking clinic might be construed as a screening test for genetic disease, an ultrasound of the kidneys might detect someone with autosomal dominant kidney disease, a cholesterol test might detect someone with familial hyperlipidaemia. All these tests could be categorised as 'genetic'.

However in general a DNA based test will detect the presence of an at risk genotype in the absence of any clinical phenotype. The special concerns relating to the predictive nature of genetic tests do need to be considered. In addition it may be that the meaning attached to the genetic test by a patient means that their reaction or future health related behaviour is different to that when a more traditional medical test is used. It may also be that genetic tests are less acceptable to the public because of concerns surrounding DNA technology ('designer babies', cloning, GM foods) or because of concerns about possible insurance implications (Reilly, 2000). All these factors need to be evaluated in a screening programme in addition to the sensitivity, specificity and clinical utility of any screening test.

2.2 Screening for haemochromatosis

Since the predisposition to haemochromatosis is common and there are presymptomatic tests and treatment, population screening has been considered as a possible strategy for early case identification in this condition (Edwards and Kushner, 1993; Bradley et al., 1996a). The College of American Pathologists recommended population screening using transferrin saturation level in 1996 (Witte et al., 1996) and several cost effectiveness studies have suggested that it might be an appropriate strategy . (Balan et al., 1994; Adams and Valberg, 1999; Adams et al., 1995; Phatak et al., 1994; Schoffski et al., 2000; Bassett et al., 2000). The identification of the gene led to calls for implementation of genetic screening programmes (Allen and Williamson, 2000). However several authors, particularly those working in public health, advised caution and more careful evaluation before implementation of screening strategies utilising either genetic or biochemical testing (Cogswell et al., 1998; Burke et al., 1998; Cogswell et al., 1999; Haddow and Bradley, 1999; McCullen et al., 2002).

2.2.1 Characteristics of haemochromatosis screening programmes

The questions that are commonly asked when reporting on screening programmes relate to the sensitivity or specificity of a particular test and the resultant positive predictive value given the prevalence of the condition in any particular population. The performance of the screening test also is affected by uptake, acceptability and the characteristics of the group accepting screening. In screening for haemochromatosis these may differ between biochemical and genetic screening tests. The performance of the two tests will also differ in terms of the characteristics of the populations that screen positive given that measurements of iron indices will be higher amongst males whereas the frequency of the genetic predisposition will not differ between males and females. Evaluation of screening programmes should consider all the constituent components of the programme including the effectiveness of subsequent treatment strategies.

Review of haemochromatosis screening studies

A purposive review of screening studies for haemochromatosis was conducted. The aims of the review were:

1. To identify data on the characteristics of those accepting screening and the uptake of screening.
2. To identify the tests used, their performance and the number of cases identified.
3. To identify studies determining the effect of screening for haemochromatosis on health related outcomes.

These data could then be used to inform the design of early diagnosis and treatment trials.

Method

Search strategy:

The electronic databases searched were Medline (1966 to 05-2002) and Embase (1980 to 05-2002). The search excluded non-English language papers since Haemochromatosis is rare in populations originating from outside of Europe and therefore the prevalence would be low and not comparable with the population in the proposed study. The reference lists of the identified papers were hand searched, as was the Journal of Medical Screening since its inception

The search terms used included mass screening (the preferred search term for population screening) as free text and in title abstract and mesh terms together with iron overload/deficiency, haemochromatosis and prevalence. The search strategies were examined by two investigators and a sub sample of the extracted abstracts and papers were also examined by two reviewers for consistency of inclusion, exclusion and data extraction.

Examples of the search strategy are in appendix 2.

Inclusion and exclusion criteria

Inclusion criteria:
1. Studies that were pragmatic screening trials explicitly offering screening for haemochromatosis or iron overload to a general adult population sample.

Exclusion criteria:
1. Studies in paediatric populations

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2. Studies that offered screening to particular disease groups e.g. patients with diabetes or restrictive populations e.g. elderly males.

3. Studies using blood donors since blood donors are not representative of the population as a whole in that they are preselected or may be more likely to be in good health. In this setting the screening was also performed on samples taken for a purpose other than screening for haemochromatosis. In addition the process of blood donation has the potential to deplete body iron stores and therefore modify the expression of the haemochromatosis genotype.

4. Studies where the screening for haemochromatosis was performed on samples not taken specifically for that purpose, e.g. as part of a general health screening, other risk factor research, patients attending a hospital or having blood taken for another reason. These studies would not evaluate the whole screening process since the screening test for haemochromatosis was added to another clinical encounter.

5. Studies establishing reference values for laboratory tests or studies primarily designed to investigate the prevalence and penetrance of haemochromatosis.

6. Studies that were not primary research.

Results

310 abstracts were retrieved, none of which were reports of randomised controlled trials of screening. 25 papers were identified for detailed review together with a further 3 papers identified by hand searching of the reference lists. There were no data on the health outcomes in the unscreened group or in the screen negative individuals in any of these studies. After detailed review five papers were identified for the purpose of this study (Figure 2.2).

Figure 2.2 Details of excluded studies
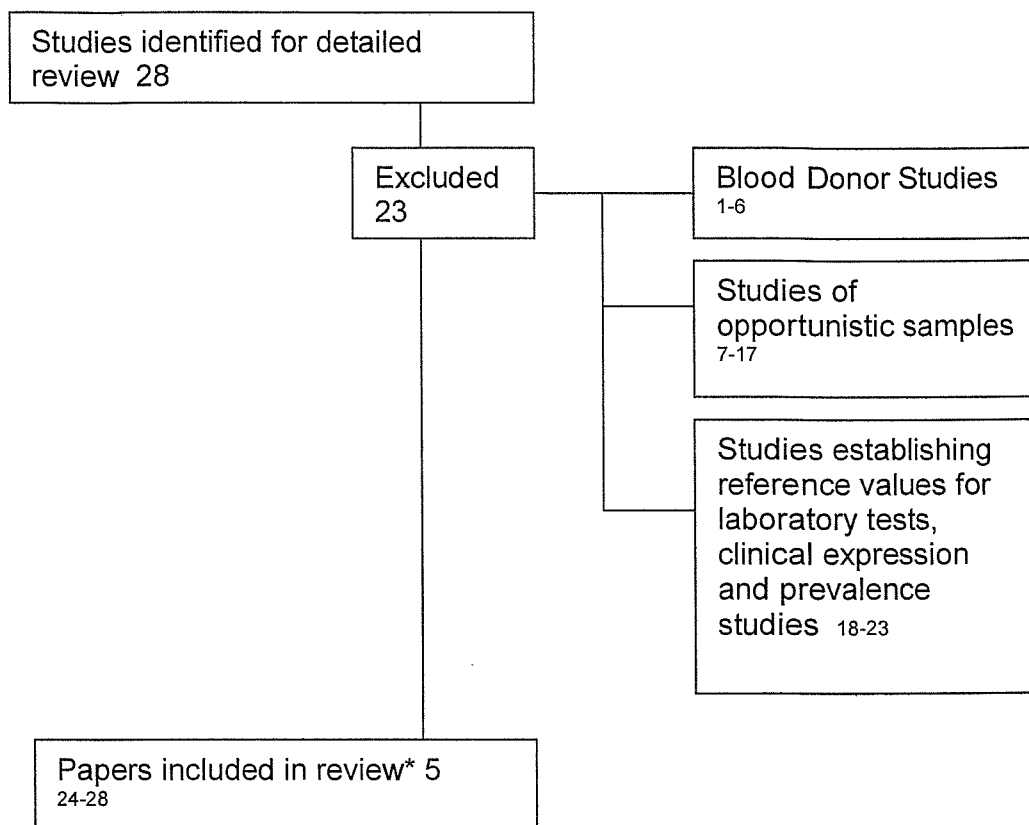
1-6 (Edwards et al., 1988; Velati et al., 1990; Wiggers et al., 1991; Bell et al., 1997; Andrikovics et al., 2001; Adams et al., 2000b) 7-17 (Olsson et al., 1983; Karlsson et al., 1988; Lindmark and Eriksson, 1988; Hallberg et al., 1989; Jonsson et al., 1991; Balan et al., 1994; Baer et al., 1995; Phatak et al., 1998; McDonnell et al., 1998; Stave et al., 1999; Asberg et al., 2001) 18-23 (Elliott et al., 1986; Porto et al., 1992; Bradley et al., 1996a; Olynyk et al., 1999; Cassanelli et al., 2001; Beutler et al., 2002a) 24-28 (Smith et al., 1997; McDonnell et al., 1999a; Niederau et al., 1998; Burt et al., 1998; Leggett et al., 1990)

* In two studies (Smith et al., 1997; Niederau et al., 1998) screening was performed both on a prospective samples of employees and either on samples from patients attending a doctor's clinic or on stored samples taken as part of a previous study. The employee samples were included for further synthesis if reported separately.

Populations and settings

Of the remaining five studies only one used a random sample selected from a general population (Burt et al., 1998). The remaining four were carried out in workplace settings. The age range of the populations studied varied from a mean age of 31 to 50 years. The majority of all populations studied were Caucasian (Table 2.3).

Uptake of screening and characteristics of screened population

The uptake of the initial screening ranged from 18% to 99% (Table 2.3). This wide variation in uptake is difficult to explain.

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Table 2.3 Characteristics of screened population and uptake of screening

Study	Population Type	Number invited	Number screened	Uptake	Age in screened sample	Ethnicity of screened sample		% males in sample screened
Leggett et al 1990	Employees	3811	1967	52%	mean 31 range 17-65	Caucasian Australian aborigine Asian	96.6% 1.8% 1.6%	48%
Smith et al 1997	Employees excluded those with known HHC or iron deficiency second samples from samples taken as part of previous PSA screening study	7118 excluding known diagnoses and previously sampled group 8087 in total sample	1331 in prospective sample 2294 in total sample	18% in prospective sample 28% in total sample	mean 49 not reported separately for prospective sample	Caucasian Black Asian/Pacific Islander Hispanic American Indian Other	86.0% 10.0% 2.0% 1.0% 0.1% 0.6%	81% in whole sample not reported separately for prospective sample
Niederau et al 1998	Employees excluded those with known HHC or iron deficiency	NR	3012	99%	NR	NR		83%
Burt et al 1998	Population randomly selected from electoral rolls	3510	1064	30%	mean 50	Caucasian Maori Pacific islanders Other	94.0% 2.2% 1.0% 2.6%	40%
McDonnell et al 1999	Employees	6000	1653	28%	mean 41 80%<50	Caucasian	97.0%	20%

There was little detailed information on the population being offered or accepting screening, or on the way in which screening was offered. There was no breakdown of uptake by age, gender or other population characteristics. The percentage of males in the screened sample ranged from 20% to 83%.

This probably reflects the opportunistic sampling strategy in four of the studies. In the one study that randomly sampled a population where it may be assumed there would have been an equal number of males and females offered screening the uptake was greater amongst females.

Screening and diagnostic strategies

No studies were identified that offered genetic testing as the initial screening strategy. All the studies utilised a two stage screening process employing transferrin saturation as the initial screening test either alone or in combination with ferritin. The cut-offs for reporting a screen positive result varied between 45% and 60%. In all studies transferrin saturation was calculated from iron and iron binding capacity. Only one study reported using fasting samples (Burt et al., 1998) as the first screening test and in this study genotyping was also performed although not as a screening test. The second screening test was repeated fasting biochemistry in all studies.

Although the initial uptake of screening was low, compliance with further screening and diagnostic tests was high. No information was reported on symptoms that might be associated with iron overload or on environmental modifiers such as alcohol consumption which would be needed to confirm or refute the diagnosis of haemochromatosis.

Liver biopsy or quantitative phlebotomy was reported as the diagnostic test in all studies, apart from one study where persistently raised transferrin saturation and a raised ferritin was accepted as evidence of haemochromatosis if other investigations were not possible(McDonnell et al., 1999a). The case definition for these studies was evidence of iron overload on liver biopsy (Hepatic iron index >=2) or by quantitative phlebotomy.

In these studies haemochromatosis would appear to be a diagnosis of exclusion once other causes for hepatic siderosis had been eliminated (table 2.4).

Prevalence of haemochromatosis in screened population.

The prevalence per thousand of haemochromatosis in the populations screened ranged from 2.2 to 4 per thousand (mean 3.2). The number needed to screen to detect one case of haemochromatosis ranged from 301 to 1617. This number is of course dependent on the uptake of screening.

Since no study reported the number of cases in the screen negative group it is not possible to assess the sensitivity and specificity of the screening test, however the positive predictive value of a first positive screening test was between 7% and 91%, of a second positive screening test was between 23% and 100%. This appeared to have no relation to the cut-off employed in the screening test, although the very high positive predictive values obtained in one study were associated with a screening strategy that included ferritin and high cut-offs for transferrin saturation (Table2.4).

Table 2.4 Screening and diagnostic strategies and yield of cases

Study	First Screening Test	Screen positive	Second Screening Test	Number screened Uptake%	Screen positive	Diagnostic Test	uptake of diagnostic test	Case definition	Positive Diagnostic	Positive predictive value *first screening test ** + second screening test	Prevalence of iron overload consistent with HHC per 1000 in screened population	Number needed to screen	Prevalence of iron deficiency per 1000
Leggett et al 1990	Transferrin saturation (TS) >45%	46 2.3%	TS >45% serum ferritin >200 males >150 females	41 90%	12 29.3%	Liver biopsy	92%	Hepatic iron index >=2	7	*15% **58%	3.6	544	54
Smith et al 1997	TS >45% and ferritin>300 or TS.55%	70 3.1% prospective sample not reported separately	Repeated fasting TS and ferritin	66 94%	15 22.3%	Liver biopsy	80%	Hepatic iron index >=2 HLA haplotyping quantitative phlebotomy	5 all male	*7% **33%	2.2 prospective sample not reported separately	1617	NR
Niederau et al 1998	TS>=50% women TS>=60% men And ferritin >250 women ferritin>350 men	11 0.4%	Repeated fasting TS and ferritin	10 91%	10 100%	Liver biopsy or quantitative phlebotomy	40% liver biopsy 60% quantitative phlebotomy	Hepatic iron concentration consistent with iron overload	1 Female 9 Male	*91% **100%	3.3	301	5 in males 60 in females
Burt et al 1998	fasting TS>55%	39 3.7%	Fasting TS > 55%	38 97%	13 33.3%	Liver biopsy if ferritin>160 female 300 male	100%	Hepatic iron index >=2	2 Female 1 Male	*8% **23%	2.8	355	49
McDonnell et al 1999	TS>=50% female >=60% male	60 4%	Fasting TS >=50% Female >=60% Male	58 97%	13 22%	Serum ferritin quantitative phlebotomy or liver biopsy	100%	Two raised TS + serum ferritin<95% for age and sex HHC without iron overload two raised TS + serum ferritin>=95% for age and sex HHC with iron overload	4 HHC without iron overload 4 HHC with iron overload	*7% **31%	4 with iron overload 8 total	413	35

Prevalence of iron deficiency in the screened population

Of the studies that reported prevalence of iron deficiency the prevalence ranged from 27 per thousand to 54 per thousand (Table 2.4)

Discussion

No studies were found that were trials of screening versus non-screening in which changes in health related outcomes could be determined. All the studies investigated screening in relation to detection of cases. No study utilised genetic testing as the initial screening strategy and all screened for iron status and identified cases of iron overload due to haemochromatosis.

Prevalence of iron overload and haemochromatosis

The definition of what constitutes a case of haemochromatosis and an estimate of disease prevalence is central to determining the effectiveness of screening or case detection strategies.

Since the expression of the haemochromatosis genotype may be modified by age, sex, and other genetic and environmental modifiers, screening programmes utilising assessment of iron status will have significantly different case detection rates depending on the age/sex profile of the screened population. None of the reported studies give detailed data on the age/sex profile of their population. The prevalence rates described would be at the lower limits because the study populations included individuals from populations of differing ethnicity where the incidence of haemochromatosis would be expected to be lower, and women in whom the expression of the at-risk genotype would also be expected to be lower.

Estimates of prevalence depend on case definitions. A stringent definition will lead to a low estimate of prevalence whereas if the definition of a case is more loosely defined the prevalence estimates will be higher. In four of the five studies assessed in detail the case definition of haemochromatosis was dependent on the presence of iron deposition in the liver. This strict definition may lead to under ascertainment of cases that might benefit

from treatment since the aim of screening for haemochromatosis is to detect cases prior to the development of organ damage and if the assumption is made that iron deposition in the liver has important clinical consequences these studies have detected individuals who may have benefited from earlier diagnosis.

Despite these the caveats the prevalence of iron overload assumed to be due to haemochromatosis is approximately 1 in 300 and this is similar to the prevalence of the at-risk genotype within Northern European populations (Hanson et al., 2001). In this synthesis the prevalence rates are not adjusted for drop out from the screening process once the initial screening was performed they thus reflect the number of cases detected in an intention to screen population cohort. The fact that the studies reviewed here use a strict case definition of iron overload would seem to provide evidence against the argument that the genotype is rarely expressed. There was however a lack of information as to how thoroughly other reasons for iron overload were excluded and prospective studies of the natural history of iron overload have not been done. Individuals identified with the at-risk genotype and those identified through biochemical screening studies may not be the same individuals although there will be considerable overlap between the two groups.

One of the crucial issues about the effectiveness of screening for haemochromatosis is the uncertainty about the natural history of the condition in terms of the burden of clinically significant disease. The morbidity and mortality of haemochromatosis is uncertain given that hospital admission rates and mortality figures do not accord with the estimate of prevalence above. The degree of the association between the at-risk genotype or biochemical indicators of iron overload and clinically important disease is not answered by any of the studies identified.

Prevalence of iron deficiency
The prevalence of iron deficiency identified by the screening strategies used was nearly ten times that of iron overload. This may reflect the characteristics of the populations in whom it was reported. There may have been a predominance of younger females studied

(Table 2.1). The use of serum ferritin as a screening strategy would be expected to detect iron deficiency more sensitively than transferrin saturation alone. The costs and benefits of detecting iron deficiency need to be included in the evaluation of such a programme.

Uptake of screening

The uptake of screening programmes depends on many factors. The studies that specifically offered screening for haemochromatosis reported uptake of approximately 30%. It was not possible in the reports of these studies to ascertain how much information was given about the screening programme or the disease itself and there were no data on uptake amongst different sub-groups. If the target population for screening for iron overload is males aged 30 - 50 consideration has to be given to how best to access that population. The number needed to screen to detect one case varied widely and is dependent on uptake of screening as well as the performance of the screening test. This would impact on recruitment to future studies designed to determine the risk of disease for individuals identified through screening programmes for haemochromatosis.

Conclusion

The evidence from these studies relates to ascertainment of cases of possible haemochromatosis through population screening programmes utilising assessment of iron status. None of the studies evaluate the health benefits of population screening programmes for haemochromatosis. The prevalence reported using a strict case definition suggest approximately 1 in 300 to 1 in 400 individuals is at risk of iron overload due to haemochromatosis, although it is not possible from these studies to determine the benefits or otherwise of early diagnosis and treatment. In all studies haemochromatosis was a diagnosis of exclusion once other causes for iron overload had been ruled out.

In order to establish the effectiveness of screening programmes for haemochromatosis further studies are needed to elucidate the risk of disease and the effectiveness of treatment, recruitment of cases of early iron overload or genotype positive individuals to these studies will require screening trials. The determination of the risk of disease and the effectiveness of treatment needs an operational definition of a case of haemochromatosis

to provide a clinically important endpoint for the studies. In addition the identification and management of other conditions such as iron deficiency or other liver disease needs to be considered and fully taken account of in the evaluation of such programmes. The design of these studies has to be informed by other research investigating such factors as uptake of screening, the characteristics of the population accepting screening, the setting in which the screening is carried out and the performance of the tests used, whether genotypic or phenotypic.

2.3 Evaluation of screening for haemochromatosis and justification for the study.

It has been suggested that haemochromatosis is a condition where population screening as a case identification strategy could be considered. Discovery of the gene has led to a more careful evaluation of the desirability of population screening as a case finding strategy. In order to advance the understanding of the necessity for screening for haemochromatosis further information is needed not only on the natural history of the condition but also on the likely performance of screening strategies in routine health care settings. An assessment of the performance of the two alternative screening strategies (phenotypic and genotypic) should include an assessment of the feasibility, acceptability and uptake, which may differ according to the test used, the way in which the screening test is offered and the characteristics of the patients it is offered to. It is considered that the use of genetic tests may raise specific concerns that make them less acceptable which would affect the uptake of the screening test. In order to provide the most credible evidence for the effectiveness or otherwise of screening strategies, any studies should be well designed with sufficient statistical power to detect the effect of interest and their design should be informed by additional research to establish the parameters of the screening programmes.

Chapter 3 Aims and objectives of the study

3.1 Aims

The overall aim of this study is to compare genetic strategy and biochemical strategy for screening for haemochromatosis, examining issues of uptake, acceptability, feasibility, yield of cases and cost.

The two screening strategies that will be investigated are:

1. Biochemical screening using transferrin saturation measured on a blood sample at the patient's general practitioners surgery.

2. Genetic screening using PCR, testing for disease associated mutations in a DNA sample obtained from a mouthwash sample, performed by the patient at home.

3.2 Objectives

The main objectives of the study are:

1. To compare the two strategies in terms of their overall uptake and the characteristics of persons accepting screening. This will be used to determine the feasibility of offering screening to a general adult population.

2. To determine the acceptability of offering screening for haemochromatosis to a general adult population by assessing uptake, psychosocial factors such as anxiety, perceptions of health and understanding of test results.

3. To compare the feasibility of the two strategies.

4. To evaluate the consequences of screening in terms of the yield of cases requiring further investigation or management, their resource use and compliance with further diagnostic tests and treatments. To evaluate the additional consequences of offering screening including detection of pathology other than that which was being screened for.

3.3 Research questions

3.3.1 Primary research question

Is the genetic screening strategy non-inferior to the biochemical screening strategy in terms of uptake, acceptability and feasibility?

3.3.2 Secondary research questions

1. What are the characteristics of the individuals accepting screening and do they differ between the two strategies?

2. Do age, sex and social deprivation affect uptake?

3. What is the yield of screen positive case with each strategy?

4. What is the yield of cases requiring further management with each strategy?

5. What is the psychological impact of each strategy as measured by health anxiety and representation of the test results?

6. What are the costs associated with each strategy?

Chapter 4 Methods

4.1 Overview of design and hypotheses

The primary outcome measure in this study was the uptake of screening. In order to establish the absence (or presence) of a clinical difference in uptake between the two screening strategies a randomised controlled trial was the preferred design. As is well established the process of randomisation with concealed allocation removes the potential of bias in allocating individuals to either treatment group, it produces roughly comparable groups balanced for known and unknown confounders particularly if the groups are large. The validity of statistical tests of significance is therefore maintained. It is considered the 'gold standard' design to demonstrate clinical efficacy (Sackett et al., 2000; Altman, 1991; Friedman et al., 1998).

The comparison in this study was between screening for haemochromatosis utilising a traditional strategy i.e. on a blood test at the doctor's surgery and a novel strategy i.e. genetic testing with the sample being taken by the patient at home. The two strategies were pragmatic and included components of the setting as well as the test. It is suggested that genetic testing may be less acceptable than other medical testing therefore the trial was set up to refute this and demonstrate that uptake of screening offered by a pragmatic genetic screening strategy was not significantly lower than that offered in a more traditional manner.

In equivalence/ non-inferiority trials the objective is to test whether a novel intervention is as good as an established one. This raises particular issues in relation to sample size calculations. Absolute equivalence would require an infinite sample size and is therefore impossible to demonstrate. The approach that is used is to predefine a range for the possible difference between two treatments within which the difference is of no clinical significance. If the confidence interval for the observed difference in the trial falls within this range equivalence is said to have been demonstrated (Friedman et al., 1998; Jones et

al., 1996; Blackwelder, 2002). This does correspond to a significance testing procedure in which the roles of the usual null and alternative hypotheses are reversed.

In this study the hypotheses are set up as follows:

Null hypothesis:

> Genetic testing is less acceptable than conventional testing therefore the uptake of screening will be less in the genetic screening arm.

Alternative hypothesis:

> Uptake of screening will be no worse in the genetic screening arm than in the biochemical screening arm.

4.2 Detailed methods

The process of the study is outlined in Figure 4.1 and discussed in detail in the following sections. The structure of this section is in accordance with the CONSORT statement recommendations for the reporting randomised controlled trials (Moher et al., 2001).

The Local Research Ethics Committee gave ethical permission and copies of the patient information sheets, letters and consent form are in Appendix 3. The readability of these were checked, the Flesch readability ease score was 63 and the grade level was 8. Within the word processing package (Microsoft Office XP) it is recommended that a standard document should have a readability ease score of 60 to 70 and a grade level of 7 or 8. In addition guidelines produced by the Plain English Campaign were followed (Plain English Campaign, 2003).

Figure 4.1 Flowchart of
study

GP Practice

Generate list age 30-70
Random sample stratified by age group and sex

Generate letters, pass letter to relevant GP GP reviews list - checks for people known
to have moved or died or who they do not want to be contacted

Randomise, send information sheets and invitation to participate.

Biochemical arm

Clinic at GP surgery

Questionnaire 1

Questionnaire 2

Genetic arm

Send sampling pack

Transferrin saturation (TS): blood test

Genotype: mouthwash

| 15%<TS>45% | TS>45 | TS<15 | YY | YD | YN,DN,NN |

'Normal' letter
patient and GP

Letter GP
& patient

Letter GP
& patient

Letter GP
& patient

'Normal' letter
patient and GP

Questionnaire 3

Hospital
(research clinic)

Genotype, LFT's
Fasting TS, Ferritin
Clinical examination

| 'Normal' | At risk of iron overload Monitor | Treatment Venesection | Treatment Venesection | At risk of iron overload Monitor | 'Normal' |

Letter GP
& patient

Letter GP
& patient

NHS Care

Questionnaire 4

4.2.1 Sample size

As discussed in section 4.1 the nature of a trial of equivalence/non-inferiority means that a different approach has to be taken to sample size calculations. The parameters that are required are an estimate of the proportion of 'successes' that it is anticipated will pertain to the standard treatment, an estimate of the difference between the two treatments beyond which they would not be deemed to be equivalent and the probability of a type 1 error and type 2 error that would be acceptable. It is normal in non-inferiority trials to use a one sided significance level since the aim is to show that a new treatment is not significantly worse than the standard treatment (Wiens, 2002; Jones et al., 1996; Machin et al., 1997). The parameters used to determine the sample size for this study are discussed below.

Proportion of individuals accepting screening: the uptake of screening is very variable as discussed in the literature review. The Department of Health performance indicator for breast cancer screening is 69% (Department of Health, 2002a). The uptake of screening for haemochromatosis derived from the literature review was approximately 35%. Because of the nature of the sample size calculation proportions adding to 100% would give identical sample sizes for a similar power. Sixty five percent was used as the proportion to calculate the sample size.

Maximum allowable difference: Again this is difficult to determine since the variation in uptake that would produce a clinically important effect in screening would depend on the condition being screened for, the costs associated with the screening programme and the clinical consequences of detecting or missing a case. If the maximum allowable difference is set at 5% this would mean that providing the uptake of screening in the genetic arm was no more than 5% different from that in the biochemical screening arm they would be said to be equivalent.

The sample size was determined as follows from statistical tables (Machin et al., 1997). When the sample size in each group is 1427, a test of proportions with a one sided significance level of 0.025 will have 80% power to reject the null hypothesis that the

standard treatment and the new treatment are not equivalent (the difference in proportions being 5% or more from zero) in favour of the alternative hypothesis that the proportions in the two groups are equivalent, assuming that the expected difference is zero and the proportion in the standard treatment group is 65%. The sample size that was chosen was 1500 in each screening arm, i.e. 3000 individuals would be invited for screening.

Other considerations:

The study is not designed to detect sufficient cases of haemochromatosis to draw conclusions relating to the clinical effectiveness of screening for haemochromatosis. However in order to gain some information about compliance with further diagnostic strategies it will be necessary to detect some screen positive individuals and follow them through the study process.

Estimates can be derived from combining results of a number of prevalence studies examining the frequency of the genotype and the frequency of raised transferrin saturation of the possible number of screen positive individuals (Bradley et al., 2000; Olynyk et al., 1999; McDonnell et al., 1998).

The number will depend on the uptake of screening and the number of individuals screened. Table 4.1 gives estimates for 35% uptake and 65% uptake assuming 3000 people are offered screening (1500 in each arm). This shows that with an uptake of 35% approximately 46 individuals might be screen positive and need to be followed up, with an uptake of 65% 84 individuals might be screen positive.

This would be sufficient for the study, allow individuals to be excluded and also fulfil the sample size required above.

Table 4.1 Estimates for the number of individuals who are screened positive.

Percentage with initial raised TS	Percentage with repeated raised TS	Percentage with low TS	Percentage homozygote C282Y	Compound heterozygote C282Y H63D
5.7	2	2.4	0.5	2.4
Numbers of individuals by uptake				
Uptake 35%				
30	11	13	3	13
Uptake 65%				
56	20	23	5	23

4.2.2 Study Sample

A random sample of the population aged between 30 and 70 selected from GP practice registers. The age range of 30 -70 was chosen in order to include those individuals who would be young enough to initiate treatment before the development of complications and also extend the range to those individuals who may benefit from treatment if they had the condition. Screening older individuals also has the advantage that cascade screening of their adult family is easier. The sampling was stratified by sex and two age groups (30-50 and 51-70). Three thousand individuals were selected from two GP practices that were chosen to reflect a diverse population.

Exclusion criteria:

Individuals unable to give informed consent, with current serious illness or any patient that the General Practitioner did not want contacted.

Recruitment of practices

Two practices were recruited to take part in the study. The initial approach was by personal contact. In one practice a presentation was made to a partner's meeting in the other detailed information was sent which was presented to the partners on the research team's behalf.

Median Townsend scores and Jarman under privileged areas (UPA) scores for each practice were obtained from health authority records (prepared in 2000) and the ranking and percentile of the two practices were checked. The scores are calculated from 1991 Census data at ward level. These are standard indices of deprivation (Jarman, 1984; Townsend et al., 1988). With both indices low scores indicate less deprivation. This was done to ensure that the sample for the study came from areas with a range of deprivation. The practice characteristics are shown in table 4.2.

Table 4.2 GP Practice characteristics.

Range for Southampton & S.W. Hants Health Authority	Townsend score -5.74 to 9.95 N=81	Jarman UPA score -29.56 to 49.54 N=81		
	Median Townsend score (ranking)	Median Jarman score (ranking)	Number of partners	Type of area
Practice 1	3.57 (13)	31.0 (6)	4	Urban practice
Practice 2	-0.46 (41)	9.1 (42)	7	Mixed urban semi-rural and rural

Sampling procedure

The GP practices provided a list of their patients aged between 30 and 70 with their sex and dates of birth. Each patient was allocated a unique identification number and this number together with their age group and sex was used to generate the population from which the study sample was drawn. The sample included equal numbers of males and females and equal numbers in each age group. The sampling was random and was performed by computer with the assistance of the Department of Medical Statistics. After the study sample was selected, the identification number was linked back to identifiable information, letters were generated to be sent from the practice and the General

Practitioner was asked to exclude anyone they did not wish to be contacted giving reasons if appropriate.

4.2.3 Randomisation

Randomisation to screening arm was performed after the GP had been given an opportunity to exclude individuals from the random sample. Randomisation to treatment arm was stratified by age group and sex. In addition individuals living at the same address were randomised to the same treatment arm. This was to avoid contamination that might happen if people at the same address were randomised to different treatment arms. The randomisation was performed by computer on the study identification number, with the assistance of the Department of Medical Statistics and treatment allocation could not be influenced by the researcher.

4.2.4 Interventions

The two screening arms are described below.

Biochemical screening arm

> The participant was invited to take part in a study investigating different ways of testing for haemochromatosis. They were offered a blood test to measure iron levels, performed on a sample of blood taken at the GP's surgery by the researcher. The sample was analysed by the hospital chemical pathology service. A transferrin saturation equal to or greater than 45% was categorised as a positive screening test. Participants with a positive result were invited back to a research clinic at the hospital for further evaluation. If transferrin saturation was less than 15% the patient and their GP were informed.

Genetic screening arm

The participant was invited to take part in a study investigating different ways of testing for haemochromatosis. They were offered a test for the genetic predisposition for haemochromatosis to be performed on a saliva sample collected by using a mouthwash sampling method. This was done by the participant at home and posted to the genetics laboratory. Homozygosity for the C282Y mutation or compound heterozygosity for the C282Y mutation together with the H63D mutation was categorised as a positive screening test. Participants with a positive result were invited back to the research clinic at the hospital for further evaluation.

4.2.5 Measures used in the study

Copies of the questionnaires are in Appendix 4.

Primary Outcome Measure

The primary outcome measure was uptake of screening i.e. the proportion of individuals in each arm who provided a sample for analysis.

Laboratory tests

Initial screening tests:

Transferrin saturation:

Five to ten mls. of venous blood was taken by routine venepuncture and analysed in the NHS pathology service at Southampton University Hospitals Trust. Transferrin saturation was calculated from iron and transferrin by the researcher using the following algorithm.

TS %= $\dfrac{\text{iron (umol/L)}}{\text{transferrin (g/L)}\times 20} \times 100$

Transferrin saturation greater than 45% was categorised as a screen positive result. In previous studies 45% has been used to maximise sensitivity without compromising specificity (Bradley et al., 1996a; Hanson et al., 2001).

Genotyping:

The mouth wash sample was collected using a standard protocol developed for use in large scale population studies (Ring et al., 2001) and sent by post to a genetics research laboratory. DNA was extracted again using a standard protocol (Spanakis and Day I, 2001) and analysed for the presence or absence of the two mutations by SSSP-PCR (Sequence Specific Primer-Polymerase Chain Reaction) (Mullighan et al., 1998).

This method uses sequence specific primers to detect the presence or absence of the C282Y and the H63D mutation by producing a PCR product for both the wild type and the mutant allele. Therefore for each DNA sample there are four PCR reactions identifying either the mutation or the wild type allele for C282Y and H63D. Internal control is provided by amplifying a fragment of the human growth hormone gene in order to ensure the reaction is working (Figure 4.2).

Homozygosity for the C282Y mutation (YY) and compound heterozygosity for the C282Y and H63D (YD) mutation was categorised as a positive screening test. At the time of the design of the study there was considerable debate about the penetrance of these mutations, the majority of patients with haemochromatosis are homozygous for the C282Y mutation, a minority are compound heterozygotes for the two mutations. As in the biochemical screening arm a decision was made to maximise the sensitivity of the screening test. The predictive value of compound heterozygosity is less than homozygosity for C282Y, however some individuals will develop iron overload.

Figure 4.2 SSSP-PCR for C282Y and H63D: gel shows normal result for one patient (provided by Maria Gomez)
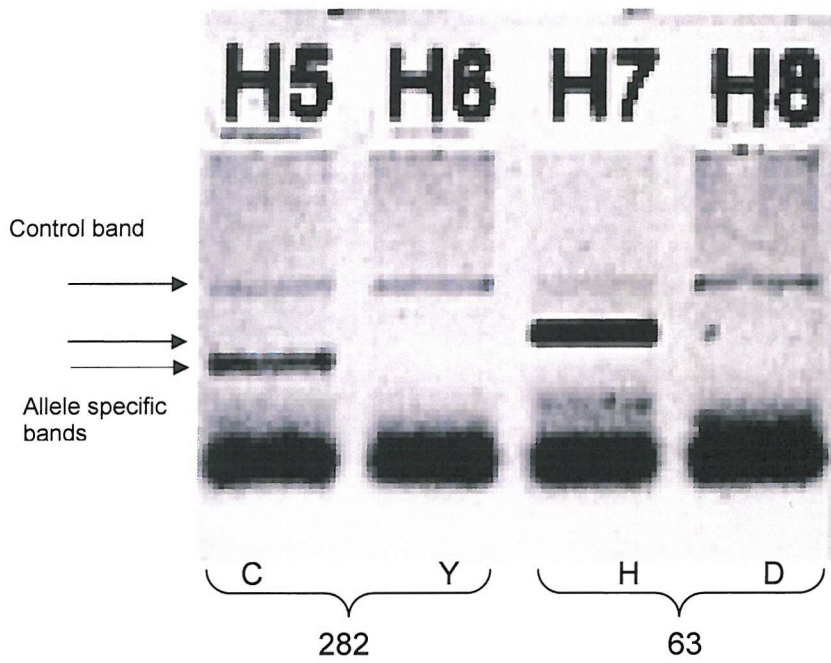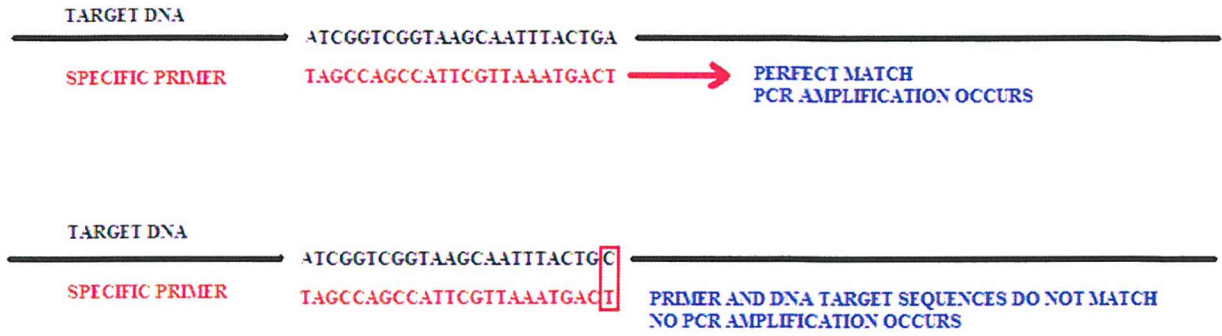
Screen positive individuals:

Individuals who screened positive and were seen in the follow-up clinic had routine blood tests performed on fasting samples obtained for:

- transferrin saturation
- ferritin
- liver function tests, specifically alanine aminotransferase (ALT) which is a marker for chronic hepatocellular disease. ALT was used in preference to aspartate aminotransferase since it is considered to be more sensitive to chronic liver disease, but less likely to be elevated in alcoholic cirrhosis (Bircher et al., 1999).

These were analysed in the NHS pathology laboratory that is subject to accreditation and quality control procedures. In addition a blood sample was taken for genotyping and this was sent to the laboratory that provided the service for NHS patients. It is normal practice to repeat genetic analyses performed in a research setting in a laboratory that provides a clinical service.

Measures of participant characteristics

As discussed previously the data collected prior to sampling were the sex and age group as derived from the general practice registration list. This was used to stratify the sample. In addition postcodes were used to derive ward level deprivation indices for each person in the sample. Using data from the Census website postcodes were mapped to Census wards, which allowed the Townsend deprivation score derived from the 1991 Census data to be used (Census dissemination unit, 2003).

At baseline the following data were collected by self-completed questionnaire

Previous experience of clinical genetics	2 questions
Experience of haemochromatosis	1 question

It might be possible that previous contact with the clinical genetics service particularly if a genetic test had already been performed, would affect the likelihood of accepting screening.

Age of leaving full time education	1 question
Employment	1 question taken from 2001 UK Census questionnaire
Ethnicity	1 question taken from 2001 UK Census questionnaire.

Education and employment may affect choices about screening. Since haemochromatosis is a disorder of Northern Europeans, it is important to establish the ethnicity of the screened population in order to draw generalisable conclusions.

Measures of anxiety, depression and health status

All assessments were made by self-completed questionnaire. Validated tools were used where possible; however attention was paid to the length of the tools used and their relevance to a well population. These issues of feasibility, acceptability and appropriateness have started to be given as much importance in choosing instruments for assessment of health related quality of life as the traditional concerns of reliability and validity derived from psychometric research methodologies (Campbell et al., 2000; Fitzpatrick, 2000).

Anxiety and depression

Assessment of anxiety was made using a six item short form of the Spielberger State-Trait Anxiety Inventory (STAI). The full STAI consists of 20 items assessing trait anxiety (how anxious one is generally), and 20 items assessing state anxiety (how one feels at the moment), and is one of the most widely used assessments of anxiety in psychological research (Bowling A, 2001). A 6 item short form assessing state anxiety has been developed for use in situations where the full 40 item form would not be appropriate (Marteau and Bekker, 1992). This

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6-item form was shown to produce scores similar to the full 20 item STAI with acceptable reliability and was able to distinguish between groups with differing levels of anxiety. It has been used in studies investigating responses to antenatal screening for Down syndrome and was able to detect changes in anxiety similar to those detected using the full form (Marteau et al., 1992). This measure has also been used in other studies investigating antenatal screening for chromosome abnormalities and cystic fibrosis carrier status (Marteau et al., 1997; Axworthy et al., 1996).

The Hospital Anxiety and Depression scale was developed as an attempt to assess depression and anxiety distinct from physical symptoms (Zigmond and Snaith, 1983). The tool was developed from clinical experience and consists of two sections one assessing anxiety, one depression. It has been widely used and is considered to have good validity (it correlates with other depression and anxiety scales and clinical assessment) and internal consistency (Bowling A, 2001). The psychometric properties have not been widely tested. It has been widely used in clinical research and most studies report the depression and anxiety scores separately. There is debate about the factor structure of the tool, however it does appear as if a two factor structure is appropriate with the depression subscale being the most robust (Dagnan et al., 2000). For the purpose of this study the depression subscale was used.

Health status

The participants in this study were selected from an ambulatory general practice population. Therefore measures of general health status that include an emphasis on mobility or self care were not considered to be appropriate, since there would be ceiling effects i.e. respondents would always gain a maximum score since they had no mobility or self care problems. In addition it was felt that the questionnaires had to be short and easy to complete and therefore a multi-item rating was not appropriate. However the SF-36, although considered too long in its full validated form does have a number of domains including a general health

scale. All items from the general health scale of the UK version of the SF36 were used. The SF-36 is a generic measure of health status which has been widely used in a variety of health care settings and in general population surveys (Bowling A, 2001). It is reported to have good psychometric properties with good construct validity, internal consistency and reliability. It has also been used to measure change in health status over time. There are population norms for both U.S. and UK populations including some derived from a postal survey (Bowling et al., 1999). The whole instrument consists of 36 items measuring eight different dimensions. For the purpose of this study only the general health perception dimension was used. The intention was to gain a global assessment of perception of health in a form that was simple to complete. The items in the domain have been developed from the general health rating index which has been validated empirically in a number of studies (Ware et al., 2002).

Measures of understanding

Measures relating to the understanding of the test result and feelings related to the test result were derived from measures used in studies investigating carrier testing in cystic fibrosis and serum screening for Down syndrome. (Hall et al., 2000a; Marteau et al., 1999; Axworthy et al., 1996; Marteau et al., 1997). These measures are also being used in study comparing genetic versus non-genetic diagnosis in families with autosomal dominant hypercholesterolaemia (GRAFT study, Marteau, personal communication). The measures consist of one item assessing understanding of test result, a series of words relating to feelings about the test result, one item assessing confidence in the test result and two items from a new measure being developed assessing the perceived coherence of the result.

The measures are summarised in table 4.3

Table 4.3 Summary of measures

Measure	Baseline *Invitation*	Time 2 *Testing*	Time 3 *Result*	Time 4 *Follow-up*
Employment	X			
Ethnicity	X			
Age completed full time education	X			
Previous experience of genetics	X			
Previous knowledge of haemochromatosis	X			
Deprivation score (derived from postcode)	X			
Perceived health	X	X	X	X
HAD depression scale	X	X	X	X
Short state anxiety	X	X	X	X
Understanding and feelings about result			X	X

Clinical assessments

A clinical assessment was offered to all participants who screened positive. A brief medical history was taken and a clinical questionnaire completed. The questionnaire was developed in other projects relating to haemochromatosis genotypes in patients with specific clinical diseases such as osteoarthritis (Field-Smith, 2000). It elicited information relating to diet, alcohol intake, clinical disease, blood loss and family history (Appendix 4). These factors will affect iron status. Biochemical assessments of iron status and genetic analysis for the two mutations were performed on fasting samples.

Other data

Information relating to subsequent management of persons identified with low transferrin saturation was also collected. A data collection form was designed based on guidelines for the management of iron deficiency anaemia produced by the British Society of Gastroenterology (Goddard et al., 2000) (Appendix 4). The patient's general practice and hospital records were reviewed and the data collection form completed.

4.3 Procedure

The procedure for the study is shown in figure 4.1 as before. For ease of reading this is reproduced in this section.

The Local Research Ethics Committee gave ethical permission for the study and research governance arrangements of the NHS trust were complied with.

After treatment allocation participants were sent an invitation to take part in the study. The invitation was addressed from the surgery and signed by the patient's General Practitioner or the senior partner at the practice. Information about the study and questionnaire 1 were included with this invitation together with a reply paid envelope. Participants were asked to complete the questionnaire and indicate if they would be prepared to take part in the study. They were asked to return the questionnaire even if they did not want to have screening.

> **Biochemical screening arm:**
> If they agreed to screening participants were sent an appointment at the surgery for a blood test. At this appointment signed consent was asked for, the sample was taken and questionnaire 2 was completed. If they failed to attend one other appointment was sent.

Genetic screening arm:

If they agreed to screening participants were sent a pack including a mouthwash sampling kit, the consent form, questionnaire 2 and a letter re-iterating that the sample was for a genetic test for the predisposition for haemochromatosis and inviting them to phone with any questions. In the pilot sample reminders were sent if the sample was not returned. This was difficult logistically and resulted in no more samples being returned. In the main study reminders were not sent if the sample pack was not returned.

Results of the screening were sent by post together with questionnaire 3 and a reply paid envelope for return. Persons who screened positive were given an appointment for the research clinic. Person with low iron levels in the biochemical screening arm were informed of this and advised to seek advice from their general practitioner. The G.P received a copy of the results and it was at the request of both practices that the subsequent management of individuals with low transferrin saturation was decided by the patients own GP

Individuals who screened positive were seen in the research clinic. If subsequent tests were normal they were written to by the consultant and informed of this. If further investigations were necessary they were also written to with an explanation and offered an appointment in the consultant's out patient clinic. Correspondence also went to their GP

A follow-up questionnaire, questionnaire 4, was sent 4 to 6 months after the initial screening result or after the research clinic appointment for those who screened positive. With this questionnaire participants were offered the opportunity to be sent a report when the study was completed.

Figure 4.1
Flowchart of study

GP Practice

Generate list age 30-70
Random sample stratified by age group and sex

Generate letters, pass letter to relevant GP GP reviews list - checks for people known to have moved or died or who they do not want to be contacted

Randomise, send information sheets and invitation to participate.

KEY to Abbreviations:

TS — Transferrin saturation
LFT — Liver function test
HHCC — Haemochromatosis
YY — Homozygous C282Y
YD — Compound heterozygote C282Y H63D
YN — Heterozygous (Carrier) C282Y
DN — Heterozygous (Carrier) H63D
NN — No mutation

Biochemical arm

Questionnaire 1

Questionnaire 2

Clinic at GP surgery

Transferrin saturation (TS): blood test

| 15%<TS>45% | TS>45 | TS<15 |

'Normal' letter
patient and GP

Letter GP
& patient

Letter GP
& patient

Questionnaire 3

Genetic arm

Send sampling pack

Genotype: mouthwash

| YY | YD | YN,DN,NN |

Letter GP
& patient

'Normal' letter
patient and GP

Hospital
(research clinic)

Genotype, LFT's
Fasting TS, Ferritin
Clinical examination

| 'Normal' | At risk of iron overload Monitor | Treatment Venesection | Treatment Venesection | At risk of iron overload Monitor | 'Normal' |

Letter GP
& patient

Letter GP
& patient

NHS Care

Questionnaire 4

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4.3.1 Pilot

The questionnaires were designed to be suitable for electronic scanning. They were shown to a convenience sample of ten different individuals of different ages, sex and background to check for readability and acceptability. They were judged as being acceptable.

A pilot of the procedures was carried out using an initial sample of 250 in the first practice. There were no substantial changes made as a result of this pilot therefore this sample was included in the study sample. However it was apparent that the processing of the DNA samples was taking longer than initially envisaged. This was due to the laboratory having to batch samples to maximise the efficiency of the technique. Because of this a research assistant was employed to work in the research genetics laboratory and perform the DNA analysis, and participants in the genetic screening arm were informed that the result of the screening test would take about a month. Two minor protocol amendments were also made which were approved by the ethics committee.

- Participants expressed some concerns to why they had been selected to take part in the study; therefore a sentence was included in the invitation letter informing them that they had been selected randomly.
- Permission was also given to re-mail non-respondents to see if this increased the response rate. It did not and re-mailings were not done in the rest of the sample.

4.3.2 Data management

Data were managed in accordance with the Data Protection Policies of the NHS trust in which the research was carried out. These data were kept and managed electronically.

The names and addresses of participants were kept separately on a disc that was locked away and only used when personal details were needed to generate letters. Participants were identified by a unique study identification number that linked them to the other

databases. A number of databases were created to manage the data and separate databases were maintained for each GP practice.

An administrative database was created in Microsoft Access to record when letters were sent, when individuals were seen and when questionnaires were returned. This was maintained manually by the researcher.

Laboratory results and information about uptake of screening were entered manually into standard statistical software (SPSS for WindowsV11.5).

Patient questionnaires were directly scanned into standard statistical software (Teleform Elite©).

Paper records of the consent forms, laboratory results and appointment letters were filed individually for each person and kept in a locked filing cabinet.

4.3.3 Data entry and validation of results

All information collected from the study was entered into databases held in the Health Care Research Unit based at Southampton General Hospital. Data were single entered and a variety of checks was made for accuracy and consistency as below.

Data validation included range and consistency checks for each variable to exclude errors in entry.

In previous studies, electronic scanning had been shown to be as accurate as double entering data using a punching agency (Dr T Bryant, Information and Computing Division, personal communication). All questionnaires from the pilot study were checked manually for accuracy of scanning. This check showed that all data that were picked up by the scanner were scanned correctly, however if data were missing it could be due to the scanner not detecting the data point rather than the question not being completed.

Therefore, for all the scanned questionnaires, missing items were checked and those items not scanned were entered manually.

A sample of 10% of the data in each manually entered database was checked for accuracy. It was decided that all the data would be rechecked if there was more than 5% incorrect entry.

Scores for the Hospital Anxiety and Depression Scale, the State Anxiety Scale and the General Health Profile were calculated according to published protocols including following guidelines for the handling of missing data (Marteau and Bekker, 1992; Zigmond and Snaith, 1983; Ware et al., 2000; Bowling A, 2001). As a measure of the internal consistency of each of these scores reliability coefficients were calculated (Cronbach's alpha). These were greater than 0.8 for all three scales consistent with published protocols (Marteau and Bekker, 1992; Zigmond and Snaith, 1983; Ware et al., 2000; Bowling A, 2001). Correlation coefficients were also calculated to check that the scores were correlated in the right direction. The anxiety and depression scores were positively correlated with each other (high scores equivalent to higher anxiety or depression) and negatively correlated with general health score (high scores equivalent to better health) (p<0.01).

4.4 Statistical methods.

Statistical analyses were conducted in SPSS for Windows (V11.5). Standard statistical methods were used for comparing groups. The chi-squared test was used to test the null hypothesis of no association between categorical variables. Fisher's exact test was applied when the sample was not sufficiently large (the smallest of the four expected numbers<5). For continuous data, t-tests were used to compare means. Two tailed p-values are reported. Because the sample size was large when comparing means, the assumptions of the central limit theorem were met and parametric techniques were used. If the distribution was particularly skewed, non-parametric tests were used (Mann Whitney U).

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In order to test for equivalence/non-inferiority the method recommended by Jones et al was used (Jones et al., 1996). If an acceptable range of difference is predefined and if the confidence interval centred on the observed difference falls with the predefined range, equivalence can be said to have been demonstrated.

Since the outcome of uptake of screening is dichotomous, logistic regression techniques were used to estimate predictors of outcome. Analysis of covariance was used for comparing repeated measures between groups adjusting for baseline measurements as recommended by Vickers and Altman (Vickers and Altman, 2001).

4.5 Economic methods

In order to compare the cost of each screening strategy a partial economic analysis was performed. This was from the perspective of the N.H.S., patient and societal costs were not included in the evaluation.

The cost of each screening arm was derived by constructing a decision tree using commercially available software (Data 3.5). Probabilities were all derived from the study and national and local N.H.S. unit costs were used. The unit was one person. The base year for costs was 2000-2001. Discounting was not relevant in this analysis since all costs refer to the same year. The outcome assessed in this analysis was the expected cost (value) for each screening arm. From this figure a cost per case was calculated using the uptake and the detection rate (path probability) for a case requiring treatment or monitoring in the two arms. The downstream costs of identifying these cases and the outcomes are not considered in this preliminary analysis. A full cost effectiveness or cost utility analysis is beyond the scope of this study.

The resource use categories were identified by examining the processes in the two screening arms fig 4.3, the costs associated with these categories, and their sources are described in table 4.4.

Figure 4.3 Process and resource categories

Process   Resources

```
                    ┌──────────┐ ◄───────────── ┌────────────────────┐
                    │          │                │ generate invitation,│
                    │          │                │ clerical, checking  │
                    │Invitation│                │ practice lists      │
                    └──────────┘                └────────────────────┘
                   ╱          ╲
                  ╱            ╲
  ┌────────────────────────┐◄────────────────── ┌────────────────────┐
  │                        │                     │ Consumables,        │
  │Send sample pack(Genetic)│                    │ postage             │
  └────────────────────────┘        ┌──────────┐ └────────────────────┘
              │                      │ Send     │
              │                      │appointment│
              │                      │(Biochemical)│
              ▼                      └──────────┘
  ┌────────────────────────┐◄────────│────────── ┌────────────────────┐
  │                        │         │           │ DNA extraction,     │
  │Sample attempted        │         │           │ processing          │
  └────────────────────────┘         ▼           └────────────────────┘
              │              ┌──────────┐◄─────── ┌────────────────────┐
              │              │ Sample   │         │ Nurse clinic,       │
              │              │ attempted│         │ consumables         │
              ▼              └──────────┘         └────────────────────┘
  ┌────────────────────────┐◄───────│─────────── ┌────────────────────┐
  │Sample analysis/Result  │        │            │ DNA analysis        │
  └────────────────────────┘        ▼            └────────────────────┘
              ╲              ┌──────────┐◄─────── ┌────────────────────┐
               ╲             │ Sample   │         │ Biochemical         │
                ╲            │analysis/Result│     │ analysis            │
                 ╲           └──────────┘         └────────────────────┘
               ┌──────────┐◄───────────────────── ┌────────────────────┐
               │ Screen   │                        │ Consultant clinic,  │
               │ positive │                        │ biochemical tests   │
               └──────────┘                        └────────────────────┘
```

Table 4.4 Categories of resources, costs and their sources.

Category	Resource item	Unit Cost (£)	Source
Invitation	Clerical time checking practice lists and generating invitation	0.81	(Richards et al., 2001)
Sending sampling pack (Genetic)	Consumables, Postage	2.74	From study
Sample attempted (Biochemical)	Nurse clinic (15 minutes)	5.50	Unit Costs of Health and Social Care(Netten and Curtis, 2002)
	Consumables	0.14	From study
Sample analysis/Result (Genetic)	DNA extraction, processing	11.20	From study
	DNA analysis	11.20	
Sample analysis/Result (Biochemical)	Biochemical analysis	6.00	From study
Screen positive	Consultant Clinic (20 minutes)	30.00	Unit Costs of Health and Social Care(Netten and Curtis, 2002)
	Biochemical tests	18.00	From study

Assumptions

Probabilities

The probability of each outcome was calculated directly from the study (see fig 5.1). No allowance was made for resending mouthwash samples to those who failed to return the sample. When this was piloted in the study it did not increase the number of packs returned. In the biochemical screening arm individuals were sent a maximum of two

appointments and the probability of attending includes those who failed to attend one appointment but who attended the second appointment.

Resource categories

The unit costs for invitation are derived from a randomised controlled trial comparing two strategies for inviting women for breast cancer screening, letter alone versus letter and reminder in notes. The costs in the study allocated to primary care in the letter alone arm were extracted and adjusted for inflation using Department of Health inflation indices (Department of Health, 2003a).

Postage of appointment letters was not identified as a separate cost since this administrative task is included in the unit cost for nurse and consultant clinic. However postage for the DNA sampling pack is included since this is an extra cost.

The unit cost of the nurse clinic was based on fifteen minutes of practice nurse time as observed in the study. The unit cost of the consultant clinic was based on twenty minutes of medical consultant time as observed in the study.

Chapter 5 Results

Summary of chapter contents

For ease of reading a summary of the content of the results chapter is presented here.

The recruitment of the study is presented first (5.1) followed by a summary of the study responses, including the response rates for the questionnaires (5.2).

Comparisons of baseline characteristics are made between the two screening arms, the responders and non-responders and by response to invitation and screening arm (5.3). Reasons for not accepting screening (5.4) and additional baseline data are presented (5.5).

Descriptive data on the outcome of screening in the biochemical and genetic screening arms is presented (5.6) including data on the screen positive group. This addresses questions of feasibility and consequences of screening. The performance of the screening strategies is presented in section 5.7 and the outcomes in the low iron group in section 5.8.

In order to address questions of acceptability, uptake and psychological consequences, reasons for not accepting screening are presented (5.4), uptake of screening in the two study arms is presented, equivalence tested (5.9) and factors associated with accepting screening are examined (5.10).

The effect of the screening arm on psychological assessments is examined both over time and between screening arm (5.11).Comparison between the screening outcomes and knowledge and feelings about the test result are made.

A decision tree is presented to compare the costs of detecting cases that require further monitoring or management (5.12).

5.1 Recruitment

A sample of 1500 individuals was randomly selected from the current age/sex register of the two GP practices as per the protocol.

- In practice 1, the individual General Practitioners reviewed the letters and excluded patients they did not want contacted.

- In practice 2, the general practitioners notified the research team of current patients they did not want contacted and in addition, the practice's death and adverse incident records were checked prior to the invitation letters being sent.

  In this practice it was noted that inadvertently, twelve residents of a home for people with learning difficulties had been selected and subsequently randomised to a treatment arm. These individuals should have been excluded and after discussion, they were not contacted and were replaced in the sample by further random selection.

Of the 3000 individuals selected 2938 were randomised and 2930 invitations were sent. Eight patients were randomised and allocated to screening arms that should not have been included. Despite the practice lists and the lists of those recently deceased being checked this included six patients who had died. The practice records in these cases were out of date.

In addition, two patients were allocated to screening arms who were subsequently discovered to have severe learning difficulties. Their carers notified the research team that it was not appropriate for them to be included since they felt they would not understand the implications of the research. Details of excluded patients are given in table 5.1.

Table 5.1 Reasons for exclusion

Reason excluded (N=70)	Not randomised	Post randomisation	
		Genetic	Biochemical
Known to have died	3	5	1
Known to have moved	8		
Does not understand English	11		
Current illness	30		
Unable to give informed consent	7		2
Other	3		
Total	62	5	3

5.2 Summary of study response

The response rates in the study are shown in figure 5.1 in accordance with the
CONSORT statement recommendations (Moher et al., 2001).

Figure 5.1 Flowchart of study

| 3000 in original sample |
| 62 excluded pre randomisation by GP |
| 2938 randomised |

| **Biochemical arm** **1461** **1458 invited** | Excluded 3 | | Excluded 5 | **Genetic arm** **1477** **1472 invited** |

Moved 87		Moved 71
No reply 730 Late reply 2		No reply 742 Late reply 2
Refuse screening 137		Refuse screening 83

| Accept screening 502 | | Accept screening 574 |

| Did not attend clinic 58 | | Did not return pack 76 |
| Withdrew 2 | | Withdrew 1 |

| Sample attempted 442 | | Sample attempted 497 |

| Failed to bleed 6 | | Sample failed in lab 32 |

| Results 435 1 requested mouthwash | | Results 465 |

| TS <15% 28 | TS Normal 351 | TS >=45% 55 | HDCC 1 |

| HHYY 3 | HDCY 4 | DDCC 5 | HHCY 59 | HDCC 103 | HHCC 291 |

Seen in clinic
Fasting TS
Ferritin
Liver function tests
Clinical history

2 withdrew
1 in prison
2 other possible explanation
  kidney transplant
  ? recurrence of
  cancer

Seen in clinic
Fasting TS
Ferritin
Liver function tests
Clinical history

| Discharged 44 | Monitor 3 Genotype DY 3 | Start venesection 3 Genotype YY 1 CY 1 DC 1 |

| Start venesection 2 Genotype YY 2 | Monitor 3 Genotype YY 1 DY 2 | Discharged 2 Genotype DY 2 |

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Table 5.2 shows the response rates for questionnaires. The denominator in each case is the number of questionnaires sent out which changes throughout the study.

Table 5.2 Response rates for questionnaires

	Genetic	Biochemical
Invitations sent	1472	1458
Questionnaire 1 returned (%)	657 (44.6)	639 (43.8)
Sample attempted	497	442
Questionnaire 2 returned (%)	467 (94.0)	415 (93.9)
Result given	465	435
Questionnaire 3 returned (%)	396 (85.2)	356 (81.8)
Questionnaire 4 returned (%)	356 (76.6)	337 (77.5)

The characteristics of those who returned follow-up questionnaires at the end of the study and those who did not are shown in table 5.3. There were statistically significant differences between those who returned questionnaires and those who did not. Those who did not return questionnaires more likely to be registered in practice 1 (the more deprived practice) and to have lower self rated health at baseline. However the actual differences were small. There was no effect of screening arm. Therefore the characteristics of those who did not return questionnaires were similar in each screening arm.

Table 5.3 Characteristics of those who returned follow-up questionnaires and those who did not.

		Returned n=693	Not returned n=207
Age (mean,SD) *		53 (11)	51 (11)
Sex %	male	38	42
	female	62	58
Age finished education (mean,SD)		18 (7)	18 (7)
Townsend deprivation score (median)		-0.29	-0.29
Baseline depression (median)		3.0	3.0
Baseline anxiety (median)		33.3	35
Baseline self rated health (median) *		72	67
Working %	yes	63	70
	no	37	30
GP practice % *	1	50	29
	2	50	71
Screening group %	biochemical	51	53
	genetic	49	47

*p<0.05

5.3 Characteristics of sample

The characteristics of the sample that were randomised are shown in table 5.4. There are no significant differences between the two screening arms.

Table 5.4 Characteristics of whole sample (N=2938)

	Genetic N=1477	Biochemical N=1461	p-Value
Age mean (SD)	49.6 (11.34)	49.7 (11.5)	0.69
Sex (%)			0.94
male	49.9	49.8	
female	50.1	50.2	
GP practice (%)			0.76
1	49.2	48.7	
2	50.8	51.3	
Townsend Deprivation Score (median interquartile range)	0.68 -0.58 to 3.53	0.68 -0.29 to 3.53	0.88

Table 5.5 compares baseline characteristics of those returning invitations and those who did not return invitations. Those who did not return invitations exclude those in whom it was known that they had moved away (158) and those who were randomised in error (8) (Total=166). There are significant differences with individuals who returned the invitation, whether or not they actively refused screening, being more likely to be older, female, to have a lower deprivation score and to come from practice 2, the less deprived practice.

Table 5.5 Characteristics of responders and non responders (N=2772)

	Returned invitation n=1300	Did not return invitation n=1472	p-Value
Age (mean,SD)	52.29, 11.0	47.61, 11.3	<0.001
Sex (%)			<0.001
male	39.7	56.3	
female	60.3	43.8	
GP practice (%)			0.027
1	46.6	50.8	
2	53.4	49.2	
Townsend Deprivation Score (median, interquartile range)	-0.29 -0.58 to 3.53	0.68 -0.29 to 0.68	<0.001

Baseline characteristics by response to invitation

Baseline data for the study sample organised by response and screening arm are shown in tables 5.6 and 5.7. Comparisons were made between screening arms in the response categories: accept screening, actively refuse screening and no reply. The actively refuse screening group were those who returned the questionnaire but declined the offer of screening. The numbers in this group are small and there is a significant proportion of missing data therefore no further statistical comparisons using these data were performed. The characteristics of this group are similar to those who accepted screening.

There are no differences in any of the baseline measures. There is a statistically significant difference in the mean HAD score at baseline in the group that accepted the offer of screening. However there is no difference in the median and when accepted cut-offs for 'possible' and 'probable' depression are examined there is no statistically significant difference.

Table 5.6 Baseline characteristic by response and screening arm

	Accept screening		Refuse screening		No reply	
	Genetic N=574	Bio-chemical N=502	Genetic N=83	Bio-chemical N=137	Genetic N=742	Bio-chemical N=730
Age (mean SD)	51.9 11.1	52.6 10.8	52.7 10.8	52.5 11.5	47.8 11.1	47.5 11.5
Sex (%)						
male	39.4	39.4	36.1	42.3	56.9	55.6
female	60.6	60.6	63.9	57.7	43.1	44.4
GP practice (%)						
1	46.3	46.2	45.8	51.1	52.3	49.3
2	53.7	53.8	54.2	48.9	47.7	50.7
Townsend (median interquartile, range)	-0.29 -0.58 to 3.53	-0.29 -0.36 to 3.53	-0.29 -0.29 to 3.53	0.68 -0.58 to 3.53	0.68 -0.29 to 3.53	0.68 -0.29 to 3.53
Ethnicity (%)						
white	95.3	93.8	67.5	78.8		
other	2.3	3.4	1.2	0.0		
missing	2.4	2.8	31.3	21.2		
Employment (%)						
working	65.2	65.1	45.8	47.4		
retired	19.7	21.1	12.0	19.0		
other	12.9	12.0	8.4	13.9		
missing	2.3	1.8	33.7	19.7		
Age left full time education (%)						
<15	25.4	27.1	22.9	16.1		
16-18	19.7	21.3	10.8	20.4		
>18	26.0	23.7	18.1	20.4		
Missing	28.9	27.9	48.2	43.1		

Table 5.7 Baseline psychological assessments by screening arm and response

	Accept screening		Refuse screening	
	Genetic n=574	Biochemical n=502	Genetic n=85	Biochemical n=139
HAD mean (SD), median (IQR)	3.8 (3.3) 3.0 (1 to 6)	4.3* (3.5) 3.0 (1 to 6)	3.0 (2.4) 3.0 (1 to 5)	3.3 (3.2) 2.0 (1 to 5)
Missing (%)	0.5	1.2	30.1	16.1
HAD				
non case %	94.9	92.3	69.9	78.1
probable case %	5.1	7.7	0.0	5.8
State anxiety mean (SD), median (IQR)	35.8 (12.2) 33.3 (26.7 to 43.3)	36.9 (11.0) 36.7 (28 to 43.3)	35.2 (9.8) 36.7 (28 to 40)	35.4 (10.9) 33.3 (26.7 to 40)
Missing (%)	8.4	12	43.4	29.2
GHP mean (SD) median (IQR)	69.3 (21.9) 72.0 (57 to 85)	67.5 (20.0) 72.0 (57 to 82)	71.3 (17.7) 72.0 (60.8 to 83.2)	69.9 (21.8) 77.0 (62 to 82)
Missing (%)	6.8	6.6	39.8	27.7

*p=0.016 genetic versus biochemical

5.4 Reasons for not accepting screening

Reasons for refusing screening are shown in Table 5.8. The categories were not mutually exclusive and the denominator is the total number of questionnaires returned. The most frequent response was 'not interested' or 'not enough time' and this was the same for both biochemical and genetic screening arms. There did not appear to be a difference in the percentage of individuals who said they did not want a genetic test in the genetic screening arm or who did not want a blood test in the biochemical screening arm. A greater percentage of individuals cited not wanting to know if they had haemochromatosis in the genetic arm than in the biochemical arm.

Table 5.8 Reasons for not accepting screening

Reason for not accepting screening (% of those who responded))	Genetic N=83	Biochemical N=137	p-Value
Not interested	17 (21.0)	30 (22.1)	0.853
No time	29 (35.8)	48 (35.3)	0.940
Do not want a genetic/blood test	22 (27.2)	33 (24.3)	0.635
Do not want to know if I have haemochromatosis	16 (19.8)	13 (9.6)	0.033
Other (Other health concerns, not applicable, moving away, insurance)	9 (11.1)	10 (7.4)	0.343
No reason given	0	3	

5.5 Additional baseline data.

Additional data were collected at baseline relating to previous experience of genetics services, genetic testing or knowledge of haemochromatosis. There were no significant differences between those accepting and those refusing screening. Approximately 2% of

people had been seen previously by a genetics department, 1% had had a genetic test and 9% had previously heard of haemochromatosis (Table 5.9).

Table 5.9 Previous experiences of clinical genetics and knowledge of haemochromatosis.

		Accept screening N=1076	Refuse screening N=220	p-Value	Overall N=1296
Seen by genetics department %				0.506	
	Yes	1.8	0.6		1.6
	No	98.2	99.4		98.4
Previous genetic test				0.149	
	Yes	1.5			1.3
	No	98.5	100		98.7
Heard of haemochromatosis				0.260	
	Yes	8.6	11.2		8.9
	No	91.4	88.8		91.1

5.6 Outcome of Screening

The results of the screening tests are presented in figure 5.2.

Figure 5.2 Results of screening tests.

TS <15% 28	TS Normal 351	TS >=45% 55	HDCC 1	HHYY 3	HDCY 4	DDCC 5	HHCY 59	HDCC 103	HHCC 291

Seen in clinic Fasting TS Ferritin Liver function tests Clinical history	2 withdrew 1 in prison 2 other possible explanation kidney transplant ? recurrence of cancer		Seen in clinic Fasting TS Ferritin Liver function tests Clinical history

Discharged 44	Monitor 3 Genotype DY 3	Start venesection 3 Genotype YY 1 CY 1 DC 1	Start venesection 2 Genotype YY 2	Monitor 3 Genotype YY 1 DY 2	Discharged 2 Genotype DY 2

The frequency of transferrin saturation in the biochemical arm is shown in figure 5.3. The percentage of those who screened positive as a percentage of successful sampling is shown in table 5.10 together with the percentage of those who required further management or treatment. There were a higher percentage of people who screened positive in the biochemical screening arm, but a lower percentage of individuals were advised to have further treatment or management. If the cut-off for the screening test had been set at 55% only 14 individuals would have screened positive. However one of the individuals who were advised to start treatment would have been missed as would the three who were advised to be monitored.

Table 5.10 Percentage screened positive and requiring further management and treatment.

	Biochemical		Genetic	
	Number (% of row above)		Number (% of row above)	
Sample attempted	442		497	
Result given	435	(98.4) (One genetic result)	465	(93.5)
Screen positive	55	(12.6)	7	(1.5)
Monitor or treat	6	(10.9)	5	(71.4)

The frequency of transferrin saturation in the biochemical screening arm is shown in figure 5.3.

Figure 5.3 Transferrin saturation in biochemical screening arm

Std. Dev = 11.87
Mean = 31.9
N = 434.00

Transferrin Saturation
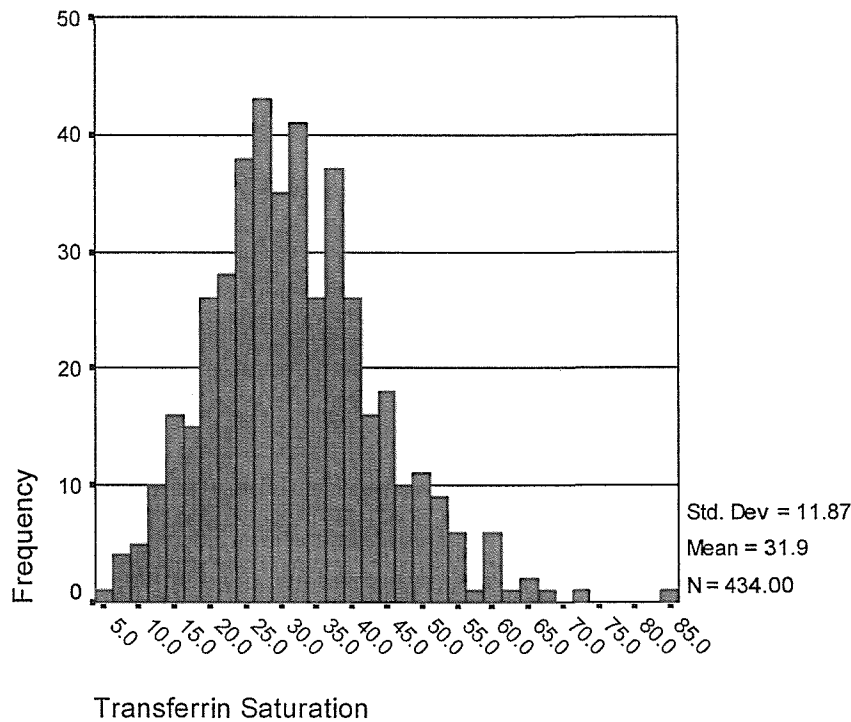
There is a difference in the mean transferrin saturation by sex with males having higher transferrin saturation (Table 5.11). The relationship between transferrin saturation and age was investigated using Pearson product-moment correlation coefficient. There was no association for males and a weak association for females with older age being associated with higher transferrin saturation. This is shown graphically in figure 5.4.

Table 5.11 Transferrin saturation by age and sex

	Males n=174	Females n=260	p-Value
Transferrin Saturation mean (SD)	34.8 (11.9)	29.0 (11.5)	<0.001
Pearson Correlation (Transferrin saturation with age)	0.051	0.118	

Figure 5.4 Transferrin saturation by age and sex.
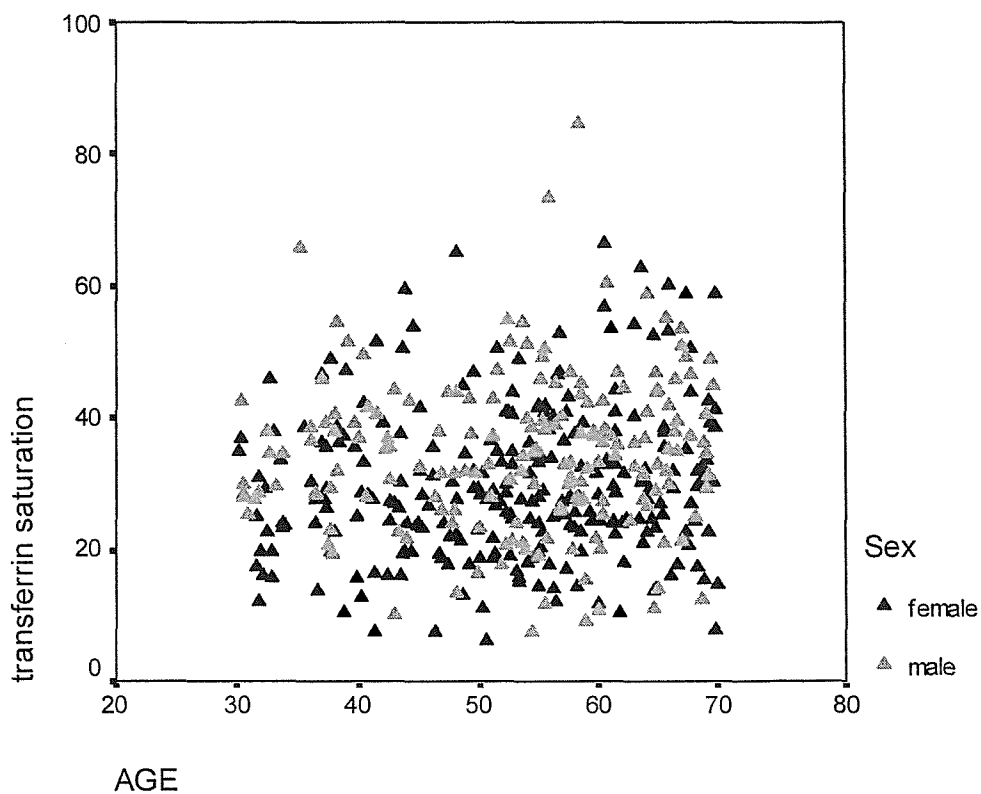
Genotype frequencies in the genetic screening arm are presented in table 5.12 together with reported genotype frequencies for the UK excluding Ireland. Allele frequencies for the study are compared with reported allele frequencies in table 5.13 (Merryweather-Clarke et al., 1997; Hanson et al., 2001). Allele frequencies are the frequency of alleles in the genome taking account of the fact that any individual allele at a specific location on an autosome can have two forms. For example in a hypothetical population of four individuals that have the following genotypes for the C282Y mutation :(Y=mutation present, N=Normal) YY, YN, NN, NN, the genotype frequency for homozygous C282Y (YY) is 25% (one in four), the allele frequency for C282Y is 37.5% (three out of eight). Allele and genotype frequencies in the study population appear to be similar to those reported previously in the general UK population apart from the HDCY genotype. However the numbers are small.

Table 5.12 Genotype frequencies in genetic screening arm

Genotype	Number	Percent	UK genotype frequencies n=368
HHCC	291	58.4	67.7
HDCC	104	20.9	20.9
DDCC	5	1.0	0
HHCY	59	11.8	7.6
HDCY	4	0.8	3.3
HHYY	3	0.6	0.5
failed sample	32	6.4	
Total	498	100.0	

Table 5.13 Allele frequencies

Allele	Ireland	UK	World	Study
C282Y	10.0	6.0	1.9	6.9
H63D	18.9	12.1	8.1	11.8

5.6.1 Outcome of screen positive group

A pragmatic clinical decision was made in individuals who were screen positive and they were assigned to one of three management pathways. The factors that influenced that decision were genotype, iron status, age and sex. Any individual who was homozygous for the C282Y mutation was venesected if their ferritin was raised or monitored if their ferritin was normal. Individuals with other genotypes were venesected if their ferritin was raised and they had disturbed liver function tests. Compound heterozygotes were monitored if they were male with raised iron indices or were female but still menstruating. All other individuals were discharged as being at low risk of developing progressive iron overload.

These decisions are summarised below:

1. Start venesection
    a. YY homozygote, raised transferrin saturation raised ferritin.
    b. Other genotype raised transferrin saturation, raised ferritin, and raised ALT ("idiopathic iron overload").
2. Monitor
    a. YY homozygote normal iron indices
    b. Compound heterozygote, raised transferrin saturation, raised ferritin, male
    c. Compound heterozygote, raised transferrin saturation, menstruating female.
3. Discharge

The characteristics of individuals in each of these three outcome groups are presented in tables 5.14 and 5.15. Table 5.14 presents summary data for the discharged group, full data are presented in Appendix 5. Table 5.15 presents individual data for the other two groups. The decision column refers to the clinical management categories outlined above.

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Table 5.14 Summary characteristics of discharged group n= 46

Age mean (SD)	Sex		Transferrin saturation % Mean (SD)	Ferritin µg/L Mean (SD)	ALT IU/L Mean (SD)	Genotype%	
	Male%	Female%					
54.3 (11.2)	50.0	50.0	44.5 (14.7)	78.2 (49)	44.0 (27)	HHCC	28
						HDCC	35
						DDCC	2
						HDCY	12
						HHCY	23
						HHYY	0

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Table 5.15 Characteristics of non-discharged group

Decision	Outcome	Sex	Age	Genotype	Transferrin Saturation %	Ferritin µg/L reference range (Males 20-250 Females (10-120)	ALT IU/L reference range (5-42)
1a (genetic arm)	Start venesection	female	64	HHYY	108.97	627	38
1a (biochemical arm)	Start venesection	female	64	HHYY	55.34	289	33
1a (genetic arm)	Start venesection	female	33	HHYY	92.04	344	20
1b (biochemical arm)	Start venesection	female	67	HDCC	70.00	1,162	174
1b (biochemical arm)	Start venesection	male	52	HHCY	88.54	611	46
2a (genetic arm)	monitor	female	38	HHYY	31.45	8	10
2b (biochemical arm)	monitor	male	55	HDCY	45.45	359	30
2b (genetic arm)	monitor	male	47	HDCY	73.37	132	31
2c (biochemical arm)	monitor	female	42	HDCY	47.06	60	15
2c (biochemical arm)	monitor	female	33	HDCY	46.03	34	24
2c (genetic arm)	monitor	female	30	HDCY	80.65	29	36

5.6.2 Clinical questionnaire

Clinical questionnaires were completed by those individuals who attended the research clinic (n=57). Four patients did not complete questionnaires; the remaining 53 were fully complete. The results are summarised in table 5.16. In addition assessments were made by self-completed questionnaires of anxiety, depression and self-rated health.

The numbers are too small to conduct statistical comparisons. There was a high frequency of self-reported joint problems in the whole group. Data from the 2001 census suggests that the prevalence of self reported arthritis and rheumatism in the community is 11% in women aged 45 to 64 and 18% in women aged 65 to 74. The prevalence in men is 9% and 12% respectively (Census dissemination unit, 2003). In this study the prevalence of self reported joint problems was 57% overall regardless of the eventual outcome. There was no statistically significant difference between the mean transferrin saturation in those who reported joint problems compared to those who did not (mean transferrin saturation in both groups 49%).

Table 5.16 Summary of clinical questionnaire (n=53)

	start venesection N=5	monitor N=6	discharge N=42
Iron supplements	1	0	0
Vitamin C	1	0	7
Multi-vitamins	2	2	9
Meat eater			
never	0	0	1
<= 3 times per week	2	2	12
>=4 times per week	3	4	29
Mean units of alcohol (SD)	5.4 (5.1)	18.3 (18.3)	9 (9)
Blood transfusion	1	0	8
Blood loss	1	0	1
Heavy periods n=27	1	1	8
Medical history			
liver disease	1	0	1
diabetes (non-insulin dependent)	1	0	1
heart	2	0	3
joints (%)	3 (60)	4 (67)	23 (55)

5.7 Performance of the screening strategies

The outcome of the two screening strategies is shown diagrammatically in figures 5.5 and 5.6.

Combining the data from the two figures means that a 2 by 2 table for each strategy can be constructed and the values in the missing cells estimated (tables 5.17 and 5.18). The

sensitivity, specificity and positive and negative predictive values of the two screening strategies are presented in table 5.19.

For the purposes of these tables the 'case' is defined as a case identified that requires treatment. This conservative definition had been chosen in order not to overstate the performance of the strategies   ·

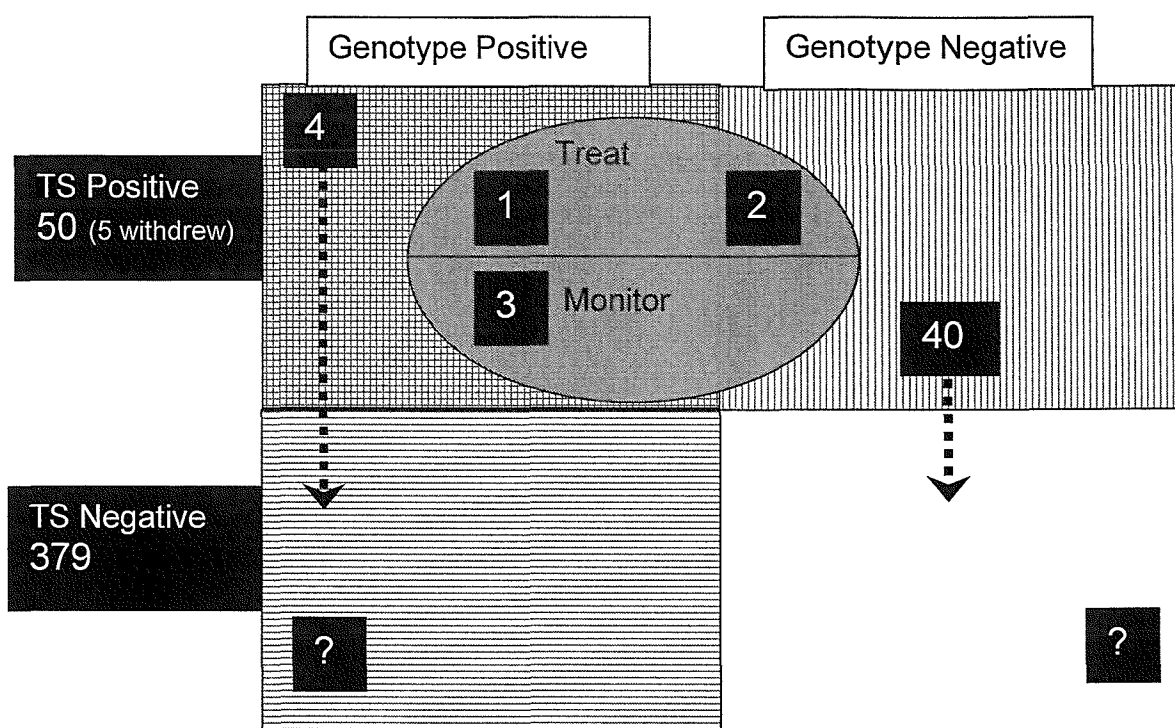Figure 5.5 Cases detected with the biochemical screening strategy.

Figure 5.6 Cases detected with genetic screening strategy
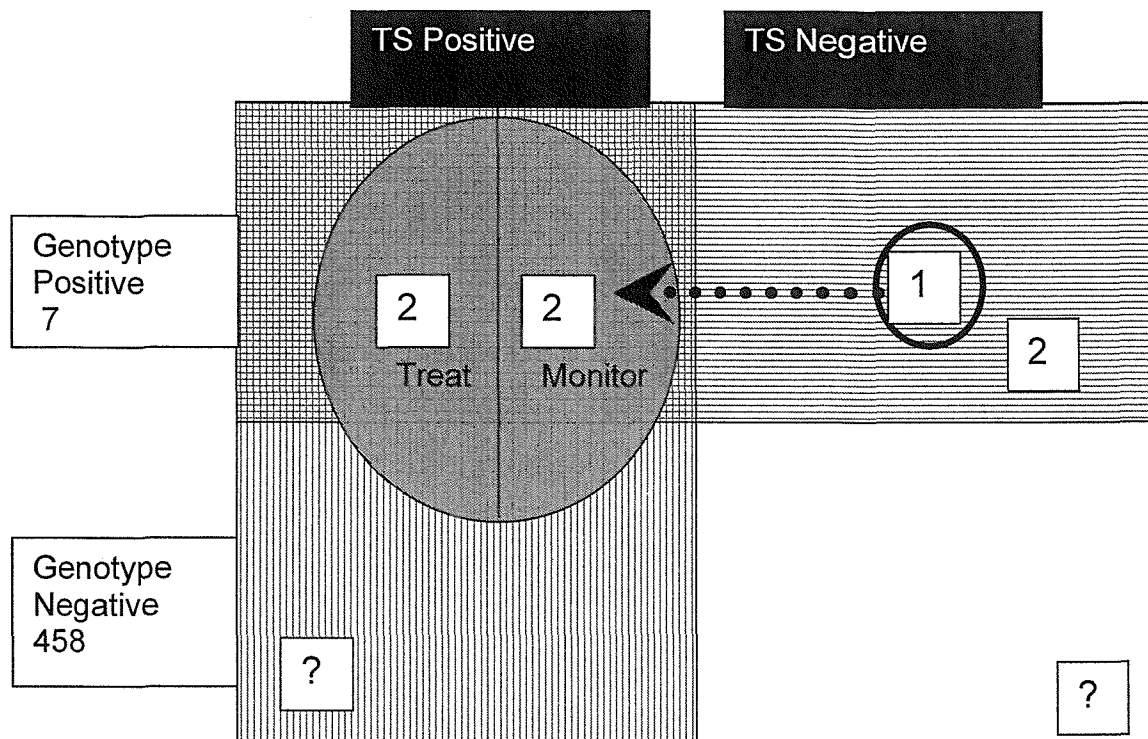

Table 5.17 Detection of cases requiring treatment in biochemical screening strategy

	Case positive	Case negative	
Test positive	3	47	50
Test negative	0	379	379
	3	426	429

Table 5.18 Detection of cases requiring treatment in genetic screening strategy

	Case positive	Case negative	
Test positive	2	5	7
Test negative	3[1]	455	458
	5	460	465

[1]expected value derived from biochemical strategy

Table 5.19 Performance of the two screening strategies

	Biochemical screening strategy	Genetic screening strategy
Sensitivity	100%	40%
Specificity	88%	99%
Positive predictive value	5%	28%
Negative predictive value	100%	99%
Prevalence (95% confidence interval)	0.7% (0.2 to 2.0)	1.1% (0.5 to 2.5)

The number of cases detected requiring treatment in the two arms was similar. If the case definition of a 'case requiring treatment' is used the number needed to screen to detect one case in this study was 586. If the case definition is a 'case requiring treatment or monitoring', the number needed to screen would be 266.

5.8 Out comes in low iron group

One consequence of using assessments of iron status as a screening test will be the identification of individuals with low iron levels. In this study individuals and their GP were notified if the transferrin saturation was less than 15%. In order to determine the consequences of these data relating to the subsequent management of the patient by the GP were collected. These are summarised in table 5.20 and figure 5.7.

Table 5.20 Outcomes in low iron group

Age Mean (SD)	Sex		
	Male	Female	Total
54.3 (10.6)	9	19	28

There were more females in the low iron group; however there was no difference in the mean age. Of the group where data was available ten had a current diagnosis that might have contributed to the normal iron indices. These diagnoses are listed below
:

- Aortic valve replacement on warfarin
- Breast cancer
- Bladder cancer
- Lung cancer
- Psororiatic arthropathy
- Unstable angina severe arthritis
- Oesophagitis
- Pregnant
- Bulbar palsy
- Known iron deficiency anaemia

Figure 5.7
Outcomes in low iron group
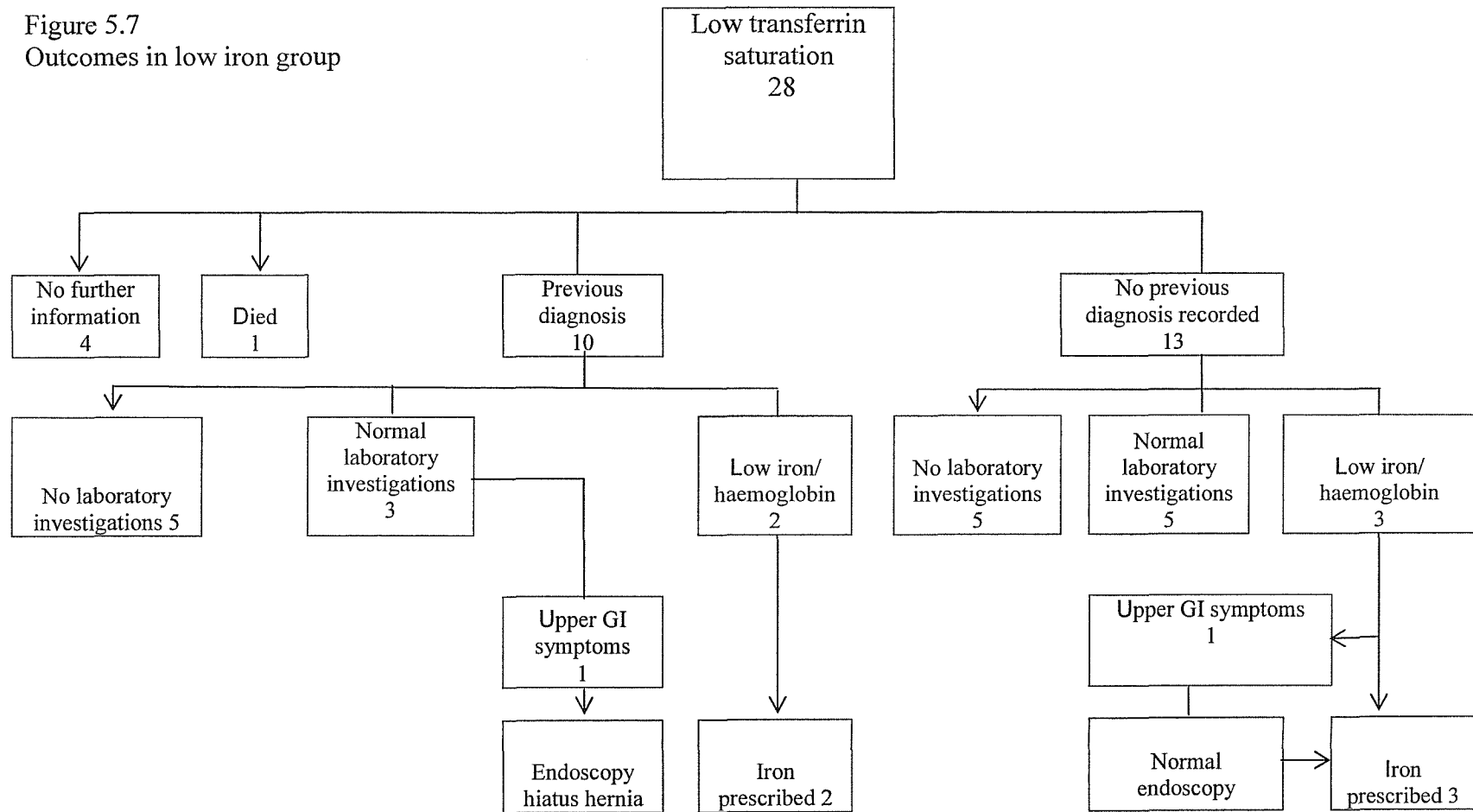
The most common further laboratory investigation was ferritin. Two individuals had endoscopies; however both of these were symptomatic. Two new diagnoses were made, one of iron deficiency anaemia (no cause identified after endoscopy) and one person had a hiatus hernia. Five individuals were prescribed ferrous sulphate.

5.9 Uptake of screening: analysis of equivalence/non-inferiority

The study was designed to test the hypothesis that there would be no difference in the uptake of screening between the two screening strategies, using uptake as a marker for acceptability. The primary analysis is therefore an analysis of equivalence/non-inferiority.

5.9.1 Choice of data and patients to include in the analysis.

It was decided to do two analyses of equivalence/non-inferiority. The choice of data and patients to analyse was derived from the research question, which was to compare two approaches to screening for haemochromatosis offered in a pragmatic and realistic way. An intention to screen analysis was performed, the denominator for the analyses being the number of participants randomised to each arm.

Uptake of screening was defined in two ways:

- people attending for sampling or returning sampling packs i.e. those who showed a clear intent to partake of screening.
- people who received a result. This would take account of the success or otherwise of the sampling procedure and analysis.

Table 5.21 shows the difference in proportions between the screening arms for these two groups. There was a statistically significant difference between the two groups in those in whom the sample was attempted, 34% in the genetic screening arm and 30% in the biochemical screening arm. There was no significant difference when the outcome was result given. This reflects the higher sample failure rate in the genetic arm.
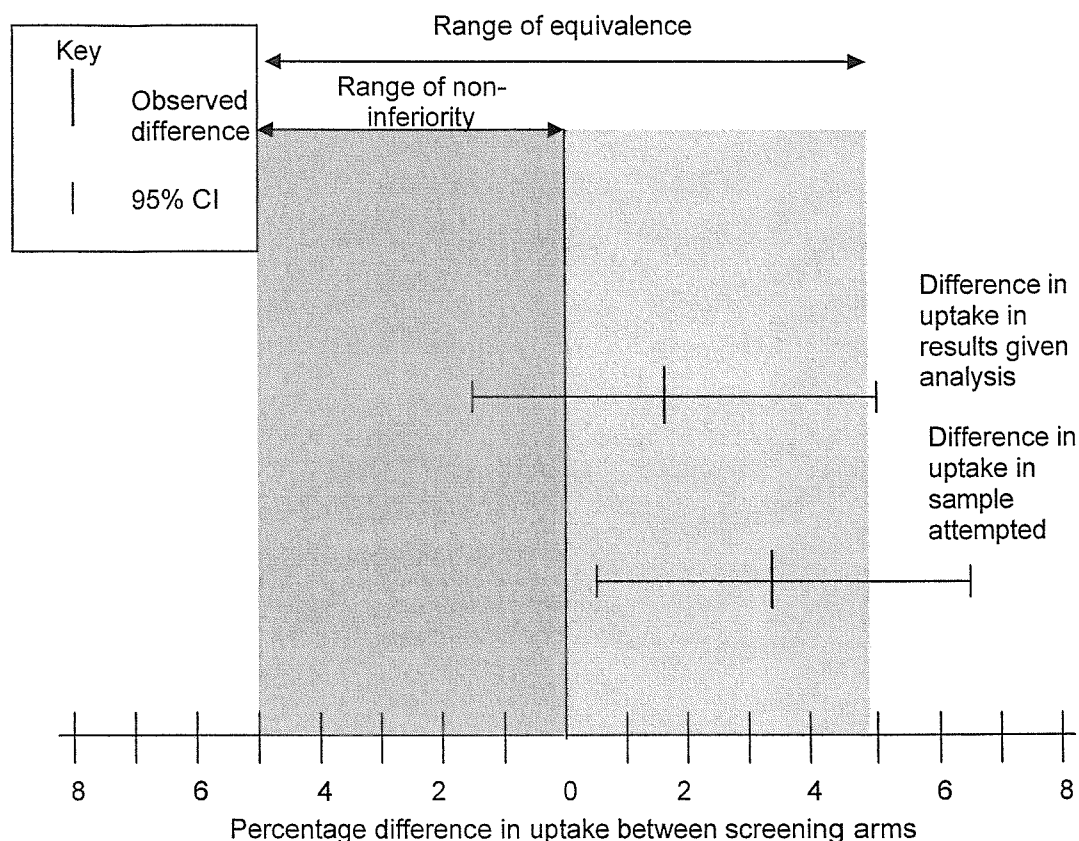
Table 5.21 Uptake of screening

		Genetic	Biochemical	p-Value	Difference in proportions (95% CI)
Sample attempted	Yes	497	442	0.048	
	No	980	1019		
Percentage sample attempted		33.6	30.3		3.4 (0.5 to 6.8)
Result given	Yes	1012	1026	0.303	
	No	465	435		
Percentage result given		31.6	29.8		1.7 (-1.6 to 5.0)

Figure 5.8 represents the results of testing for equivalence/non-inferiority. The confidence interval when the outcome is sample attempted falls outside the previously defined range of equivalence of 5% either side of a null difference. The confidence interval when the outcome is result given is at the limits of the range of equivalence. It should be noted that conventionally equivalence/non-inferiority studies are designed to demonstrate that a new treatment, in this case genetic screening is no worse than standard treatment, a one sided test.

The uptake for both outcome measures in the genetic screening arm was no worse than in the biochemical screening arm. When the outcome measure was sample attempted the difference was 3.4% in favour of the genetic screening strategy (p=0.046).

Figure 5.8 Test for equivalence/non-inferiority

5.10 Factors affecting the uptake of screening

Logistic regression techniques were used to estimate odds ratios using 'sample attempted' as the binary outcome variable. Sampling attempted was used since this reflects an intention by the participant to have screening. Table 5.22 presents unadjusted and adjusted odds ratios for the factors that were associated with the outcome variable. All factors on which data was available were included in the initial model. A significance level of less than 0.05 on a univariate analysis was used as the inclusion criterion. GP practice and interaction terms of age and sex, age and screening arm and sex and screening arm did not contribute and were excluded in the final analysis. Figure 5.9 illustrates graphically the effect on uptake of screening of age and sex.

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Table 5.22 Factors affecting uptake of screening (sample attempted)

N=2938		Univariate (Unadjusted)			Adjusted		
Factor		p-Value	Odds Ratio	95%CI	p-Value	Odds Ratio	95%CI
Townsend Deprivation Score Above vs. below median		0.002	1.282	1.093 to 1.504	0.004	1.272	1.079 to 1.498
Age	<40		1			1	
	40-49	0.021	1.336	1.044 to 1.708	0.012	1.378	1.074 to 1.768
	50-59	<0.001	2.169	1.739 to 2.706	<0.001	2.279	1.821 to 2.852
	60-70	<0.001	2.892	2.293 to 3.647	<0.001	2.930	2.316 to 3.707
Sex Female vs. Male		<0.001	1.871	1.598 to 2.191	<0.001	1.926	1.639 to 2.264
Study Group Genetic vs. Biochemical		0.048	1.169	1.001 to 1.366	0.038	1.181	1.06 to 1.386

Figure 5.9 Sample attempted by age and sex (percentage)
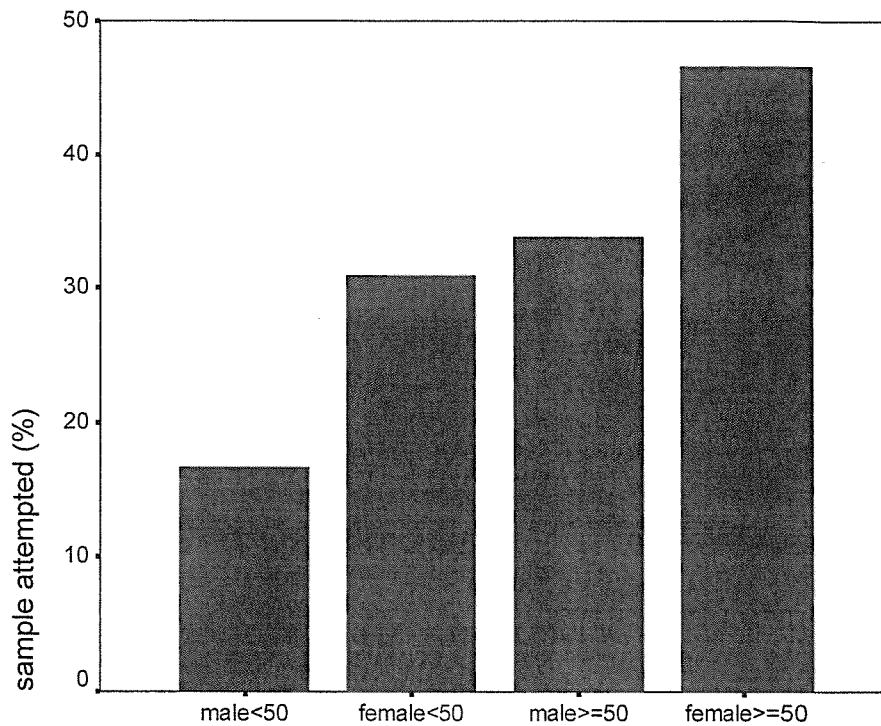
5.11 Analysis of psychological assessments

To answer questions relating to the acceptability of the two screening strategies and in order to determine if there was any difference between them a series of psychological assessments were performed. These were the depression subscale of the Hospital Anxiety and Depression scale (HAD) the State Anxiety Inventory (STAI) and the General Health Profile of the SF36 (GHP). They were self-completed at baseline, testing, and result giving and three to six months after the result. In addition the screen positive group completed the assessments at the follow up clinic. With the HAD and the STAI higher scores indicate higher levels of anxiety and depression, with the GHP higher scores are a measure of better health.

5.11.1 Comparison between screening arms

In order to determine if there was a difference in psychological state between the two screening arms during the process of screening comparisons of the psychological assessments at each time point are shown.

Figures 5.10 to 5.12 show box plots of each of the measures over the four time points split by screening arm.

Figure 5.10 Depression score by screening arm over time (higher scores=more depressed)
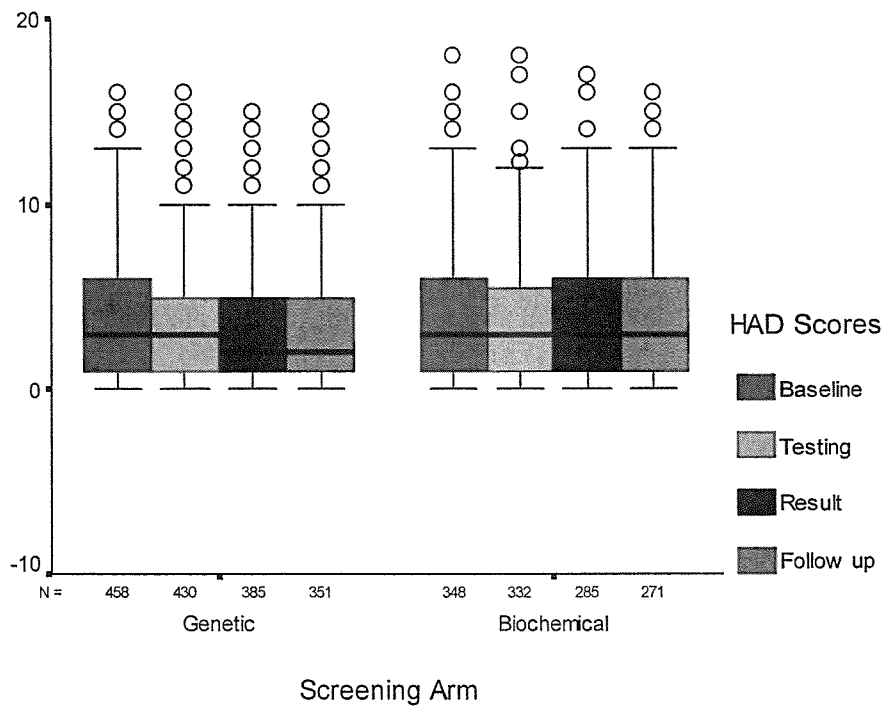
Figure 5.11 State anxiety score by screening arm over time (higher scores=more anxious)
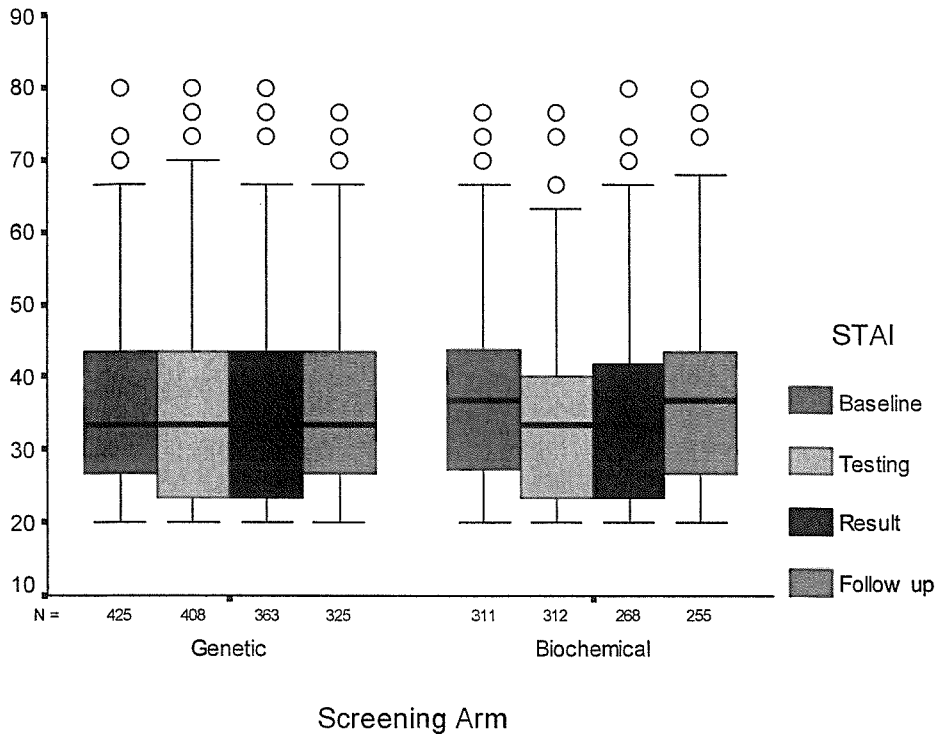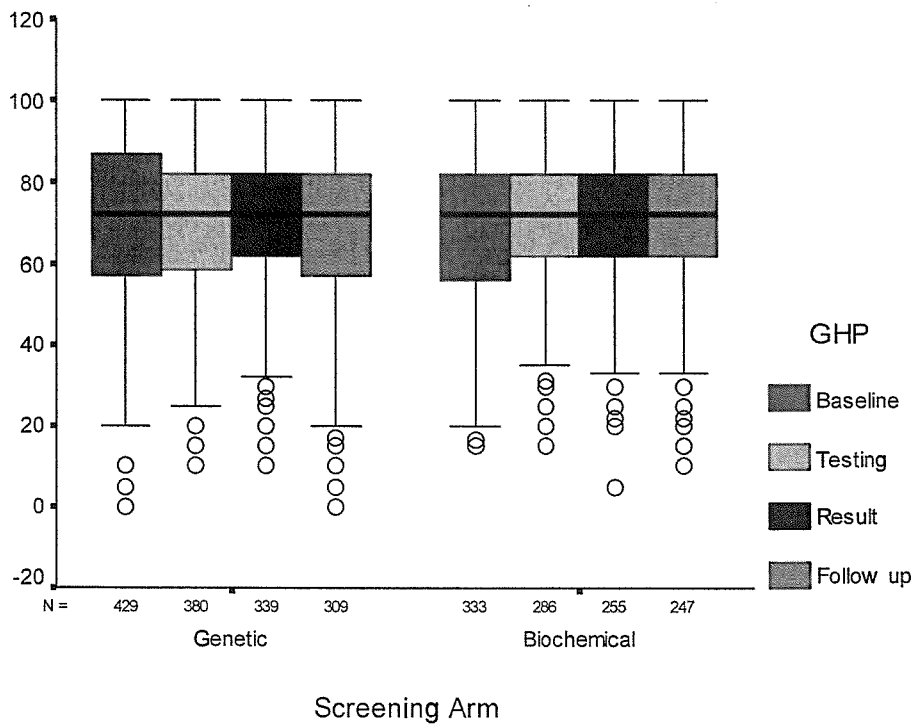

Figure 5.12 General health profile by screening arm over time (higher scores=better health)

A one-way between groups analysis of co-variance was conducted to compare the psychological assessments between the two screening arms at the four time points. The independent variable was the screening arm and the dependent variable was the particular psychological measure at testing, result or follow up. The psychological assessment at baseline was used as the covariate in each analysis.

Table 5.23 presents significance tests for each analysis at each time point. Adjusted means and confidence intervals are presented for those assessments where there was a statistically significant difference between the two screening arms.

There is a suggestion that there was a small statistically significant difference between the genetic and biochemical screening arms at the time of the testing with the participants in the biochemical screening arm scoring lower on the depression and anxiety scales and higher on the health profile. There was a 1 point difference on the health profile at result giving with the biochemical screening arm having higher scores. These differences had disappeared by follow-up.

At follow up there was no significant difference between the two screening arms on the depression and anxiety scales or in the self-rated health scale. Equivalence was tested using the same method as before. The limit of difference was set at plus or minus ten percent of each scale. The general health profile subscale of the SF 36 is divided into bands of ten points for the purpose of comparison with other scales. The depression scale of the Hospital Anxiety and Depression scale and the State Anxiety Inventory are divided into three bands for clinical comparisons. The choice of plus or minus ten percent of each scale as the limit of equivalence is therefore conservative. This is presented in table 5.24 and graphically in figure 5.13

Table 5.23 Psychological assessments over time by screening arm

Assessment Time	Assessment Type	p-Value	Adjusted Means (95% CI)	
			Genetic	Biochemical
Testing	HAD	<0.001	3.9 (3.8 to 4.1)	3.4 (3.2 to 3.6)
	STAI	0.005	34.8 (33.9 to 35.7)	32.9 (31.0 to 33.9)
	GHP	<0.001	69.0 (68.0 to 70.1)	72.0 (70.9 to 73.1)
Result	HAD	0.327		
	STAI	0.057		
	GHP	0.017	69.5 (68.4 to 70.3)	71.5 (70.3 to 72.6)
Follow up	HAD	0.986		
	STAI	0.693		
	GHP	0.057		

Table 5.24 Difference in psychological assessments by screening arm at follow up (questionnaire 4).

	Adjusted means			95% CI	Limits of equivalence (±10% of scale)
	Biochemical	genetic	difference		
HAD	3.839	3.842	-0.003	-0.513 to 0.507	±2.100
STAI	35.646	35.966	-0.320	-2.283 to 1.643	±8.000
GHP	70.860	69.034	1.826	-1.364 to 5.016	±10.000

Figure 5.13 Equivalence test of psychological assessments at follow up.

Difference in means of psychological assessments at end of study by
screening arm (Biochemical-Genetic)

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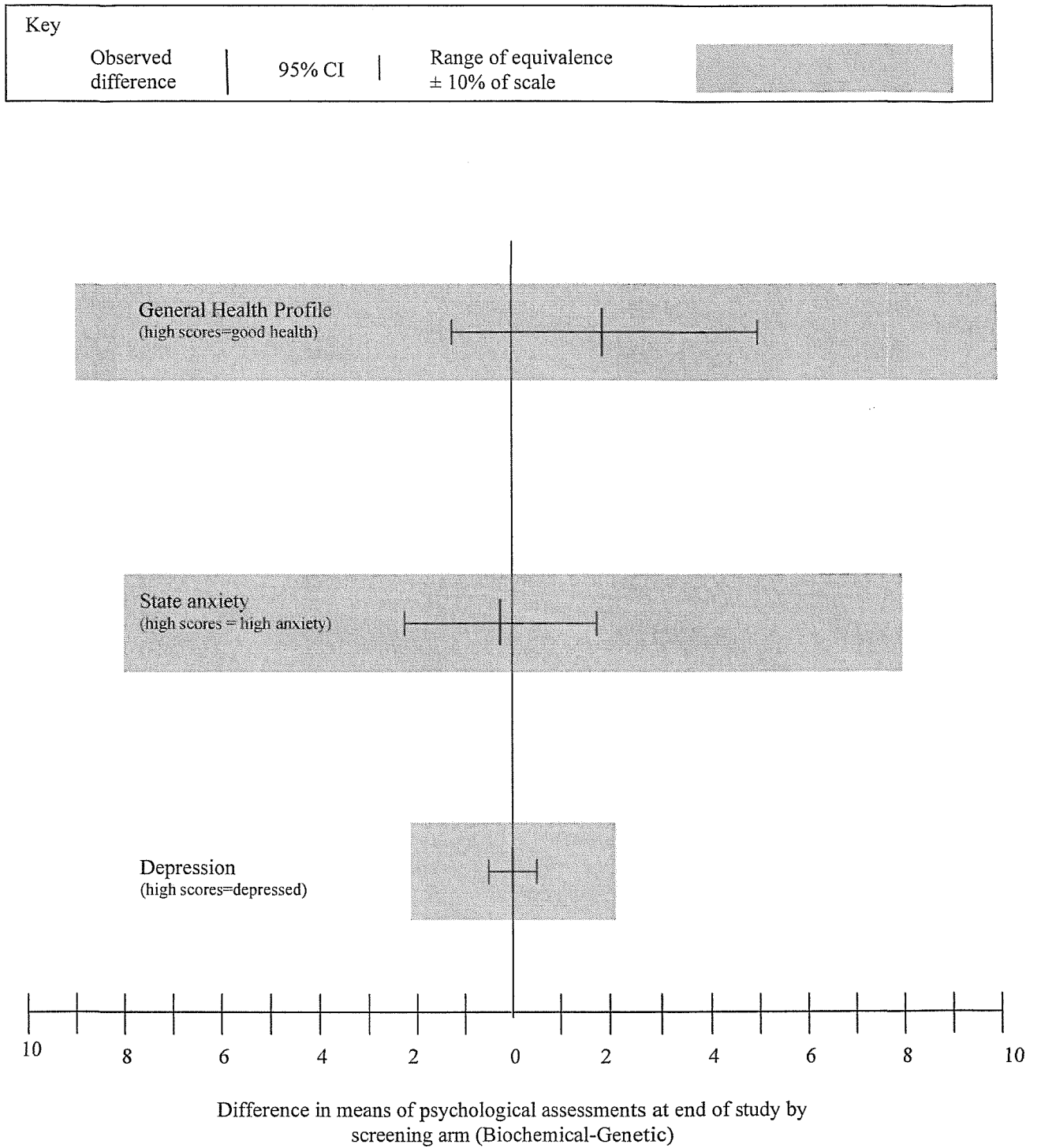
5.11.2 Comparisons between screening outcomes

In order to assess if there were any differences in psychological adjustment by screening outcome (low iron, screen negative and screen positive) a one-way between groups analysis of co-variance was conducted to compare the psychological assessments between the screening outcomes at the four time points. The independent variable was the screening outcome and the dependent variable was the particular psychological measure at testing, result or follow up. The psychological assessment at baseline was used as the covariate in each analysis. There were no significant differences.

The same analysis was performed to test if there were any differences between the screen positive and screen negative groups. There were no significant differences.

5.11.3 Changes in psychological assessments between baseline and follow up (3-6 months).

In order to assess the impact of the intervention on individuals, change scores were calculated for each psychological assessment by subtracting the final score from the baseline score. Figures 5.14, 5.15 and 5.16 show the distribution of these change scores.

A negative score reflects an increase in the depression and anxiety scores i.e. a negative change, whereas a positive change score of the general health profile reflects a decrease in self-rated health i.e. a negative change.

Figure 5.14 Change in depression score between baseline and follow-up

change in depression score
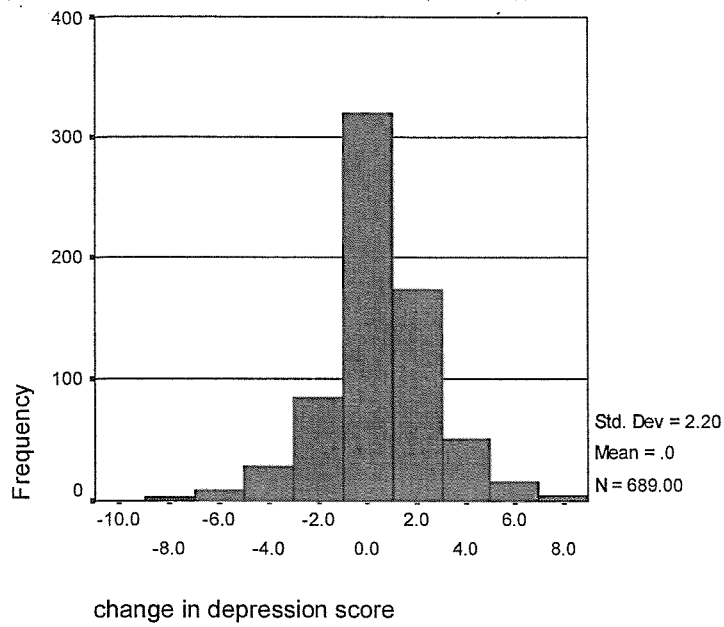
Figure 5.15 Change in anxiety score between baseline and follow up

change in anxiety score
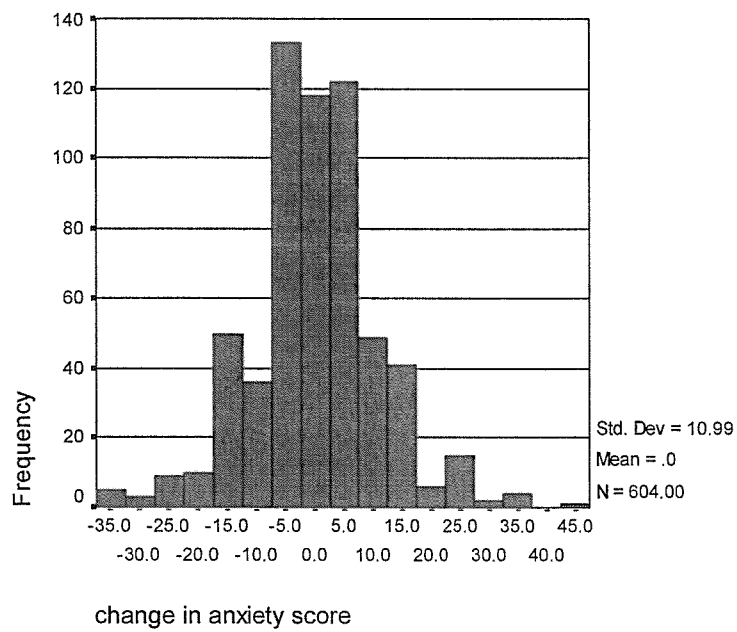
Figure 5.16 Change in self-rated health between baseline and follow-up
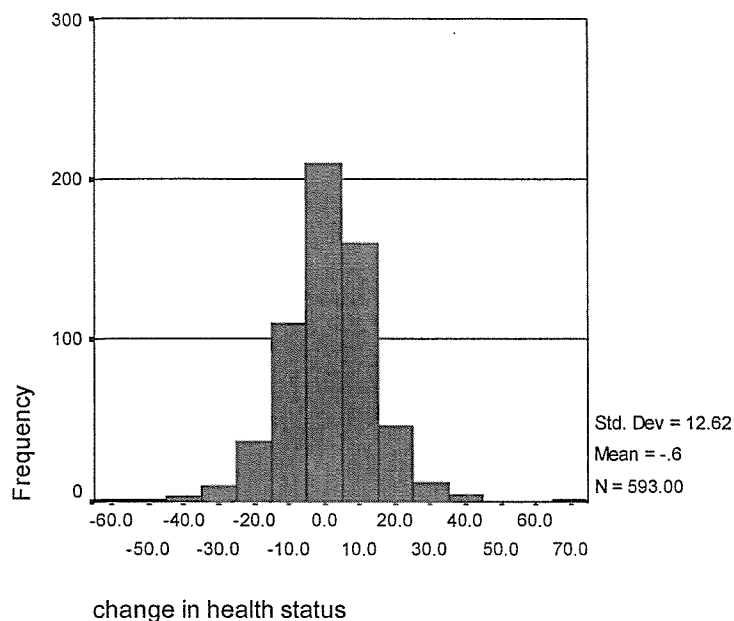
The change scores were categorised into two categories, a change in a negative direction and no change or a change in a positive direction. Table 5.25 summarises these results.

Table 5.25 Categories of change scores by psychological measure

	HAD	STAI	GHP
No/positive change (%)	65.3	59.1	60
Negative change (%)	35.7	40.9	40

Approximately two thirds of individuals show either no change in these assessments or a change in a positive direction, t- tests were performed to compare the mean change in each assessment by screening arm and by screening outcome. There were no significant differences.

5.11.4 Feelings about test results.

The emotional responses to the test results were assessed by asking participants to tick words that best described their current feelings about the test result at result giving and follow-up. For ease of presentation these were divided into positive, negative and neutral statements. Figure 5.17 presents the percentage that responded in each category, the denominator being the number of returned questionnaires in each group multiplied by the total number of statements in each category. The categories were not mutually exclusive and the non-responses are not included therefore the percentages do not add up to 100.

Figure 5.17 Feelings about test results at result giving by screening outcome.
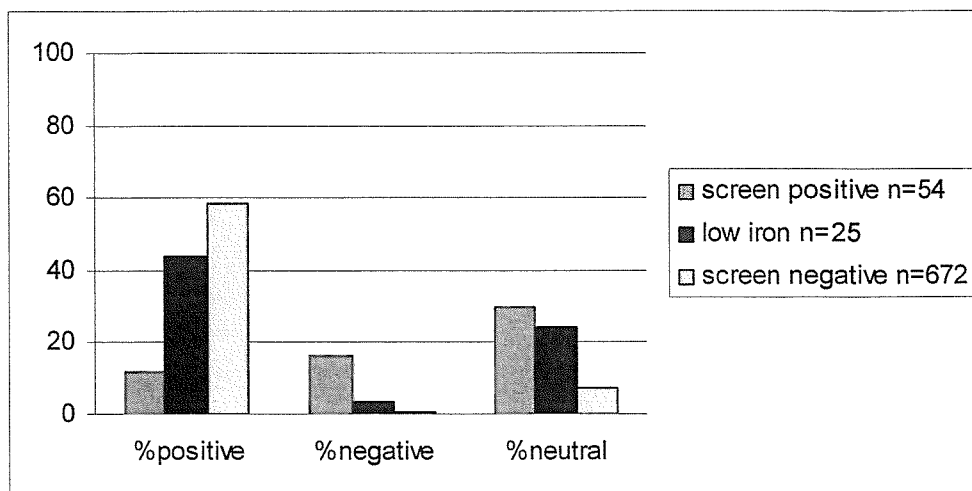
There are differences in the pattern of feelings about the test result depending on the screening outcome, the screen positive group producing more negative statements than the other groups. Further analysis of the actual statements showed that those in the screen positive group were more likely to report feeling surprised and worried and less likely to report feeling happy or pleased (P<0.001).

The same questions were asked at follow up and the results were broadly similar. In the screen positive group the numbers in each outcome are too small to perform statistical tests. However those who start venesection or who are monitored were more likely to

report negative feelings and less likely to report positive feelings, than those who were discharged.

Feelings about the test result at the time of the results are compared between the genetic and biochemical screening arms in the same way as previously. The outcome of screening in the two arms is different in that more people in the biochemical screening arm screened positive and would be expected to be more worried. Therefore only the screen negative group is analysed. These data are presented in figure 5.18.

Figure 5.18   Feelings about test result by screening arm
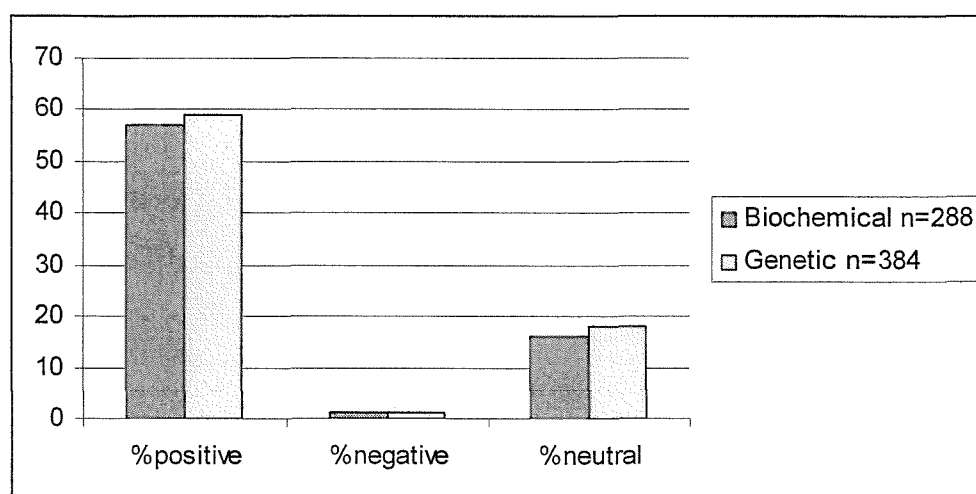
There appears to be no difference in the percentage of positive and negative feelings by screening arm in the screen negative group who returned questionnaires.

5.11.5 Knowledge and understanding of test results.

Participants were asked to indicate what they believed was the result of the test when the result was received and at follow-up. Table 5.26 cross tabulates the result that was given with the belief about the result. There are 18 (3%) individuals in the screen negative group who chose the statement saying they almost certainly did have haemochromatosis. The same table is presented for the belief about test results at follow-up (Table 5.27). Again 18 of the screen negative individuals believed they had haemochromatosis and a

further five thought they probably had haemochromatosis. By follow-up 9% of people who responded said, they either had not been given or did not remember their result.

Table 5.26 Beliefs about result of screening by actual result at result giving

| | | Screening outcome | | |
		Low iron	screen positive	screen negative
knowledge at result	almost certainly do have haemochromatosis	0	0	18
	probably do have haemochromatosis	1	9	5
	probably do not have haemochromatosis	7	32	125
	almost certainly do not have haemochromatosis	17	4	505
	have not been given a result	0	6	0

Table 5.27 Beliefs about result of screening by actual result at follow-up

		start venesection	monitor	normal	low iron	screen negative
Knowledge at follow up	almost certainly do have haemochromatosis	4	0	1	1	18
	probably do have haemochromatosis	0	1	0	0	5
	probably do not have haemochromatosis	0	3	8	5	76
	almost certainly do not have haemochromatosis	0	1	23	14	429
	have not been given a result	1	0	2	1	33
	do not remember result	0	0	0	0	25

In order to test whether the screening arm, age group or sex affected the likelihood of non-agreement between belief about results and actual result, chi-squared tests were conducted at result giving and follow-up. There were no significant differences.

Only two individuals in the screen negative group expressed the belief that they almost certainly/probably did have haemochromatosis at both result giving and follow up. Of the remaining sixteen, four did not return follow up questionnaires and twelve said they did not have haemochromatosis at follow up. In addition there was only one individual in this group who appeared to have non-agreement between the actual result and their belief about the result who indicated they had any negative feelings about the result, the remainder had positive feelings about the result. This might suggest that this question had not been correctly filled in for some individuals.

A further measure had been included in the questionnaire relating to how confident, puzzled or whether the result made sense to the participant. These questions were not from a validated questionnaire. The question relating to how confident the respondent felt in the result was analysed despite a non-completion rate the questionnaire of 18% at result giving and 29% at follow up. There was no significant difference between the two screening arms, genetic and biochemical. Non-completion rates were greater than 30% for the second two domains, these were therefore not analysed.

5.12 Economic analysis

The model that was constructed is shown in fig 5.19.

Figure 5.19. Decision tree for screening decision
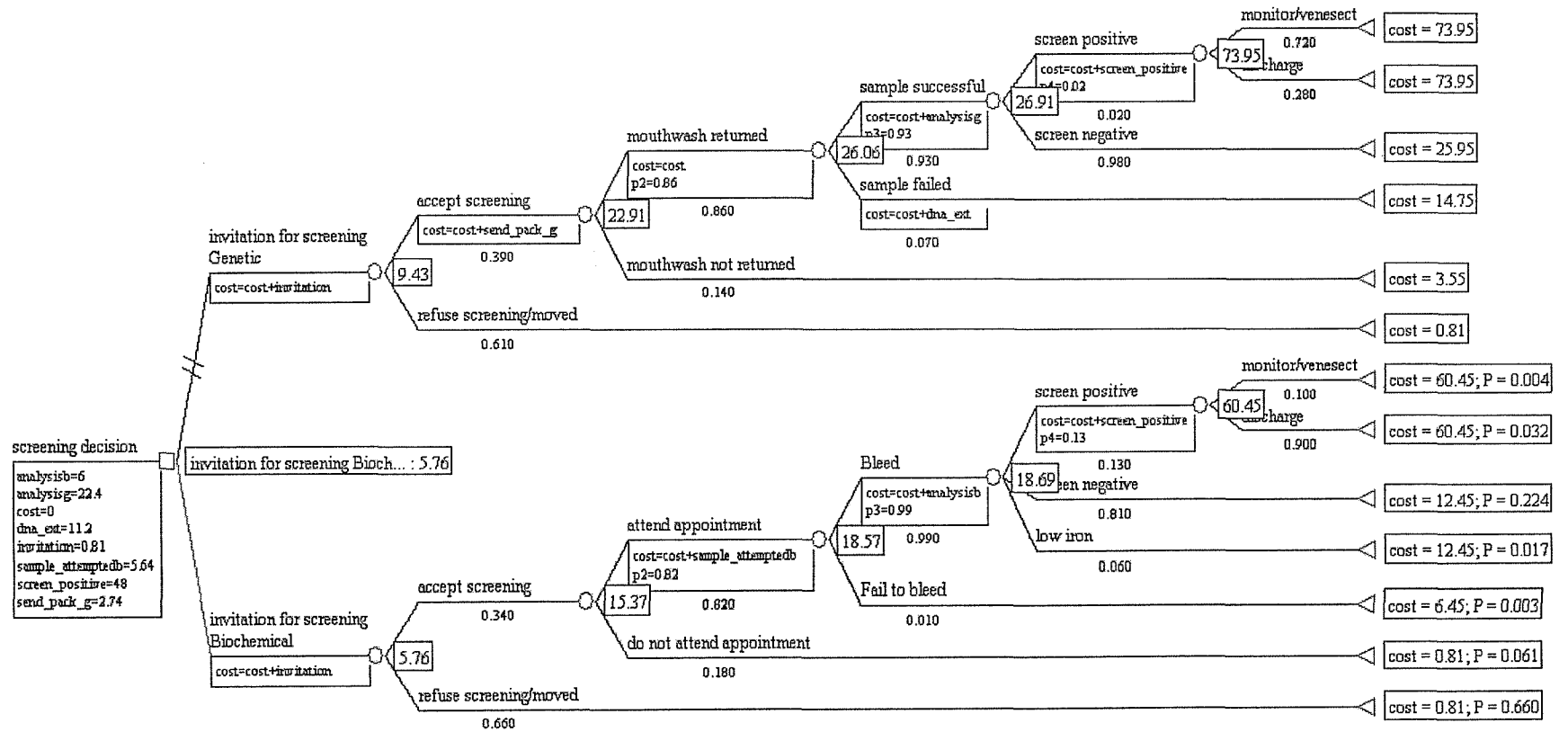
The biochemical screening strategy is shown to have the lowest expected cost per case detected using the parameters derived from the study. In order to assess the implication of varying uptake of screening the model was run using an uptake of 75%. An uptake of approximately 75% is achieved in screening programmes for breast and cervical cancer (Department of Health, 2002a). However it should be remembered that the cost estimates refer to the uptake that was achieved in the study. The cost of achieving an uptake of 75% is not known in this context and caution should be exercised in interpreting these extrapolated results. The results are shown in table 5.28.

The biochemical screening arm still produced the lowest expected cost. A one way sensitivity analysis was conducted to establish the threshold for the cost of DNA analysis.

Table 5.28 Results of decision tree analysis

	Expected cost of screening one person taking into account all outcomes.		One way sensitivity analysis. Threshold value for cost of genetic analysis	Cost per case (expected value*1/path probability)	
	Genetic screening arm	Biochemical screening arm		Genetic screening arm	Biochemical screening arm
Uptake in study	£ 9.43	£ 5.76	£10.64	£2357.50	£1440.00
Path probability	0.004	0.004			
Uptake 75% NB No extra costs included.	£17.38	£11.73	£11.70	£1931.111	£1466.25
Path probability	0.009	0.008			

The threshold of the cost of DNA analysis at which genetic screening is equal to biochemical screening is £10.64 when the uptake is as in the study (approximately 34%) and £11.73 when the uptake of screening is 75%. The cost per case in the biochemical screening arm is higher when the uptake is 75%. This is despite the extra costs of achieving this uptake not being included in the analysis. It probably reflects the lack of specificity of the biochemical test i.e. there is a high false positive rate. The main driver that affects the decision evaluation appears to be the cost of DNA analysis.

5.13 Summary of results

- Uptake of screening was equivalent when the test was one sided, i.e. the difference in uptake and the lower limit of the confidence interval fell within the previously defined limit of 5%. Uptake of screening in the genetic arm of the study was no worse than in the biochemical arm. Uptake in the genetic screening arm was 33.6%, in the biochemical screening arm it was 30.3%. This difference was statistically significant p=0.048.

- There were no significant differences in psychological assessments between the two screening arms over the time of the study. The psychological assessments at follow up were equivalent in the two screening arms.

- The screening strategies did not appear to cause significant changes in psychological status over time as assessed by the measures used.

- Both strategies were feasible and detected cases. The number needed to be screened to detect one case was 586 if a case was defined as a case requiring treatment and 226 if a case was defined as a case requiring monitoring or treatment

- There were no statistically significant differences in the characteristics of those accepting screening between the two strategies.

- Being female, aged over 50 and being less socially deprived was associated with uptake of screening.

- When the case was a 'case detected requiring treatment', the sensitivity of the biochemical screening strategy was 100% compared to 40% in the genetic screening strategy. The positive predictive value of the biochemical screening

strategy was 5% compared to 28% in the genetic screening strategy. In the biochemical screening arm three cases required further monitoring and three treatment. Only one of the cases requiring treatment had the at-risk genotype. In the genetic screening arm three cases were identified who required monitoring and two who required treatment.

- There were no significant changes from baseline in the psychological assessments in either strategy. There was a lack of congruence in the result given and the result as it was assessed by the participant in 3% of the sample.

- Of the 28 individuals who were detected as having low transferrin saturation levels 10 had previous diagnoses which could have contributed to the low transferrin saturation. Twelve had further investigations and two new diagnoses were made.

- The cost per case detected in the biochemical screening arm was £1440 and £2357 in the genetic screening arm.

Chapter 6 Summary of findings.

The study was a randomised controlled equivalence/non-inferiority trial comparing a biochemical and a genetic screening strategy for haemochromatosis. The main and secondary findings will be reported and then discussed in chapter 7.

6.1 Main findings

- The primary research question was whether the biochemical and genetic screening strategy were equivalent in terms of the uptake and acceptability and feasibility of screening. The study showed that uptake of screening offered using the genetic screening strategy was no lower than when it was offered using the biochemical strategy. The overall uptake was approximately 34%. The assessments of psychological state at the time of follow up were also equivalent.

- There was no difference in the acceptability of the two strategies as assessed by uptake of screening, changes in psychological assessments and reasons given for not wanting screening.

- Both strategies were feasible and detected cases. The study showed that the genetic screening strategy was successful and amenable to high volume testing. The biochemical screening strategy did not result in an unmanageable clinical load of either iron overload or iron deficiency.

- The factors that were predictive of accepting screening were age, gender and social deprivation. Older women living in less deprived areas were more likely to accept the offer of screening. Uptake in males under the age of 50 was 16%, in comparison to an uptake of screening of 46% in women over the age of 50.

- The numbers of cases requiring further monitoring or treatment were similar in each arm: six cases with the biochemical screening strategy and five in the genetic screening strategy. The biochemical screening strategy was more sensitive than the genetic screening strategy and had a lower positive predictive value.

- The cost per case requiring further management in the study was less in the biochemical arm than in the genetic arm.

6.2 Secondary findings

- Compliance with further treatment and management was high with only two individuals in the screen positive group declining to be followed up.

- The biochemical screening arm detected 28 people (6% of those sampled) with transferrin saturation less than 15%. These were possible cases of anaemia. Two new diagnoses were made in this group, although they were not fully evaluated.

- Three percent of those in the screen negative group indicated that they believed they might have haemochromatosis despite having been told they were very unlikely to develop haemochromatosis. There appeared to be no association with age, gender or screening arm. This belief was inconsistent between result giving and follow-up and did not appear to be associated with negative feelings.

Chapter 7 Discussion

7.1 Study design

As discussed in the introduction the most widely accepted description of the principles and theory of screening is the 1968 World Health Organisation report by Wilson and Jungner in which it is made clear that the purpose of screening is to identify previously unrecognised disease in order to intervene and reduce the risk of adverse outcomes associated with that disease (Wilson and Jungner, 1968). This has now been expanded in order to take into account more rigorous requirements for evaluation which includes negative as well as positive outcomes and consideration of effectiveness.

The UK National Screening Committee's criteria for evaluating screening programmes are in Appendix 1 and were discussed in relation to haemochromatosis in section 2.1. (NSC, 1988) . In this study we were evaluating two possible screening strategies for haemochromatosis addressing questions relating to:

The test:-it should be simple, safe, precise, validated and acceptable to the population

The screening programme:- the complete screening programme (test, diagnostic procedures, treatment/ intervention) should be clinically, socially and ethically acceptable to health professionals and the public. The benefit from the screening programme should outweigh the physical and psychological harm (caused by the test, diagnostic procedures and treatment).

At the time of the design of the study it was considered that there were two possible ways of screening for haemochromatosis: using a genetic test or using a biochemical test. In the context of screening the specific test is only one part of the screening intervention, the setting and the way in which the test is offered should also form part of the evaluation. A

pragmatic approach was taken to the design of the study and we compared two different screening strategies rather than simply comparing the tests.

Transferrin saturation is considered to be 'gold standard' (Witte et al., 1996; Edwards and Kushner, 1993) for biochemical screening in haemochromatosis and since it is measured on blood, the participant has to be seen in a clinical setting. The majority of screening takes place in a primary care or community setting and therefore the strategy in this arm of the study was to take the blood in the GP surgery.

It was considered that an advantage of a genetic test would be that it could be done on samples other than blood and therefore did not require clinical intervention. Again a pragmatic screening strategy was designed in which the participants were able to take the sample themselves at home and post it directly to the laboratory.

Since the time of the design of the study the Human Genetics Commission has produced a report on direct access to genetic tests that raises concerns about genetic tests where the sampling is performed at home and posted to a laboratory. These concerns include issues of understanding, consent and sample identification (Human Genetics Commission, 2003).

The remit of this report is tests supplied directly to the public without a medical intermediary so the situation is not directly comparable to this haemochromatosis study, but there are similarities that might mean that ethical concerns would be raised if the study was being designed now. However the UK colorectal cancer screening pilot used a similar approach, sending out sampling packs (faecal occult blood) which were posted back to a central laboratory , with the results being sent by post (The U.K.CRC Screening Pilot Evaluation Team, 2003). In this pilot there have been no ethical concerns raised and the strategy appeared to be acceptable.

In summary the study addressed the evaluation and comparison of two different screening strategies for haemochromatosis. The discussion will follow the structure outlined below

which addresses the evaluation and comparison initially followed by the strengths and weaknesses of the study. The study will then be put into a wider context followed by suggestions for future research.

- Comparison of the two strategies
    - o Uptake and acceptability.
    - o Feasibility

- Outcomes of screening in this study
    - o Performance of the screening tests
    - o Detection of other disease
    - o Negative outcomes

- Strengths and weaknesses of the study

- Screening for haemochromatosis

- Haemochromatosis in the context of the 'new genetics'

- Suggestions for future research

7.2. Uptake and acceptability and feasibility of the two screening strategies.

7.2.1 Uptake and acceptability.

Criteria for the evaluation of screening emphasise that the screening programme must be acceptable to both the participant in and the provider of the programme.

Uptake of the components of a screening programme can be a measure of acceptability in that if the programme is not acceptable people will not take part. However acceptability also involves the concept of not causing psychological harm or negative consequences. It is difficult to disentangle the two components. For ease of reading the findings of the study relating to acceptability will be discussed followed by those relating to uptake, although as said before this is a rather arbitrary distinction.

In addition to uptake, acceptability can be assessed by examining reasons for not participating in the screening programme. In this study, for both screening strategies, the main reason given for not taking part was lack of interest or time, not reasons relating to the programme itself. The response rate was low and there is no information as to what individuals who did not return questionnaires thought about the screening programme.

It would have been interesting to have been able to interview a proportion of these people who did not respond in any way to the invitation for screening. However given that they had chosen not to take part in the study it would have been unethical to contact them. In the pilot study a further invitation was sent to those who did not reply to the first invitation, but this did not increase the response rate. This illustrates the difficulty of finding out reasons for non-response, a difficulty that is common to most clinical research.

The acceptability of the programme may also be affected by the type of screening test offered and the way in which it is offered. In this study two different screening strategies

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were used and comparisons were made between the two strategies. The differences in the strategies are summarised below.

Table 7.1 Differences between the two strategies

	Screening Strategy	
	Biochemical	**Genetic**
Setting	GP surgery	Home
Type of test	Biochemical assessment of iron	DNA analysis
Method of obtaining sample	Blood test	Buccal cells from mouthwash

Because of the way in which the study was designed it is difficult to separate out which were the major factors which contributed to the overall uptake and acceptability of the two strategies.

One question that can be considered is the acceptability of a genetic test compared to a biochemical test. As discussed in the introduction it is considered by some commentators that genetic tests require special consideration because of their predictive nature, the potential for causing anxiety, the implications for the rest of the family , the complex nature of the information and the potential for discrimination . It may also be that genetic tests are less acceptable to the public because of concerns surrounding DNA technology. In the Human Genetics Commission's public consultation for the report on genetic tests supplied directly to the public there was a belief expressed that genetic tests are somehow different from other medical tests although acceptability was not directly questioned (Human Genetics Commission, 2003).

In this study when people were questioned about why they would not have screening 27% of those who responded in the genetic screening arm said they did not want a genetic test and 24 % in the biochemical screening arm said they did not want a blood

test. The frequency of responses to the other questions was the same apart from more people in the genetic screening arm saying they did not want to know if they had haemochromatosis. Only one person commented that they were concerned about insurance issues, they were randomised to the biochemical screening arm and had recently been discovered to have raised cholesterol leading to problems with an insurance company.

The response rate was so low in this part of the study that it is difficult to interpret the findings. Nevertheless, they would tentatively support the conclusion that there is little difference in acceptability between a biochemical test and a genetic test as offered in this study. It is interesting to note that the only concern raised about insurance was as the result of the previous identification of a biochemical risk factor not a genetic one.

A further assessment of acceptability is whether the programmes themselves caused significant increases in anxiety or depression or caused a reduction in self-rated health. In the assessments used in this study there were no significant changes either between screening arms or by outcome of screening. People who screened positive did report more negative feelings about the result than the people that screened negative. These differences disappeared in those people who screened positive and had further tests that resulted in them being discharged. The screening programme appears to be acceptable in that it did not cause long term psychological disturbance. Again we do not know the outcome in those that did not return questionnaires. Those who did not return questionnaires differed in some characteristics from those who did but were not different between the two arms. Therefore although the study may have underestimated the psychological disturbance that might have been caused overall, the non-return rate would have had little difference on the comparison of the two strategies.

The uptake of the screening in both arms was low (overall 34%). The uptake was slightly higher in the genetic screening arm than the biochemical screening arm also supporting the view that the genetic testing was no less acceptable than the biochemical test. Although this result is confounded by the fact that the setting in which the test was

offered was different, the setting effect would have had to be significant in order to alter this interpretation.

Evaluation of the uptake of screening should include the initial screening test and any consequent test and treatment. Compliance with further investigations and management was high, only two people actively withdrew from the study after they screened positive.

Non-compliance with further investigations in other examples of screening programmes including data from the literature review is summarised in table 7.2. The screening programmes are U.K. based programmes offering screening to an adult general population

Table 7.2 Non-compliance rates in U.K. screening programmes

Haemochromatosis screening study. Did not attend follow up clinic.	3%
Review of haemochromatosis screening studies (Table 2.4) Did not attend for second screening test.	6%
Review of haemochromatosis screening studies (Table 2.4) Did not attend for diagnostic test (liver biopsy/phlebotomy).	14%
Colorectal cancer pilot screening study. Did not attend for colonoscopy(The U.K.CRC Screening Pilot Evaluation Team, 2003).	13%
Aortic aneurysm screening study. Did not attend for clinical follow up (Scott, 2002).	8%
Breast and cervical cancer screening programmes (Office of National Statistics, 2003a; Office of National Statistics, 2003b)	not reported

Acceptability as assessed by compliance with further investigations would seem to be similar in this study to the other haemochromatosis screening studies and the study of aortic aneurysm screening. Where the further investigation is invasive, such as liver biopsy or colonoscopy, compliance is lower.

The above discussion addresses issues of acceptability for the patient. Acceptability of the screening programme to the provider was not directly addressed by this study. One consequence of a screening programme might be to increase the workload of the patient's GP by initiating more consultations or investigations. We did not collect data on this apart from in the group who were identified with low iron. This did lead to further consultations, investigations and prescription of iron. The quality of the data is very poor and it is difficult to assess whether this extra workload was burdensome to the GP. If screening for haemochromatosis were to be initiated in a primary care setting then the extra workload would probably fall on the primary care team.

In the evaluation of the costs of the two strategies, despite the biochemical strategy requiring 15 minutes of nurse time, the cost per case detected was still lower than that in the genetic strategy. This was due to the cost of the DNA test. But it should be noted that in terms of the GP workload the nurse time is a cost that they might bear, the cost of the test being borne by the laboratory. It is possible to speculate that a biochemical strategy may therefore become less acceptable to the primary health care team if it were not properly funded. However there are not enough data from this study to address questions of acceptability to the provider.

The review of studies offering screening for haemochromatosis reported in the background literature review found no papers that reported factors that affected the uptake of screening (section 2.21). The uptake of screening in this study is similar to the uptake in previous studies of the general population where individuals were randomly selected to be invited for screening for haemochromatosis (Burt et al., 1998). The screening strategy had a small effect on the uptake of screening. The uptake in the genetic screening strategy was 3.4% higher in than the biochemical screening arm.

The factors that were shown to affect the uptake of screening in this study were:

- sex   women were more likely than men to accept the offer of screening
- age   older people were more likely than younger people to accept the offer of screening.
- deprivation   people from less deprived areas were more likely to accept the offer of screening than those from more deprived areas.

For the studies and programmes that were reported in table 7.2 factors affecting the uptake of screening and the overall uptake are presented in Table 7.3. The review of haemochromatosis screening studies is not included, since these studies included diverse populations and it is not possible to extract the relevant information or it is not reported.

The overall uptake of screening is calculated and the numbers having screening broken down by age and sex are reported where it was possible to do this from data in the published reports. For ease of comparison these data are also presented for this haemochromatosis screening study.

In the results section for this study, the data for age and sex have been presented as the uptake in that particular group in order to compare uptake, rather than as a percentage of those accepting screening. The percentages by age and gender in those having screening are presented table 7.3.

Table 7.3 Factors affecting screening

Study	Overall uptake	Proportions having screening by sex and age		Deprivation
Haemochromatosis screening study.	34%	males 30-50 female 30-50 male 51-70 female 51-70	13% 24% 26% 36%	less deprived>more deprived
Colorectal cancer pilot screening study (The U.K.CRC Screening Pilot Evaluation Team, 2003).	53%	male 50-59 female 50-59 older>younger	52% 61%	less deprived>more deprived
Aortic aneurysm screening study (Scott, 2002).	80%	male 65-74	80%	not reported
Breast cancer screening programme (Office of National Statistics, 2003a)	70%	female 50-64	70%	not reported
Cervical cancer screening programme (Office of National Statistics, 2003b)	82%	female 25-64	82%	not reported

The data in this table are not directly comparable in terms of the actual uptake however they do support the findings in our study that age, gender and social deprivation were the factors that affected the uptake of screening.

A systematic review of the uptake of screening found that age, previous attendance at screening programmes and insurance status affected uptake. The effect of age was not consistent between studies i.e. in some studies being older was associated with higher uptake, in others being younger (Jepson et al., 2000). However this review included a heterogeneous group of studies that were for different conditions and in different countries.

Uptake of screening is also affected by knowledge of the condition being screened for and the threat posed by that condition. Psychological models of health related behaviour incorporate these factors in explanations of screening decisions (Cameron, 1997; Leventhal et al., 1997). Haemochromatosis is a condition that is not well known, unlike other conditions such as bowel cancer, and this could affect the decision to have screening. In addition care was taken in the patient information sheets not to overstate the benefits of screening for this condition since there is still uncertainty. These factors would tend to contribute to a low uptake in this programme in contrast to programmes screening for conditions that are high on the public agenda such as cancer.

Access to and participation in health is reported to be associated with both gender and deprivation, with women and individuals from areas of less social deprivation participating more in health care than men do and individuals from areas of higher social deprivation. (Baker, 2001; White and Cash, 2003; Townsend et al., 1988; Department of Health, 2002b). The mechanisms by which this occurs however are not known and are likely to be complex. There are no national screening programmes within the UK that are addressed specifically at men and if programmes for men are to be developed, exploring why men might not participate would be important.

In this study it was clear that younger men were less likely to accept the offer of screening for haemochromatosis. In addition younger men from areas of higher social deprivation may have a higher prevalence of other risk factors that are associated with the development of complications from this condition such as alcohol consumption. Although the benefit of screening for haemochromatosis is now under question, one hypothetical modelling study suggested that screening in younger men would be cost effective (Asberg et al., 2002). If haemochromatosis screening programmes were to be designed, particular care would have to be taken in order to access the population of younger men that might derive most benefit from such programmes.

- In summary, the two screening strategies do appear to be acceptable as assessed by the outcomes in this study.

- The uptake of screening was low. In common with other screening programmes and studies, the factors affecting the uptake of screening were age, sex and deprivation. The uptake was lowest in the section of the population that might be most at risk from the complications of haemochromatosis i.e. younger more socially deprived men. This has implications for the design of screening programmes in this condition.

7.2.2 Feasibility

The question of feasibility relates to practical issues about the process of screening.

The biochemical screening strategy did not involve any novel methods of sampling or analysis. Although transferrin saturation is considered to be the gold standard for screening for this condition, the review by McCullen highlights problems with standardisation of measurement and assays (McCullen et al., 2002). Transferrin saturation is a two-stage assay combining measurements of iron with either transferrin or total iron binding capacity; a screening programme would require standardisation of laboratory

assays. In this study all samples were sent to one laboratory reducing the problem of comparing un-standardised assays.

The genetic screening strategy used a novel approach to screening i.e. mouthwash sampling by post. The method had previously been shown to be feasible in research studies and proved feasible in this study. Thirteen percent of those requesting a sampling pack did not return it. When reminders were sent this did not increase the return rate. The non-attendance rate for the biochemical screening strategy was similar at 12%.

Approximately 6% of the DNA samples failed to yield a result in the laboratory. Possible reasons for the failure of samples are as follows:

- **Quality and Quantity of DNA**

  This is affected by the stability of the buccal cells in the mouthwash and the way in which the mouthwash is done. During cell death enzymes are released which degrade the DNA. This degradation is affected by temperature and the time delay between taking the sample and analysis. The yield of DNA has been shown to be significantly reduced by delay between sampling and extraction. The recommendation has been made that extraction take place no more than 5 days after collection. Also tooth brushing before sample collection has been shown to reduce the yield of DNA (Feigelson et al., 2001).

  Since the sampling was performed at home and posted to the laboratory, the way in which the sample was collected and any delay between sampling and arrival at the laboratory were factors over which we had no control. The method of sampling was chosen because it was the preferred method for the research laboratory which had systems in place for extraction of DNA upon receipt in the laboratory.

- **Inhibitors**

 The Polymerase Chain Reaction (PCR) reaction can be inhibited by a number of factors: intrinsic factors in the saliva which are not eliminated during extraction such as polysaccharides, urea, humic acids or haemoglobin or extrinsic factors such as residual chemicals from the extraction process or high concentrations of bacterial DNA. High concentrations of human DNA may also inhibit the PCR reaction (Wilson, 1997). All failed samples were repeated with the samples being diluted 1/2 and 1/5 to reduce this possibility (personal communication M Gomez Division of Human Genetics.)

- **PCR equipment and reagents**

 The quality of the reagents used in the PCR reaction could contribute to failure of samples. Temperature inconsistency across the thermal block in the PCR machine could also affect amplification of some of the samples. However the samples were run in batches and it was never the case that all the samples in a batch failed or that particular wells in the block generated failed samples.

All samples that failed were repeated, therefore when samples were run again and failed the most likely explanation for failure would be that there were problems with the quality and quantity of DNA or that inhibitory factors were present.

As with the biochemical testing strategy, quality control issues would be important if a screening programme using this methodology were to be proposed.

In the comparison of costs that was conducted the driver of the cost of the genetic screening arm was the cost of the DNA analysis. This is the same for cystic fibrosis screening (Murray et al., 1999). The cost of the DNA analysis was provided by the research laboratory and included careful cost breakdowns of the consumables and staff time required to extract DNA from one sample and analyse it. However DNA analysis is amenable to automated high through put testing which might lead to cost savings. In the decision tree the cost of a case detected with the genetic screening strategy became less

than that of the biochemical screening strategy at a DNA analysis and extraction cost of £10.00. The analysis in this study was performed using microplate array diagonal gel electrophoresis (MADGE) (Day et al., 1998). This technology allows for the typing of thousands of samples simultaneously and is compatible with the ARMS technique that was used for the mutation analysis. It would therefore be feasible to use this technology for population screening which would drive down the cost.

- In summary both strategies appeared to be feasible. Quality control of both strategies would be important. The methodology of the DNA screening strategy would allow for the development of rapid high through put testing if a screening programme were to be proposed.

7.3 Comparison of the two strategies.

The study had been designed to test whether the two strategies were equivalent with the hypothesis that the genetic screening strategy would be less acceptable and have a lower uptake.

Equivalence/non inferiority trials are designed to test a new intervention against a standard and demonstrate that the new intervention is no worse than the standard (Jones et al., 1996; Wiens, 2002). Within these parameters the genetic screening strategy was not inferior to the biochemical screening strategy.

As discussed before the study compared two pragmatic strategies, but this means that the uptake of screening will be affected by both the test and the setting in which the test was provided. The finding that uptake in the genetic strategy was higher (therefore no worse) than in the biochemical strategy is confounded by the fact that uptake may have been increased because access to the test was easier (it could be done at home). A more rigorous comparison would have been to do the genetic test on a blood test taken at the surgery in the same way as the biochemical test. However this would not have examined the other components of the screening strategy which at the time of the design of the

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study were thought to be some of the advantages of a genetic strategy i.e. that it could be done without needing a blood test and without attending a clinic.

Although there was an overall effect of some socio-demographic factors on the uptake of screening which has been discussed earlier, there was no differential effect of age group, sex, age left full time education or employment between the genetic and biochemical screening strategy. If the setting did have a significant effect it could be hypothesised that there might be different effects for some of these variables particularly employment status. Being employed full time may make it more difficult to attend an appointment therefore offering a test that could be done at home might increase uptake.

There was a 3.4% difference in uptake of screening between the two arms of the study. The lower limit of equivalence that was predefined was 5%. If effect of the setting had been to increase the uptake of screening, it would have had to increase the uptake by greater than 8% in order to make uptake in the genetic screening arm less than the predefined range of equivalence. There is therefore support for the interpretation that the nature of the test and the setting had an effect and that the genetic test was no less acceptable than the biochemical test.

The two strategies were also equivalent in terms of the psychosocial assessments. There appeared to be no difference in the assessments of anxiety, depression or self-rated health between the two strategies.

- In summary there appeared to be no difference in acceptability, feasibility and uptake between the two strategies.

7.4 Outcome of the screening

7.4.1. Cases detected

This study was not designed to be full evaluation of the effectiveness of screening for haemochromatosis. That would require a randomised controlled trial of screening versus not screening. The outcome evaluated in this study was the detection of cases that required treatment or further management.

One of the difficulties about determining the effectiveness of screening as a case detection strategy is defining what is meant by a case of haemochromatosis. The European Association for the Study of Liver Disease suggests a staged approach to case definition which moves from the genetic predisposition through early evidence of iron overload, early organ damage to irreversible organ damage (Adams et al., 2000a). This definition is careful to distinguish between the genetic predisposition and the expression of a clinical phenotype, emphasising that hereditary haemochromatosis is a clinical diagnosis based on the evidence of end organ damage due to iron overload. It is not clear from this definition how end organ damage is defined.

As molecular genetics has advanced and many of the genes that cause Mendelian disease have been identified, it is clear that the relationship between genotype and phenotype is not simple. This relationship will be even more complex in diseases where the genetic contribution is less certain. Haemochromatosis may be thought of as a condition where there is a major identified genetic risk factor (mutations in the HFE gene) which acts in combination with other genetic and environmental risk factors and modifiers which are as yet largely unidentified. The haemoglobinopathies are another example of a group of inherited conditions where the molecular genetics are well studied and while superficially they appear to be simple monogenic diseases, it is clear that the phenotype is the end result of a complex interaction between many genetic and environmental factors (Weatherall, 2000).

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All single gene disorders show clinical variability even in patients with the same mutation and as clinical and molecular genetic science advances the challenge will be to tease out the individual components of the genetic and environmental factors, their interactions and their relative contributions to the eventual phenotype.

This complexity means that when considering questions similar to those in this study that there is an attempt to clear about what is being tested for. Despite being unclear about what constitutes organ damage the European Association for the study of the liver is useful in that it distinguishes between phenotypic disease in haemochromatosis and the 'at risk' genotype. The diagnosis of a case therefore becomes a phenotypic diagnosis.

In this study we compared a genotypic strategy and a phenotypic strategy for identifying cases. A pragmatic definition of 'cases' was based on the need for further monitoring or treatment in line with clinical guidelines (Adams et al., 2000a; Witte et al., 1996). The clinical diagnosis requires a combination of genotypic and phenotypic information and this is represented schematically in figure 7.1.

Ninety percent of patients from Northern Europe with haemochromatosis have the 'at-risk' genotype, however this leaves 10% with unidentified mutations(The UK haemochromatosis consortium, 1997). Understanding of iron metabolism has increased since the identification of the HFE gene and other proteins and genes have been discovered which account for some of this 10% but not all. The penetrance of the 'at risk' genotype is not known and is currently the subject of debate as discussed earlier (Beutler et al., 2002a; McCune et al., 2002; Olynyk et al., 1999). It is clear that many people with the haemochromatosis genotype never develop clinical disease. In addition not all iron overload is due to haemochromatosis. Estimates of penetrance of the at-risk genotype range from 1% in population studies to 50% in family based studies.

If the measurement of iron status is considered in isolation, it will detect iron overload due to haemochromatosis in those with the common genotype and those without. It will also detect iron overload not due to haemochromatosis. Since the test measures iron

levels it will also detect people who have low iron levels and who may require further investigation.

Genetic testing in isolation will detect those who have the common at-risk genotype and currently have iron overload due to haemochromatosis. It will also detect those who have the genotype in whom iron levels are normal. Some of those people will go on to develop haemochromatosis, exactly what proportion is not known. This is illustrated diagrammatically below. In order not to complicate the diagram the low iron outcome is not included.

Figure 7.1 Comparison of genotypic and phenotypic testing.
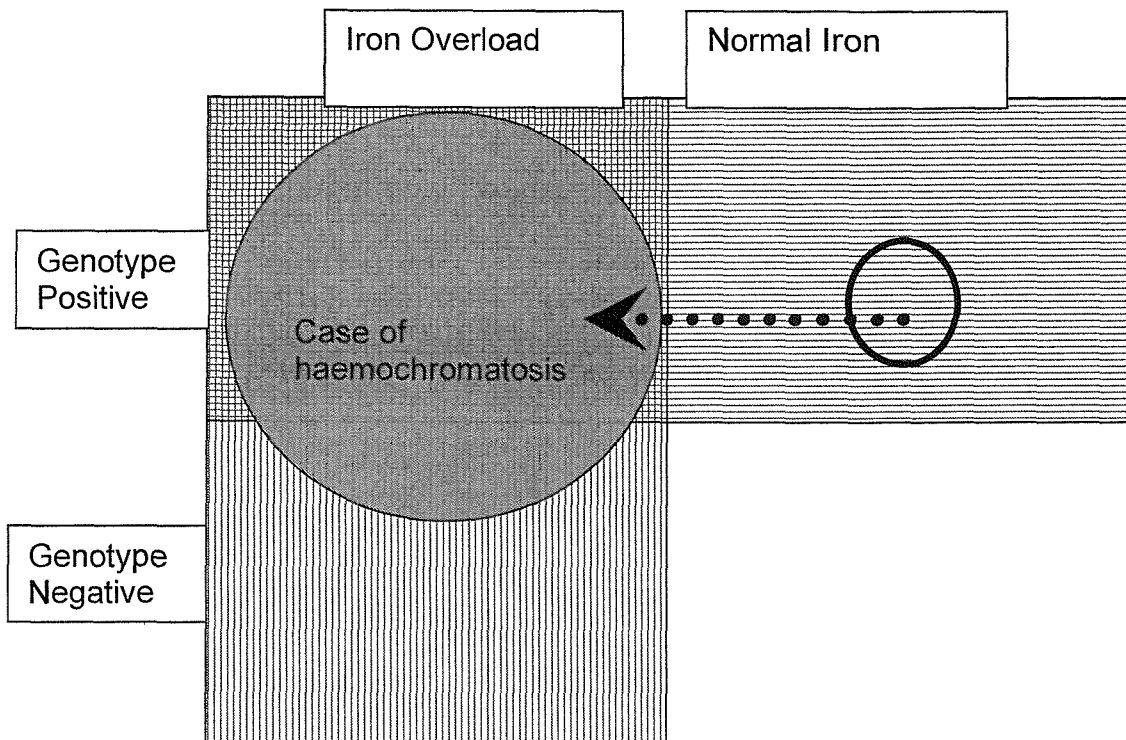
The prevalence of haemochromatosis as defined by the screening studies reviewed in the literature review is approximately 1 in 300 or 0.03%. This is higher than the prevalence in our study although it does fall within the limits of the confidence interval. As has been discussed before one of the critical questions that need to be answered in this area is how to identify the proportion of cases that will benefit from treatment.

Screening using the biochemical strategy test had a higher sensitivity and a slightly lower specificity than screening using the genetic strategy. In assessment of a test there is always a trade off between sensitivity and specificity. The sensitivity in the genetic screening strategy was only 40% meaning that 60% of cases were missed. Calculation of these parameters is of course dependent on the case definition and it is not known whether the cases that were detected in this study did benefit from treatment.

When the study was designed it was assumed that only one or two cases would be detected. Five cases were detected that started venesection treatment. Of the cases detected in this study that commenced treatment, the two individuals detected in the genetic screening arm were both women over the age of 60 with mildly raised iron parameters. It is possible to speculate that they would not have developed problems from iron overload and therefore the benefit of detecting them has to be questioned. In the biochemical screening arm the two cases that were detected that started treatment did not have two mutations in the haemochromatosis gene and are still being investigated. Both of these cases would have been missed in a genetic screening programme, but given their iron status it is possible to speculate that they both would have developed significant iron overload.

The usefulness of the clinical information collected on the screen positive cases is limited by the fact that there was no control group. In retrospect it might have been useful to collect clinical data on all participants. However given the small number of cases that were detected the numbers would have been too small to detect any effects. The symptoms associated with haemochromatosis are common within the general population

and this high background prevalence means that any studies designed to examine the relative risk associate with haemochromatosis would have to be large enough to detect small effects.

The number of cases detected in the two arms was similar. If the case definition of a 'case requiring treatment' is used, the 'number needed to screen' to detect one case in this study was 586 (which would half if the uptake of screening was doubled). If the case definition is a 'case requiring treatment or monitoring', the 'number needed to screen' would be 266. Comparing these to figures estimated from the reports of screening programmes and studies discussed earlier indicates that these estimates are comparable to most of the studies (Table 7.4).

The 'number needed to screen' is a statistic that it is suggested will allow comparison between screening strategies (Rembold, 1998). However as discussed before detection of cases is only a partial analysis of the performance of a screening programme. For the purpose of a full evaluation of screening this statistic should include an outcome or measure of effect such as life year gained. In addition evidence from a randomised controlled trial of the magnitude of the effect in the screened versus the non-screened population would be required.

Table 7.4 Comparison of number needed to screen

N=1000	Number screened	Further assessment	Cases	Case definition	Number needed to screen
Haemochromatosis screening study.	340	22	11	cases requiring venesection	266
			5	cases requiring venesection or further monitoring	586
Colorectal cancer pilot screening study (The UKCRC Screening Pilot Evaluation Team 2003).	600	10	4	polyp cancer colorectal cancer adenoma	273
Aortic aneurysm screening study (Scott 2002).	802	n/a	32	aortic aneurysm detected>3cm requiring monitoring.	31
Breast cancer screening programme (Office of National Statistics 2003a)	700	35	5	all breast cancer	213
Cervical cancer screening programme (Office of National Statistics 2003b)	820	62	4	cervical cancer, adenocarcinoma in situ CIN3	250

In the decision tree constructed to compare the costs of the two strategies, the end point was a 'case detected requiring further treatment or management'. The biochemical screening strategy was the lowest cost strategy. This was despite the lower positive predictive value of the biochemical test.

In considering the case for screening for haemochromatosis there are a number of areas where further information is needed before a full evaluation of the effectiveness of screening could be considered. These are the penetrance of the genotype, the natural history of early iron overload i.e. is progression and organ damage inevitable and the effectiveness of early treatment. All of these questions require carefully designed studies that have adequate power to answer the question of interest.

This study has given an estimate of the number of individuals that would need to be offered testing in order to detect enough cases to study. For example if the estimate from this study is used, in order to detect 100 cases that required treatment approximately 60,000 individuals would need to be offered testing. To detect 100 cases that required treatment or monitoring then approximately 30,000 individuals would need to be offered testing. Clearly considerable investment would be required to conduct studies that identified cases in this way. It does not appear from this study that the screening strategy would have an effect on this estimation.

- In summary both screening strategies detected cases that on current clinical guidelines required further management or treatment.

- The biochemical screening strategy had a higher sensitivity than the genetic screening strategy for detecting cases of iron overload requiring treatment but a slightly lower specificity.

- The biochemical screening strategy had a lower positive predictive value, however the costs per case detected was less with this strategy than with the genetic strategy.

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- The 'number needed to screen' to detect one case requiring further management o treatment was similar to other adult screening programmes.

- Further evaluation of the case detection strategies is not possible because further data are needed on:

  o Prognosis
  o Effects of treatment

- The number needed to screen in this study means that a large number of people would have to be invited to take part in studies that would have sufficient statistical power to answer these questions.

7.4.2 Detection of anaemia

One of the benefits that is claimed for biochemical screening for haemochromatosis is that the measurement of iron parameters will detect patients with undiagnosed anaemia. In the review of screening studies the frequency of low transferrin saturation detected was between 3.5 and 5.4 percent. In this study of those tested a comparable percentage had low transferrin saturation (6.4%).

In this study patients and their G.P's were notified if they had low iron levels. Nearly half of this group had co-morbidity that could have contributed to their results. The quality of the data that was collected was poor in this group. Half of the group had further investigations, mostly further iron studies. In the two patients who had upper G.I. endoscopies it was noted that they were symptomatic. Five new cases of iron deficiency anaemia were detected two of which had known clinical problems. However there were no further investigations in ten out of the twenty three people on which we had information. Guidelines have been produced for the management of iron deficiency anaemia focusing on the diagnosis of gastrointestinal disease (Goddard et al., 2000).

These guidelines, despite being produced for use in secondary care have been applied to the management of iron deficiency anaemia in primary care. Audit of the guidelines have concluded that they are not adhered to either in primary or secondary care (Patterson and Johnston, 2003; Logan et al., 2002).

Without clear guidelines for the investigation and management of iron deficiency anaemia it is not possible to determine either the yield of cases or the benefits of detecting those cases. Screening for iron deficiency should be evaluated using the same criteria that are used to evaluate screening for iron overload including that the natural history of the condition that is being screened for is understood and that there is evidence from high quality randomised controlled trials of the benefit from screening versus not screening. This has been considered by a health technology assessment panel in the United States and the conclusion is that the benefits are not sufficient to recommend screening in an adult population (Health Services Technology Assessment Texts (HSTAT), 1996). However it may still be a benefit of a screening programme that uses iron status to detect both iron overload and iron deficiency.

7.4.3 Negative outcomes.

Detecting cases that may benefit from treatment could be considered to be a positive outcome of screening. Evaluation of screening should also take account of the harms that might be caused by screening.

In this study the screening tests used in both strategies appeared to be acceptable and safe. There appeared to be minimal changes in the psychosocial assessments used, particularly in the screen negative group. This was in the context of a research study where there was a commitment to ensuring that the protocols were followed in practice there might not be the same commitment to ensuring this was so. If a screening programme were to be introduced quality management would have to be part of the programme. The study used a quantitative design that had the power to detect effects and differences at the level of the sample, but was not able to detect subtle adverse effects on individuals or rare events. A follow up qualitative study exploring the experience of

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screening in a proportion of the sample might generate information which could inform the design of future screening programmes.

In the genetic screening strategy, since it was done by post there was a higher possibility of an individual not understanding the implications of the test or the result and of organisational difficulties relating to problems with the postal system e.g. samples having been posted failing to arrive.

In the study there was little evidence that there was more anxiety with the genetic screening strategy, or that there were differences in the understanding of the result between the two screening arms. Approximately 13% of sampling packs sent out were not returned and it is possible that this was not individuals withdrawing from the study but was due to delivery problems. In the pilot study letters were sent when sample packs were not returned, this did not increase the response rate. In the biochemical screening strategy 12% of people failed to attend for the blood test, despite being offered another appointment. The interpretation of the non return of sample packs as withdrawal from the study would tend to be supported by these findings rather than being interpreted as a failure of procedure.

Part of the evaluation of a screening programme is the performance of the test. If all cases were identified then the sensitivity of the test would be 100%. In this study the sensitivity of the biochemical screening strategy was higher than the genetic screening strategy. There would therefore be fewer false negatives. However the biochemical screening strategy was less specific than the genetic screening strategy and 44 people were brought back to the research clinic for further tests and then discharged compared to 2 in the genetic screening strategy. Although the assessments of anxiety, depression and self-rated health did not change significantly, these people did report more negative feelings about the result at the time of the results. However this profile had disappeared at the time of follow up.

An abnormal result will of course be worrying and it is not surprising that there was this difference in the screen positive and screen negative group. The fact that this difference disappeared at follow-up in those who were discharged suggests that it was a temporary effect. At follow-up the people that were identified as needing treatment or further monitoring still had more negative feelings about the test than those who screened negative or were discharged.

Much attention has been paid in the literature to anxiety, morbidity and even mortality caused by false positive screening results (Black, 2000; Marteau et al., 1992). What may be of more relevance in the context of this study is that worry may have been caused in the people who are recommended to have treatment or monitoring without a clear estimate of the benefit. Until questions are answered about the natural history of haemochromatosis and the effectiveness of treatment at reducing morbidity it is not possible to determine the benefit for individuals of being identified through screening. The observational studies that are used as evidence for the benefit of treatment are concerned with cases that are identified symptomatically or through family testing. Individuals identified through screening may never have developed disease from the condition and therefore would derive no benefit from unnecessary treatment.

Another possible negative consequence of screening is misunderstanding the result that is given. In this study a small proportion of people demonstrated incongruence between the actual result they were given and the statement about the result that they selected in the questionnaire. They had all screened negative and indicated that they might have, or did have haemochromatosis. This was not consistent between result giving and follow-up and also was not associated with increases in anxiety, depression, negative feelings about results or low self rated health. It is possible that there was a problem with the way the question was phrased and how it was set out in the questionnaire. However it is of concern that for some people there appeared to be confusion. Future studies might investigate this further.

If a screening programme were to be developed attention would need to be paid to the information that was given and how results were conveyed in order to promote an informed decision about screening.

Both screening strategies identified individuals who may be at risk of iron overload at some unspecified time in the future. The consequence of managing this predictive information has not yet been thought through. Health services are designed to manage the patient who currently requires diagnosis or treatment and it is known that within current systems individuals with chronic health conditions are not managed optimally (Wagner and Groves, 2002). In haemochromatosis it is not known if diagnosis is currently delayed leading to people being unable to derive maximum benefit from treatment. It is not known whether all who should receive treatment on current guidelines do and it is not known whether people receive optimal treatment.

If an individual is predicted to have a risk of developing a disease in the future, and needs monitoring perhaps for many years there are implications for health care providers e.g. who should take responsibility for monitoring the individual and initiating further screening and diagnostic tests, for the individual who is not yet a patient e.g. potential anxiety, possible 'labelling' and lack of clarity about responsibility for future management and for the health care systems e.g. how to manage the information about the initial screening and future management. If screening for genetic risk factors were to be introduced these issues would need to be addressed.

7.5 Strengths and weaknesses of the study

The study was designed to answer the question is the acceptability and uptake of genetic screening strategy equivalent to the biochemical strategy. A pragmatic approach was taken to the two screening strategies and at the time the study was designed it was thought that one benefit of genetic testing might be that it could be performed at the participants' convenience in their own home. This may have increased the uptake in the

genetic screening arm and would have been a source of bias in comparing the genetic and biochemical strategies. It would have been advantageous to have a third arm in the study where the genetic test was performed at the GP's surgery making the two interventions more similar. However this would have been prohibitive in terms of the cost of the study and was not feasible within the resources available. The study was resourced at a hypothetical uptake of 65% and if the participants in the genetic screening arm had all had to be seen in a clinic further research nurse time would have been needed. The uptake in the genetic screening arm was higher than that in the biochemical screening arm and therefore the question of equivalence/non-inferiority was probably not compromised in that we demonstrated that uptake was no worse in the genetic screening arm.

Testing for genetic mutations lends itself to rapid throughput testing since it is possible to analyse large numbers of samples in one analysis. Within the context of this study relatively small numbers of samples were analysed in order to provide timely results. Greater efficiency could have been achieved by batching samples however this would have resulted in an unacceptable delay between receiving the sample and giving the subject a result. It was felt this would have been unethical. However the study did show that the technology was feasible and would be able to be used if large numbers of samples were analysed as would be the case in a population screening programme.

During the time this study has been conducted there has been an active debate in the literature regarding the morbidity associated with haemochromatosis and the presenting signs and symptoms. In retrospect it might have been better to ask all participants to complete a clinical symptom questionnaire, this would have enabled comparisons of symptoms to be made between those with iron overload and a control group without iron overload which may have contributed data to the debate. However for issues of time, feasibility and funding the study was not designed to detect large numbers of cases and therefore would probably not have enough power to detect a difference in the prevalence of a common symptom such as arthritis.

The strengths of the study are that it was a well conducted randomised controlled trial designed to answer a specific research question and powered sufficiently to provide that answer. Because of the nature of the design, the attention paid to the randomisation procedure and the way the trial was conducted it was possible to make a fair comparison. Validated measures of psychological status were used. The two GP practices studied differed in indices of deprivation and the old Southampton and South West area Health Authority from which they were recruited is representative of England as a whole apart from a lower ethnic minority population. This means that the findings in the study are robust.

This is the first study to make a direct comparison between a genetic screening programme and a biochemical screening programme in terms of uptake, acceptability and feasibility. The finding that there were no differences attributable to the way the test was offered is relevant to evaluating the use of genetic tests in health care.

7.6 Screening for haemochromatosis

This study has demonstrated that both screening strategies were acceptable and feasible for the detection of cases of haemochromatosis. There was no difference in uptake or acceptability, as assessed by uptake and changes in psychological assessments between the two strategies. There appeared to be few negative consequences of the actual screening interventions, which caused minimal changes in anxiety, depression or self-rated health. The biochemical screening strategy would appear to be the least costly in terms of the cases detected requiring treatment or monitoring. Because of the differential uptake between males and females the biochemical strategy would also detect more cases requiring treatment since more males have raised iron indices.

However this study did not address the benefits of screening for haemochromatosis which are not clear. In order to quantify the benefits, further information is needed on the burden of disease associated with haemochromatosis. Current data is uninformative as discussed previously. The information that is needed includes the natural history of the

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disorder when it is diagnosed prior to symptoms developing, the effectiveness of treatment and the prevalence of disease associated morbidity in the population. Cases for these studies will need to be ascertained through some kind of screening strategy. From this study it is possible to suggest that a biochemical screening strategy would be acceptable and feasible and would detect cases. Attention would need to be paid to accessing the populations at risk in order to increase uptake of the screening test, but this should not be at the expense of the participant making an informed decision about whether to take part.

In addition, before considering implementing a screening programme there should be evidence based guidelines in place for the management of at risk individuals and the treatment of affected individuals. Although there are no quantitative data from the study the experience of the clinicians in the research clinic highlights the uncertainty that is present in making clinical judgements in this condition. This is brought out in the difficulty that there was in defining strict criteria for allocation of screen positive individuals to being discharged, monitored or treated.

The question of current management was not addressed by this study. The presumption before embarking on a population screening strategy is that the management of currently diagnosed cases is optimised, that all other case identification strategies have been implemented and that population screening would be a more effective case identification strategy. The evidence to either support or refute this is not available in haemochromatosis therefore it would be premature to instigate screening before an evaluation of current case identification strategies was made.

7.7 Haemochromatosis screening in the context of the 'new genetics'

Genetic tests are currently used in a variety of different ways for diagnosis e.g. testing for Fragile X in a developmentally delayed child, to offer reproductive choice e.g. carrier testing for haemoglobinopathies or haemophilia, for prenatal or pre-implantation testing of diseases such as Down syndrome or Duchenne muscular dystrophy, in newborn screening for phenylketonuria and in predicting late onset disease such as Huntington's disease.

In the future genetic tests may be used for predicting those who would benefit from treatment or some other intervention in more common diseases. There has been a shift in considering the use to which genetic tests may be used. In the past genetic tests were used to make decisions about reproduction, particularly prenatal diagnosis and in testing for incurable diseases such as Huntingtons disease. The advent of cancer genetics shifted the focus to tests being used to identify those at increased risk in order to offer targeted surveillance. If the promises of the genetic advances are realised, in the future there may be the possibility of using genetic information, based on the risk associated with multiple combinations of genetic markers, to provide individual risk assessment which will mean targeted treatment or prevention.

If this shift is seen as a continuum then haemochromatosis falls between cancer genetics and individualised risk assessment. The major genetic risk factor is known and the purpose of testing is to identify individuals who will benefit from treatment. As such, it provides a model system where questions such as those posed in this study can be explored, i.e. was the overall performance of the screening strategies including uptake, acceptability and feasibility affected by whether they were genotypic or phenotypic. In this study it was not, in contrast to what might have been predicted by commentators who claim that genetic tests have special properties.

Traditional models of genetic practice have focused on testing for reproductive choice and predictive testing for highly penetrant single genes where there is little therapeutic

possibility. Guidelines for practice emphasis the importance of a non-directive approach to genetic testing with careful pre-test counselling. There has been the development of the view that there is something 'special' about genetic tests which mean that they have particular characteristics relating to their predictive nature, implications for the family, potential for stigmatisation and potential for causing psychological distress.

While it would be ill advised not to consider the potential harm of genetic tests the whole scale application of principles derived from traditional models of genetic practice may carry a different set of risks. This 'genetic exceptionalism' may mean the methods that are in existence to evaluate the consequences of medical information and interventions are, wrongly, not applied to genetic information.

In primary care there is a call to develop skills in genetics in order to maximise the perceived benefits from the 'New genetics' (Starfield et al., 2002). The subtext being that the generalist does not have the skills to identify and manage genetic risk appropriately. An alternative point of view is that a GP's skills are suited to managing the consequences of identifying people at risk and provide risk estimations based on biological parameters, lifestyle factors and knowledge of the patients social situation. The knowledge gap relates to exact details of how gene expression is modified by these other factors (Kumar, 1999).

In order to make sense of genotype-disease associations epidemiological principles and methods need to be applied. The fact that a genetic risk factor is being considered rather than some other risk factor does not render the techniques invalid. Of course the complexity of the multiple factors and their interactions does pose difficulties, however the principles remain the same.

In considering the use of genetic risk factors in screening for disease the same criteria for evaluation should be applied as in the context of using other risk factors or markers for disease. The primary question should be the balance of benefit and harm resulting from the screening programme (Fig 7.2).

Figure 7.2 Evaluation of screening

The benefits arising from the screening programme must exceed the harms

Benefits

Improved survival
Quality of life

Harms

False positives
anxiety
injury
False negatives
Direct costs
Opportunity costs

If the benefit of the programme is the detection of cases of haemochromatosis in which treatment is effective at reducing morbidity and mortality then evaluation of genetic or biochemical screening strategies should include assessment of the benefits and harms of each strategy. From this study it would appear as if there was no difference in harm or benefit between the two strategies in detecting cases needing further treatment or monitoring. What was not answered by this study was whether the two strategies detected cases that might gain a differential benefit from treatment. The biochemical screening strategy identified cases that already had some degree of iron overload who might be those who benefit more from treatment.

In considering screening for haemochromatosis the question of the benefit of detecting cases in this way has not been answered and this in itself makes screening premature. What has been shown in this study is that the genetic screening strategy per se was not unacceptable and did not lead to more adverse consequences as assessed by this study.

As knowledge of haemochromatosis grows it is now apparent that haemochromatosis is a polygenic disorder in which the HFE gene is permissive but not sufficient for disease. As discussed previously it could be regarded as condition in which some of the questions relating to the application of the new genetic technologies in practice can be explored.

Evaluation of these questions should apply existing methodologies and techniques from epidemiology and health services research with the aim of maximising the benefits and minimising the harm of the new genetic technologies. These include observational and analytical studies e.g. cohort and case control designs to determine the genetic and environmental factors that contribute to clinical disease, health service research to determine the burden of disease, pathways into care and whether current treatment is optimised and randomised controlled trials to determine the effects of interventions.

7.8 Implications

In the debate about implementing population screening for haemochromatosis, there remain unanswered questions about treatment and natural history. These questions require large well designed studies sufficiently powered to detect the effect of interest. It will probably be necessary to identify patients for these trials using a screening approach. This study demonstrates that with the low uptake achieved in this study a large number of people would need to be invited to take part to identify enough potential cases to follow up. In addition efforts would have to be made to recruit young men who would be the population most likely to be at risk of future complications of this condition.

The two screening strategies were feasible and acceptable and had minimal effects on the psychological status of the individual and either could be used as case identification strategies. Biochemical screening has the lowest cost per case as defined in this study and the main driver affecting that in the decision analysis is the cost of DNA analysis. Mutation analysis is amenable to high through put testing which theoretically would drive down the cost; however, this situation is unlikely to occur outside of national population screening programmes for common disease causing genetic variation. The question of

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whether such programmes will be developed will be informed by consideration of the criteria used to evaluate all screening programmes.

This study is the first study to make a direct comparison between a genetic and biochemical screening strategy. It has demonstrated that genetic testing is no less acceptable than biochemical testing, therefore in considering whether to introduce a genetic test for screening in order to initiate treatment focus should be placed on the clinical utility of that test i.e. its sensitivity and specificity, the effectiveness of treatment and the full costs and benefits of implementing a screening programme.

7.9 Future research

- Evaluation of the other case identification strategies

  o There are two other case identification strategies in haemochromatosis: earlier diagnosis and extended family testing. Neither of these has been fully evaluated. The following studies have been funded as follow on studies to this thesis.

    A study examining pathways into diagnosis in order to identify the scope for earlier intervention by seeing if there are missed opportunities for diagnosis.

    A review and a survey of the barriers to implementing extended family testing. First degree relatives will have a higher prior risk of being affected in addition to sharing other genetic and environmental modifiers.

  o Evaluation of the three case identification strategies by developing an economic evaluation model.

- Burden of disease

  o Questions about the natural history of both genotype positive individuals and individuals with a minor degree of iron overload could be answered by a cohort study of asymptomatic cases diagnosed through screening. A nested case control study could answer questions relating to the burden of disease. The HEIRS study funded by the NIH for five years will identify cases that could be followed up long term. This study is testing 100,000 individuals (genotype, ferritin and transferrin saturation) and collecting clinical information. Although this is a large study it is approaching an ethnically diverse population, therefore the yield of cases may not be particularly high.

  o Large population based epidemiological studies such as the UK Biobank may also provide data collections in which case control studies could be performed. In addition linkage of data to medical records and death certification would provide data to estimate the clinical burden of haemochromatosis.

  o We are funded for an observational study investigating the burden of disease at diagnosis in treated cases and plan that this will form the basis of an inception cohort study to answer some of these questions.

- Effectiveness of treatment

  o Although a standard randomised controlled trial with an untreated arm would be unethical in haemochromatosis, there are still major unanswered questions about the effectiveness of treatment which mean that there are still doubts about the benefits of treatment particularly in cases diagnosed with a minor degree of iron overload. It would be possible to design a trial with a 'watchful waiting' arm. This, although a difficult trial to design,

would provide data relating to the progression of early iron overload, the benefits and dis-benefits of treating early iron overload and the effectiveness of treatment.

Appendices
Appendix 1: Screening Criteria
The NSC Criteria
The Criteria for appraising the viability, effectiveness and appropriateness of a screening programme

The criteria, which are set out below, are based on the classic criteria first promulgated in a WHO Report in 1966 but take into account both the more rigorous standards of evidence required to improve effectiveness and the greater concern about the adverse effects of healthcare; regrettably some people who undergo screening will suffer adverse effects without receiving benefit from the programme.

These criteria have been prepared taking into account international work on the appraisal of screening programmes, particularly that in Canada (2) and the United States (3). It is recognised that not all of the Criteria and questions raised in the Format will be applicable to every proposed programme, but as many as possible should be answered since this will assist the NSC to make quicker and better evidence based decisions.

All of the following criteria should be met before screening for a condition is initiated:

The condition

1.1. The condition should be an important health problem.

1.2. The epidemiology and natural history of the condition, including development from latent to declared disease, should be adequately understood and there should be a detectable risk factor, or disease marker and a latent period or early symptomatic stage.

1.3. All the cost-effective primary prevention interventions should have been implemented as far as practicable.

The test

1.4. There should be a simple, safe, precise and validated screening test.

1.5. The distribution of test values in the target population should be known and a suitable cut-off level defined and agreed.

1.6. The test should be acceptable to the population.

1.7. There should be an agreed policy on the further diagnostic investigation of individuals with a positive test result and on the choices available to those individuals.

The treatment

1.8. There should be an effective treatment or intervention for patients identified through early detection, with evidence of early treatment leading to better outcomes than late treatment.

1.9. There should be agreed evidence based policies covering which individuals should be offered treatment and the appropriate treatment to be offered.

1.10. Clinical management of the condition and patient outcomes should be optimised by all health care providers prior to participation in a screening programme.

The screening programme

1.11. There must be evidence from high quality Randomised Controlled Trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an

"informed choice" (e.g. Down's syndrome, cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened.

1.12. There should be evidence that the complete screening programme (test, diagnostic procedures, treatment/ intervention) is clinically, socially and ethically acceptable to health professionals and the public.

1.13. The benefit from the screening programme should outweigh the physical and psychological harm (caused by the test, diagnostic procedures and treatment).

1.14. The opportunity cost of the screening programme (including testing, diagnosis, treatment, administration, training and quality assurance) should be economically balanced in relation to expenditure on medical care as a whole (i.e. value for money).

1.15. There must be a plan for managing and monitoring the screening programme and an agreed set of quality assurance standards.

1.16. Adequate staffing and facilities for testing, diagnosis, treatment and programme management should be made available prior to the commencement of the screening programme.

1.17. All other options for managing the condition should have been considered (e.g. improving treatment, providing other services), to ensure that no more cost effective intervention could be introduced or current interventions increased within the resources available.

1.18. Evidence-based information, explaining the consequences of testing, investigation and treatment, should be made available to potential participants to assist them in making an informed choice.

1.19. Public pressure for widening the eligibility criteria for reducing the screening interval, and for increasing the sensitivity of the testing process, should be anticipated. Decisions about these parameters should be scientifically justifiable to the public.

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Appendix 2: Search strategy

Examples of search strategy

#8	mass
#9	screening
#10	mass screening
#11	screen*
#12	screen* in ti
#13	#12 or #10
#14	IRON in TI,AB,MESH
#15	iron
#16	deficiency
#17	iron deficiency
#18	anaemia
#19	#17 or #18
#20	iron
#21	overload
#22	iron overload
#23	haemochromatosis
#24	hemochromatosis
#25	haemochromatosis or hemochromatosis
#26	#25 or #22 or #19
#27	#26 and #13
#28	HEMOGLOBINOPATH??? in TI,AB,MESH
#29	"Hemoglobinopathies"/ all subheadings
#30	H??moglobinopath???
#31	#28 or #29 or #30
#32	#27 not #31
#33	THALASSAEMIA in TI,AB,MESH
#34	"Thalassemia"/ all subheadings
#35	SICKLE CELL in TI,AB,MESH
#36	"Anemia-Sickle-Cell"/ all subheadings
#37	#33 or #34 or #35 or #36
#38	#32 not #37
#39	LA = "ENGLISH"
#40	#38 and (LA = "ENGLISH")

Appendix 3: Patient information sheets, letters and consent forms.

Letter from GP
Patient information sheet biochemical screening arm: presented to patient as an A5 booklet
Patient information sheet genetic screening arm: presented to patient as an A5 booklet.
Consent form
Letter with normal result
Letter for low iron
Letter for screen positive results

1.

XXXXXXX SURGERY

Dr xxxxxx   Address
Dr xxxxxx   Address
Dr xxxxxx   Telephone
Dr xxxxxx   Facsimile

August 2001

LREC 382/00
HFE «STUDY_ID»

«TITLE» «FORENAME» «SURNAME»
«ADDRESS1»
«ADDRESS2»
«DISTRICT», «CITY»
«COUNTY» «POSTCODE»

Dear «TITLE» «SURNAME»

Our surgery is collaborating with medical researchers from Southampton Hospital and the University in a study investigating testing for a medical condition called haemochromatosis.

Your name has been selected randomly from the practice list. We would like to invite you to take part in this study.

Enclosed with this letter is some information about the study for you to read and decide if you would be willing to take part in this interesting research.

Yours sincerely

Dr «GP»

**University
of Southampton**

School of Medicine

**Community Clinical
Sciences Division**

LREC 382/00
B December 2000

Information sheet

Haemochromatosis screening study

Why is this study being done?

We would like to tell you about a study we are doing in your GP practice that is looking at two different ways of testing for a condition called haemochromatosis. We are working with senior doctors and researchers from Southampton Hospital. We hope that this study will help us develop better ways of diagnosing this condition earlier.

Please would you take the time to read the enclosed information and then decide if you would be willing to be tested for haemochromatosis.

How common is Haemochromatosis?

About 1 in 300 people from the UK would have the possibility of developing the condition. We do not know how many of those people will actually have health problems.

What is haemochromatosis?

If you have haemochromatosis you carry on absorbing iron from your food even though you have enough iron in your body. This extra iron cannot be got rid of and is stored in various organs in the body. The build up of iron in these organs can cause damage. The main organ that can be damaged is the liver; iron can also build up in the pancreas and cause diabetes, the joints causing arthritis, the heart and the endocrine glands. If the body is storing too much iron and these organs are being damaged then this can cause serious health problems.

Is there a treatment?

Yes. The treatment is for the doctor to take blood from you regularly, in the same way as if you were being a blood donor. The body needs to use its stored iron to make new blood cells and therefore the damaging levels of iron in the body are reduced. The level of iron in the body is monitored and how often a person has to give blood depends on how quickly the iron builds up again.

Does the treatment work?.

Yes. If the treatment starts before the liver or other organs are damaged then a person with haemochromatosis will live normal life. We therefore need to try and diagnose it as early as possible.

How do you get haemochromatosis?

It is a genetic condition. There is a gene that controls how much iron we absorb from our food. We have two copies of this gene one from our mother and one from our father. (This is the same for all the other genes that we have) Someone who has haemochromatosis has two copies of a gene that does not work quite as well as it should do. This means they are at risk of developing iron overload. As far as we know somebody with one haemochromatosis gene and one normal gene is very unlikely to have problems. Recent exciting genetic research in the UK has identified the gene responsible and we are trying to understand more about it.

How do you test for the condition

You can test for the condition either by taking some blood and looking at how much iron is there or by looking for the faulty genes.

Why isn't everybody tested for this condition?

We don't know yet the best way to test for this condition early enough to prevent problems from it. We also don't know how many people who are predisposed to develop the condition will develop problems from it. This study will start to help to answer some of these questions.

Our study

We would like to offer you screening for haemochromatosis using a test to see if you are developing iron overload.

What is screening?

Screening is trying to identify people who may be at risk of a disease, or to pick up the early stages of a disease. You will probably be aware of screening programmes such as the cervical smear test that women have. It is important to understand that **any** screening test will miss some people who actually have the disease and will pick up people who may not have or develop any health problems.

What does taking part involve?

Initial Testing

The initial test would be on a blood test at a special clinic in the GP practice. You will be contacted and told whether this first screening test meant we would advise further tests, or if we would recommend no further tests. As well as picking up people who may have too much iron the blood test would also pick up people who have too little iron. This is nothing to do with haemochromatosis but may need further tests. The vast majority of people would need no further tests.

Further testing

If the first screening test showed that there was a possibility of you having too much iron in your body we would arrange to see you and organise further blood tests in a consultant clinic at the hospital. We would tell you the results of these tests and discuss what they meant with you. You would be under no obligation to have these further tests.
If the first screening test showed that there was a possibility that you had too little iron in your blood we would tell you and your GP. Your GP might want to see you to discuss that.

Questionnaires

In order to find out what you thought about the test we will ask you to complete the enclosed questionnaire, one when you get your result and six months after that. We would also like to talk to a few people in depth about the test and if you were one of those we would contact you again to ask your permission to do this.

What are the benefits and risks of taking part?

We will pick up a few people with the predisposition to develop haemochromatosis early enough to start treatment, which would prevent them having problems from it. However it is important to realise that not everyone who is found to be at risk of developing haemochromatosis would develop problems from it.

You would also have helped in developing services for the early diagnosis of haemochromatosis.

We may also pick up other conditions that cause too much or too little iron and be able to start treatment for those conditions.

For the few people we pick up who have the predisposition to develop haemochromatosis there would be implications for their family. Their family would also be at risk of having the predisposition and may wish to see someone to discuss this. This is standard practice for the family of anyone with haemochromatosis wherever they live.

At the present time you do not have to tell insurance companies about the results of any tests done for research. If you were one of the few people diagnosed with Haemochromatosis or any other condition through this study you may have to tell a life or health insurance company if you were taking out any new policies.

Will the information I give be confidential?

Your GP will receive all your results. Apart from that

Yes. The information collected on you will not be discussed or shown to anyone else apart from the research team. All paper records will be kept in secure locked files. Every one who takes part in the study will be given a study number and will be referred to by that number in any information that is kept on computer. No personal data will be stored on computer.

What will happen to my samples?

The samples will only be looked at for testing related to haemochromatosis. They will not be used for any further study without coming back to you and asking your permission.

Who is involved in this research?

Your GP practice is involved together with Christine Patch an experienced genetic counsellor/ specialist nurse and Dr William Rosenberg a consultant who is an expert on haemochromatosis, who will be doing the clinics at the hospital. Other health researchers in the university are also involved.

The research is funded with research grants from the NHS and from the European Union. We aim to publish the results of the research in major medical journals.

What do I have to do now?

If you have any questions at all then please contact Christine Patch on 023 8079 6742. If she is not there please leave a message and she will get back to you.

I would like to take part in the research

If you would like to take up the offer of screening for haemochromatosis please send back the enclosed questionnaire and put a cross in the box saying you do want an appointment for a blood test. You will be sent an appointment for a clinic at your GP's surgery to have the blood taken. When you are seen in the clinic you will be asked to sign a consent form.

I do not want to take part

Thank you for your time. It would be very helpful for us to know a bit more about people who do not want to take part. If you would be willing to fill in and return the enclosed questionnaire we would be very grateful.

We hope that you are interested in this study. Entering the study is entirely voluntary and you are free to withdraw from it at any time. Your decision will not affect your future health care in any way.

Thank you again for your time.

Christine Patch
Genetic Counsellor/Research fellow

Health Care Research Unit	Telephone	+44 (0)23 80 796742
Mailpoint 805	Fax	+44 (0)23 80 796529
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Southampton General Hospital		
Southampton		
SO16 6YD		

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University of Southampton

School of Medicine

Community Clinical Sciences Division

LREC 382/00
B December 2000

Information sheet

Haemochromatosis screening study

Why is this study being done?

We would like to tell you about a study we are doing in your GP practice that is looking at two different ways of testing for a condition called haemochromatosis. We are working with senior doctors and researchers from Southampton Hospital. We hope that this study will help us develop better ways of diagnosing this condition earlier.

Please would you take the time to read the enclosed information and then decide if you would be willing to be tested for haemochromatosis.

How common is Haemochromatosis?

About 1 in 300 people from the UK would have the possibility of developing the condition. We do not know how many of those people will actually have health problems.

What is haemochromatosis?

If you have haemochromatosis you carry on absorbing iron from your food even though you have enough iron in your body. This extra iron cannot be got rid of and is stored in various organs in the body. The build up of iron in these organs can cause damage. The main organ that can be damaged is the liver; iron can also build up in the pancreas and cause diabetes, the joints causing arthritis, the heart and the endocrine glands. If the body is storing too much iron and these organs are being damaged then this can cause serious health problems.

Is there a treatment?

Yes. The treatment is for the doctor to take blood from you regularly, in the same way as if you were being a blood donor. The body needs to use its stored iron to make new blood cells and therefore the damaging levels of iron in the body are reduced. The level of iron in the body is monitored and how often a person has to give blood depends on how quickly the iron builds up again.

Does the treatment work?.

Yes. If the treatment starts before the liver or other organs are damaged then a person with haemochromatosis will live normal life. We therefore need to try and diagnose it as early as possible.

How do you get haemochromatosis?

It is a genetic condition. There is a gene that controls how much iron we absorb from our food. We have two copies of this gene one from our mother and one from our father. (This is the same for all the other genes that we have) Someone who has haemochromatosis has two copies of a gene that does not work quite as well as it should do. This means they are at risk of developing iron overload. As far as we know somebody with one haemochromatosis gene and one normal gene is very unlikely to have problems. Recent exciting genetic research in the UK has identified the gene responsible and we are trying to understand more about it.

How do you test for the condition

You can test for the condition either by taking some blood and looking at how much iron is there or by looking for the faulty genes.

Why isn't everybody tested for this condition?

We don't know yet the best way to test for this condition early enough to prevent problems from it. We also don't know how many people who are predisposed to develop the condition will develop problems from it. This study will start to help to answer some of these questions.

Our study

We would like to offer you screening for haemochromatosis using a test for the faulty genes.

What is screening?

Screening is trying to identify people who may be at risk of a disease, or to pick up the early stages of a disease. You will probably be aware of screening programmes such as the cervical smear test that women have. It is important to understand that **any** screening test will miss some people who actually have the disease and will pick up people who may not have or develop any health problems.

What does taking part involve?

Initial Testing

The initial test would be done on a sample of your saliva (spit). If you agreed to take part we would send you a sampling kit through the post. You would take the sample yourself, complete a consent form and post it back to us. If you preferred we could see you in a clinic and take the sample then. You will be contacted and told whether this first screening test meant we would advise further tests, or if we would recommend no further tests. The vast majority of people would need no further tests. We will only advise further tests if you have two altered copies of the gene. If you have only one altered copy we will count that as a normal result.

Further testing

If the first screening test showed that there was a possibility of you having a predisposition to haemochromatosis we would arrange to see you and organise further blood tests in a consultant clinic at the hospital. We would tell you the results of these tests and discuss what they meant with you. You would be under no obligation to have these further tests.

Questionnaires

In order to find out what you thought about the test we will ask you to complete the enclosed questionnaire, one when you get your result and six months after that. We would also like to talk to a few people in depth about the test and if you were one of those we would contact you again to ask your permission to do this.

What are the benefits and risks of taking part?

We will pick up a few people with the predisposition to develop haemochromatosis early enough to start treatment, which would prevent them having problems from it. However it is important to realise that not everyone who is found to be at risk of developing haemochromatosis would develop problems from it.

You would also have helped in developing services for the early diagnosis of haemochromatosis.

We may also pick up other conditions that cause too much or too little iron and be able to start treatment for those conditions.

For the few people we pick up who have the predisposition to develop haemochromatosis there would be implications for their family. Their family would also be at risk of having the predisposition and may wish to see someone to discuss this. This is standard practice for the family of anyone with haemochromatosis wherever they live.

At the present time you do not have to tell insurance companies about the results of any tests done for research. If you were one of the few people diagnosed with Haemochromatosis or any other condition through this study you may have to tell a life or health insurance company if you were taking out any new policies.

Will the information I give be confidential?

Your GP will receive all your results. Apart from that

Yes. The information collected on you will not be discussed or shown to anyone else apart from the research team. All paper records will be kept in secure locked files. Every one who takes part in the study will be given a study number and will be referred to by that number in any information that is kept on computer. No personal data will be stored on computer.

What will happen to my samples?

The samples will only be looked at for testing related to haemochromatosis. They will not be used for any further study without coming back to you and asking your permission.

Who is involved in this research?

Your GP practice is involved together with Christine Patch an experienced genetic counsellor/ specialist nurse and Dr William Rosenberg a consultant who is an expert on haemochromatosis, who will be doing the clinics at the hospital. Other health researchers in the university are also involved.

The research is funded with research grants from the NHS and from the European Union. We aim to publish the results of the research in major medical journals.

What do I have to do now?

If you have any questions at all then please contact Christine Patch on 023 8079 6742. If she is not there please leave a message and she will get back to you.

I would like to take part in the research

If you would like to take up the offer of screening for haemochromatosis please send back the enclosed questionnaire and put a cross in the box saying you do want an appointment for a blood test. You will be sent an appointment for a clinic at your GP's surgery to have the blood taken. When you are seen in the clinic you will be asked to sign a consent form.

I do not want to take part

Thank you for your time. It would be very helpful for us to know a bit more about people who do not want to take part. If you would be willing to fill in and return the enclosed questionnaire we would be very grateful.

We hope that you are interested in this study. Entering the study is entirely voluntary and you are free to withdraw from it at any time. Your decision will not affect your future health care in any way.

Thank you again for your time.

Christine Patch
Genetic Counsellor/Research fellow

Health Care Research Unit	Telephone	+44 (0)23 80 796742
Mailpoint 805	Fax	+44 (0)23 80 796529
Level B, South Academic Block	Email	cp2@soton.ac.uk
Southampton General Hospital		
Southampton		
SO16 6YD		

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1 B «STUDY_ID»

Ethics submission number 382/00

CONSENT FORM

Title of Project: Screening for haemochromatosis

Name of Researchers: Mrs Christine Patch
Dr William Rosenberg

Please initial box

1.   I confirm that I have read and understand the information sheet
dated December 2000 for the above study and
have had the opportunity to ask questions.

2.   I understand that my participation is voluntary and that I am free to
withdraw at any time, without giving any reason, without my medical
care or legal rights being affected.

3.   I understand that sections of any of my medical notes may be looked at
by responsible individuals from the research team. I give
permission for these individuals to have access to my records.

4.   I agree to take part in the above study.

I consent to samples being taken and used for research into
disorders of iron metabolism. I understand that the research team will contact me
and ask my permission for any other studies. I understand
that I can ask for the samples to be destroyed at any time.

«FORENAME» «SURNAME»

_____   _____
Date   Signature

Researcher

_____   _____
Date   Signature

5.
ethics submission number 382/00
study ID «group» B«STUDY_ID»

«TITLE» «FORENAME» «SURNAME»
«addr1»
«addr2»
«DISTRICT» «town»
«COUNTY» «POSTCODE»

24/10/2003

Dear «TITLE» «SURNAME»

Result of your screening test for haemochromatosis

The result of your recent screening test for haemochromatosis is **normal**.

In a small number of people this test would not have detected those with a predisposition to haemochromatosis. As you may recall the test was a screening test. Any screening test will miss some people who do have the disease and will pick up some people who would never have had problems from the disease.

The result of the test we have done means it is unlikely that you would develop problems relating to haemochromatosis.

Thank you very much for your help with this study. We would be very grateful if you would complete the enclosed questionnaire and return it in the envelope provided. We will also be sending another questionnaire in a few months time and would be very grateful if you would fill that one in as well.

If you have any questions or concerns about this please do not hesitate to contact me.

Telephone 023 8079 6742

Yours sincerely

Christine Patch
Genetic counsellor/ Research fellow

Cc   Dr William Rosenberg

6.
ethics submission number 382/00
study ID 2 B«STUDY_ID»

«TITLE» «FORENAME» «SURNAME»
«addr1»
«addr2»
«DISTRICT» «town»
«COUNTY» «POSTCODE»

Dear «TITLE» «SURNAME»

Result of your screening test for haemochromatosis

The result of your recent screening test for haemochromatosis is **normal,** however it did come back slightly low.

This means that you are extremely unlikely to have haemochromatosis, but as we discussed I have sent a copy of this to your GP who may want to see you to do some further investigations for anaemia.

In a small number of people this test would not have detected those with a predisposition to haemochromatosis. As you may recall the test was a screening test. Any screening test will miss some people who do have the disease and will pick up some people who would never have had problems from the disease.

The result of the test we have done means it is unlikely that you would develop problems relating to haemochromatosis.

Thank you very much for your help with this study. We would be very grateful if you would complete the enclosed questionnaire and return it in the envelope provided. We will also be sending another questionnaire in a few months time and would be very grateful if you would fill that one in as well.

If you have any questions or concerns about this please do not hesitate to contact me.

Telephone 023 8079 6742

Yours sincerely

Christine Patch
Genetic counsellor/ Research fellow
Cc   Dr William Rosenberg

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7.

ethics submission number 382/00

study ID 2 B «STUDY_ID»

«TITLE» «FORENAME» «SURNAME»
«addr1»
«addr2»
«DISTRICT»
«town» «POSTCODE»

Dear «TITLE» «SURNAME»

Result of your screening test for haemochromatosis

The result of your recent screening test for haemochromatosis is slightly raised. This may mean nothing, but as we discussed I would like to offer you an appointment to see Dr Rosenberg and myself to discuss this. At this appointment we would like to repeat the blood test, take a medical history and do some other blood tests. In order to make this test more accurate we would like you not to eat anything from about midnight on the night before the appointment. We would also like you not to take any supplements containing iron on the morning of the appointment.

The appointment is below

Date and time   Thursday 5th September at 10.30 am

Place   **Wellcome Trust Clinical Research Facility
Level C West Wing Southampton General Hospital**

Thank you very much for your help with this study. We would be very grateful if you would complete the enclosed questionnaire and return it in the envelope provided.

If you have any questions or concerns about this please do not hesitate to contact me. Telephone 023 8079 6742

Yours sincerely

Christine Patch
Genetic counsellor/ Research fellow
Cc   Dr William Rosenberg

Appendix 4: Questionnaires and data collection sheets.

1. The self completed questionnaires were designed as A5 booklets, as were the patient information sheets. In order to save space only one questionnaire has been fully copied.

The questionnaires are as follows
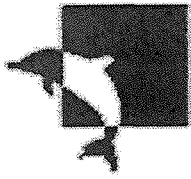
University of Southampton

Haemochromatosis Study

Questionnaire 1B

Thank you for reading the study information. It would be very helpful to us if you would consider completing and returning this short questionnaire whether or not you would like to have screening for Haemochromatosis.

Thank you for taking the time to complete this questionnaire.

☐ ☐☐☐☐☐

All the information you give is confidential and anonymous. We put a study number on the front so that no one is referred to by name.

Please fill in the questionnaire as soon as possible.

Please answer all the questions.

If you really cannot answer a question do not worry. Just leave it and carry on to the next one.

Try to answer all the questions on your own, rather than discussing them with anyone else. We are interested in what you think.

If you are considering having screening for haemochromatosis please go on to section 1 and then complete the rest of the questionnaire.

If you do not want to have screening for haemochromatosis we would be very grateful if you would take the time to answer the question below and then go on to complete the rest of the questionnaire.

This would help us understand more about why people would not want to have testing for haemochromatosis.

Please put a cross in all the boxes that apply ☒

I am not interested ☐

I do not have time ☐

I do not want a blood test ☐

I do not want to know if I have haemochromatosis ☐

Other (please give details).. ☐

....

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Section 1

1.1 Have you ever been seen by a genetics department?

Yes   ☐

No   ☐

Don't know   ☐

1.2 Have you ever had a genetic test?

Yes   ☐

No   ☐

Don't know   ☐

1.3 Have you ever heard of haemochromatosis before this letter?

Yes   ☐

No   ☐

Section 2 How you feel

2.1 A number of statements which people have used to describe themselves are given below. Read each statement then put a cross in the most appropriate box for each statement to indicate how you feel right now at this moment. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seem to describe your present feelings best.

	not at all	somewhat	moderately	very much
I feel calm	☐	☐	☐	☐
I am tense	☐	☐	☐	☐
I am upset	☐	☐	☐	☐
I feel relaxed	☐	☐	☐	☐
I feel content	☐	☐	☐	☐
I am worried	☐	☐	☐	☐

2.2 Please read each item below and put a cross in the box which comes closest to how you have been feeling in the past week. There are no right or wrong answers. Don't take too long over your replies: your immediate reaction will probably be more accurate than a long thought out reponse.

I still enjoy the things I used to enjoy

Definitely as much	☐
Not quite so much	☐
Only a little	☐
Hardly at all	☐

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I can laugh and see the funny side of things

As much as I always could ☐

Not quite so much now ☐

Definitely not so much now ☐

Not at all ☐

I feel cheerful

Not at all ☐

Not often ☐

Sometimes ☐

Most of the time ☐

I feel as if I am slowed down

Nearly all the time ☐

Very often ☐

Sometimes ☐

Not at all ☐

I have lost interest in my appearance

Definitely ☐

I don't take so much care as I should ☐

I may not take quite as much care ☐

I take just as much care as ever ☐

I look forward with enjoyment to things

As much as I ever did ☐

Rather less than I used to ☐

Definitely less than I used to ☐

Hardly at all ☐

I can enjoy a good book or radio or TV programme

Often ☐

Sometimes ☐

Not often ☐

Very seldom ☐

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Section 3 Some questions about you

This section asks a few questions about you. The answers
will be used anonymously. All the answers are confidential.

3.1 What age were you when you left full time education?
Please write your age in the box

□□ Years

3.2 During the last 30 days were you:

Employed full time   □

Employed part time   □

Self employed   □

Unemployed   □

Retired   □

Full time student   □

Looking after home/family   □

Permanently sick/disabled   □

Other (please specify)...□

3.3 How would _you_ describe the ethnic group to which you belong:

Please put a cross in one box only

White (British, Irish, any other white background) ☐

Mixed (White and Black Carribean, White and Black
African, White and Asian, any other mixed background) ☐

Asian or Asian British (Indian, Pakistani, Bangladeshi, any
other Asian background) ☐

Black or Black British (Carribean, African, any other
Asian background) ☐

Chinese ☐

Other ethnic group, please specify.. ☐

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Section 4 Your health

Please put a cross in the one box that best describes your answer for the each question.

4.1 In general would you say your health is

Excellent □

Very good □

Good □

Fair □

Poor □

4.2 Please choose the answer that best describes how true or false each of the following statements is for you.

	Definitely true	Mostly true	Not sure	Mostly false	Definitely false
I seem to get ill more easily than other people	□	□	□	□	□
I am as healthy as anyone I know	□	□	□	□	□
I expect my health to get worse	□	□	□	□	□
My health is excellent	□	□	□	□	□

Thank you very much for your time.

Please could you confirm if you would like to receive an appointment for screening for haemochromatosis

I would like to receive an appointment for screening for haemochromatosis

Yes ☐

No ☐

Your telephone number...

Please return this questionnaire in the envelope provided to:

Christine Patch
Health Care Research Unit
Level B (805)
South Academic Block
Southampton General Hospital
Tremona Road
Southampton
SO16 6YD

If you have any questions at all about this study please feel free to contact

Christine Patch: Tel: 023 8079 6742

4570328605

Section 1

1.1 Have you ever been seen by a genetics department?

Yes ☐

No ☐

Don't know ☐

1.2 Have you ever had a genetic test?

Yes ☐

No ☐

Don't know ☐

1.3 Have you ever heard of haemochromatosis before this letter?

Yes ☐

No ☐

Section 3 About your result

3.1 Please would you put a cross to indicate which words best describe your feelings about the test results today

	Yes	No
Suprised	☐	☐
Happy	☐	☐
Upset	☐	☐
Pleased	☐	☐
Healthy	☐	☐
Worried	☐	☐
Guilty	☐	☐
Unhealthy	☐	☐
Depressed	☐	☐
Relieved	☐	☐
Indifferent	☐	☐

3.2 Please put a cross in the box next to the statement that is closest to your result

I almost certainly do have haemochromatosis	☐
I probably do have haemochromatosis	☐
I probably do not have haemochromatosis	☐
I almost certainly do not have haemochromatosis	☐
I haven't been given the result	☐
I don't remember the result	☐

9663263656 ◢

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3.3 How confident do you feel about the results of this test?
(Please circle the number the best reflects your view)

not at all   0   1   2   3   4   5   6 completely
confident   confident

3.4 My test result is puzzling me

strongly   0   1   2   3   4   5   6 strongly
disagree   agree

3.5 My test result makes sense to me

strongly   0   1   2   3   4   5   6 strongly
disagree   agree

Please return this questionnaire in the envelope provided to:

Christine Patch
Health Care Research Unit
Level B (805)
South Academic Block
Southampton General Hospital
Tremona Road
Southampton
SO16 6YD

If you have any questions at all about this study please feel free to contact

Christine Patch: Tel: 023 8079 6742

3407263656

2. Haemochromatosis screening study

Clinical Questionnaire

Study Number ☐ ☐ ☐ ☐ ☐ ☐

It would be extremely helpful if you could fill in the questionnaire and hand it to Dr Rosenberg or Christine Patch when you see them. All your replies are confidential. The questions are about things that may affect your iron levels or your chance of having haemochromatosis.

1. Are you taking any of the following tablets?

	Yes	No
a. Iron supplements	☐	☐
b. Vitamin C	☐	☐
c. Multivitamins (eg Sanatogen Gold)	☐	☐

	Yes	No
2. Do you ever eat meat	☐	☐

If yes: How many times per week/month (on average) do you eat meat?
(Please tick one box only)

1-2 times per month	☐
1-3 times per week	☐
4-7 times per week (up to once per day)	☐
7-14 times per week (1-2 times per day)	☐
14+ times per week (2+ times per day)	☐

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3. We would like to know about how much alcohol you drink because alcohol can affect your iron levels.

Do you ever drink alcohol?

No ☐

Yes ☐

If yes: **How much of the following types of drink do you usually have per week (on average)?**

a. Beer/ lager/ stout/ cider .... 1/2 pints

b. Spirits or liqueurs (including gin,whisky, ... glasses
rum,brandy,cocktails)

c. Sherry or martini (including port, vermouth.................. glasses
cinzano)

d. Wine (including babycham,champagne) .... glasses

e. Shandy (excluding bottle or can) .... 1/2 pints

NB Doubles count as two single glasses.
   Do not include non and low alcoholic drinks

4. Have you ever had a blood transfusion?   **Yes**   **No**
                                                  ☐   ☐

If yes

In which year/s did you have the blood transfusion(s)? ....

How many units were you given (total number if you know) ....

5. Have you experienced any major blood loss (more than two units of blood) in the last 6 months?

                                                  Yes   **No**
                                                   ☐   ☐

6. <u>For women only</u> **Do you have, or have you ever experienced, heavy periods?**

Yes	No
☐	☐

(Heavy periods = bleeding more than 6 days per month, using more than 6 tampons/towels per day, flooding or passing blood clots)

7. Have you ever been told by a doctor that you have liver disease?

Yes	No
☐	☐

<u>If yes</u> **What diagnosis were you given?**...

....

8. Have you ever been told by a doctor that you have diabetes?

Yes	No
☐	☐

<u>If yes</u> **a. When were you first diagnosed (year)**...

   b. Do you take insulin

Yes	No
☐	☐

9. Have you ever been told by a doctor that you have problems with your heart?

Yes	No
☐	☐

If yes a. What problem(s) do you have?   . ...

b. When was the problem first discovered? (year)...........................

10. Do you have any problems with your joints including pain, stiffness and/or swelling?

	Yes	No
	☐	☐

If yes **Which joints are affected?** (e.g. left hip,both knees etc.)

11. Do you have any other major health problems?

	Yes	No
	☐	☐

If yes: **Please say what they are**

12. Has any one in your immediate blood family (your parents, brothers, sisters or your children) ever been told by a doctor that they have any of the following conditions?

	Yes	No

If yes: **Who is affected and with what?**

a. Liver disease   ☐   ☐

....

b. Heart disease   ☐   ☐

....

c. Diabetes　　　　　　　　☐　　☐

....

d. Severe arthritis　　　　　☐　　☐

....

Other major problems

....

....

Thank you very much for your time.

3. Data Collection form Low iron

Study ID [   ]

previously known pathology

History

	Yes/No	Comments
Nutrition		
Menstruation		
NSAID use		
GI symptoms		
Family history of bleeding disorders		

Laboratory investigations

	Yes/No	Result
ferritin		
haemoglobin		
iron		
Other		

Other investigations

	Yes/No	Result
upper GI endoscopy		
lower GI endoscopy		
other		

Diagnosis	Treatment

Appendix 5 : Data from discharged group.

Sex	Age	TS	Genotype	Genotype2	Repeat TS	Ferritin	ALT
female	32	.	HDCY	HDCC	39.86	18	20
female	42	.	HDCY	HDCY	48.52	58	39
male	56	45.45	.	HHCC	45.45	135	28
male	59	45.45	.	HDCC	48.61	97	37
male	66	45.89	.	HDCC	63.11	191	19
male	37	45.89	.	HDCC	77.73	100	20
male	68	46.67	.	HDCC	53.14	110	25
female	56	46.71	.	HDCC	38.33	24	9
female	37	46.73	.	HDCC	34.09	43	21
male	58	46.88	.	HHCY	33.33	73	22
female	50	46.99	.	HHCC	37.40	40	15
male	65	47.03	.	HHCC	18.60	112	29
female	57	47.17	.	HHCC	37.85	118	20
female	39	47.46	.	HDCC	16.61	10	14
male	52	47.49	.	HHCY	38.62	36	43
female	38	48.89	.	HDCC	42.22	19	21
male	69	49.02	.	.	64.61	65	21
male	67	49.42	.	HHCC	49.57	131	26
male	55	49.47	.	HDCC	39.43	43	42
male	40	49.60	.	HHCY	41.51	36	39
female	68	50.51	.	HDCC	55.02	45	29
female	44	50.51	.	HHCY	50.72	40	24
male	55	50.72	.	HHCC	50.69	19	130
female	51	50.76	.	HHCY	.	.	.
male	67	50.97	.	HHCC	38.10	109	26
male	54	51.40	.	HHCC	32.13	115	39
male	53	51.72	.	HDCC	56.87	86	18
male	39	51.72	.	HHCY	53.10	35	35
female	65	52.50	.	.	24.49	134	21
female	57	52.85	.	HHCC	81.97	76	18
female	66	53.40	.	HHCY	36.14	120	24
male	67	53.57	.	HHCY	57.50	96	41
female	61	53.72	.	HDCY	18.02	.	.
female	45	54.05	.	.	38.46	96	45
female	63	54.19	.	HHCY	61.27	56	15
male	38	54.51	.	HDCC	33.09	114	30
male	54	54.69	.	HHCC	63.38	198	39
male	66	55.28	.	HDCY	46.22	146	21
female	60	56.86	.	HHCC	22.81	66	16
female	70	58.96	.	HDCC	22.06	15	12
female	44	59.67	.	DDCC	32.47	6	20
female	66	60.39	.	HHCY	44.87	135	27
male	61	60.67	.	HHCC	51.65	144	28
female	48	65.12	.	HDCY	54.19	49	18
male	35	65.85	.	HDCY	58.58	31	15
female	61	66.67	.	HDCC	49.71	49	19

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