British Society for Haematology

British Committee for Standards in Haematology

Guidelines on diagnosis and therapy

Genetic Haemochromatosis

guidelines

guidelines

Level of evidence

Level	*Type of evidence*
Ia	Evidence obtained from meta-analysis of randomised controlled trials
Ib	Evidence obtained from at least one randomised controlled trial
IIa	Evidence obtained from at least one well-designed controlled study without randomisation
IIb	Evidence obtained from at least one other type of well-designed quasi-experimental study
III	Evidence obtained from well-designed non-experimental descriptive studies, such as comparative studies, correlation studies and case control studies
IV	Evidence obtained from expert committee reports or opinions and/or clinical experiences of respected authorities

Grade of recommendation

Grade	*Evidence level*	*Recommendation*
A	Ia, Ib	Required – at least one randomised controlled trial as part of the body of literature of overall good quality and consistency addressing specific recommendation
B	IIa, IIb, III	Required – availability of well-conducted clinical studies but no randomised clinical trials on the topic of recommendation
C	IV	Required – evidence obtained from expert committee reports or opinions and/or clinical experiences of respected authorities
		Indicates absence of directly applicable clinical studies of good quality

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British Society for Haematology

Genetic haemochromatosis

A guideline compiled on behalf of the Clinical Task Force of the British Committee for Standards in Haematology by

Dr James Dooley and Professor Mark Worwood

Methods

- Provided as a solicited draft guideline
- Based on a Medline search of world literature
- Review of existing guidelines of Expert Groups
- Presented in open forum at BSH April 1999

Clinical Task Force of BCSH: Prof AK Burnett (Chair), Drs D Milligan, R Marcus, J Apperley, P Ganly, S Johnson, J Davies (Secretary), SE Kinsey

Genetic haemochromatosis

1 Current understanding of haemochromatosis

Methods

The literature review is based on a total of forty years experience in haemochromatosis by the authors, on searching the literature using appropriate keywords (in Medline and BIDS), and a review of the existing guidelines published by Expert Groups[1,2], as well as guidelines in the process of development as a result of the International Consensus Conference on Hemochromatosis (European Association for the Study of the Liver), Sorrento, 1999.

Evidence and strength of recommendations

Randomised controlled trials

In 1976 the results of a study of venesection therapy for the removal of excess iron from patients with genetic haemochromatosis were published[3]. The authors compared the survival of treated patients with that of historical controls (patients presenting with genetic haemochromatosis but not receiving phlebotomy). For patients receiving venesection therapy, life expectancy was significantly improved compared with that for untreated patients. This result has since been confirmed and extended in larger studies[4–6]. If phlebotomy is started before cirrhosis and diabetes have developed, life expectancy is normal. However, the obvious success of this treatment has meant that it has not been ethically acceptable to compare venesection with 'no treatment' or even an alternative such as iron chelation with subcutaneous desferrioxamine which would certainly be more expensive and probably a less effective treatment.

For similar reasons there have been no randomised controlled trials of the effect of treatment in family members of probands with genetic haemochromatosis. Once it became feasible to identify siblings at risk from iron overload because they shared the same HLA-A, B haplotypes as the proband it became clear that most showed evidence of iron accumulation. Phlebotomy has been undertaken if there is evidence of iron overload and it has not been acceptable to delay treatment in order to follow the development of clinical symptoms or assess the value of phlebotomy in preventing disease.

For this reason there are no recommendations graded A.

2 Introduction

Definitions

No classification of haemochromatosis comfortably encompasses all forms of iron overload.

Haemochromatosis is the clinical condition of iron overload[7].

Genetic haemochromatosis refers predominantly to iron accumulation in the body due to the inheritance of mutations in the *HFE* gene on both copies of chromosome 6. This leads to excessive absorption of iron from food. In the UK over 90% of patients with genetic haemochromatosis are homozygous for the C282Y mutation of the *HFE* gene and another 4% are compound heterozygotes (C282Y/H63D). This is the condition previously known as HLA-linked haemochromatosis. There are other rarer forms of inherited haemochromatosis where patients have 'classical' clinical features of haemochromatosis but lack mutations in the *HFE* gene (see also juvenile haemochromatosis, below). In such families there may be no association with HLA haplotypes or other markers for chromosome 6[8].

Juvenile haemochromatosis is an inherited condition in which there is clinical onset in the second or third decade. The gene responsible is probably located on chromosome 1[9].

African Iron overload describes a syndrome originally thought to be related to the drinking of large quantities of beer brewed in iron containers, although a genetic influence has been detected[10].

Secondary iron overload (secondary haemochromatosis, haemosiderosis) describes iron overload following chronic blood transfusion for haematological conditions, including thalassaemia major and aplastic anaemia. This also includes conditions in which enhanced iron absorption is secondary to ineffective erythropoiesis with marrow hyperplasia. Thalassaemia intermedia and inherited sideroblastic anaemias are examples.

Neonatal haemochromatosis is a condition of acute liver damage with iron accumulation[11]. This encompasses severe iron overload in neonates of undefined pathogenesis.

Description and history of genetic haemochromatosis (HC)

HC is a condition caused by continued absorption of iron from the upper small intestine, despite normal and then increased total body iron. This leads to accumulation of iron in the tissues as the body has no means of getting rid of excess iron. In advanced disease, iron accumulation causes widespread tissue damage including diabetes mellitus and cirrhosis. The mean age of diagnosis for 251 patients studied between 1947 and 1991 was 46 ± 11 years (mean ± SD)[5]. The disorder is inherited in autosomal recessive fashion. The gene involved lies close to the HLA-A region on chromosome 6. Prevalences

have been estimated from 1 in 2000 to 1 in 200 (0.5%) in various populations of northern European origin[12]. The diagnosis is made by biochemical screening using serum transferrin saturation and ferritin levels. In most cases demonstration of homozygosity for the C282Y mutation in *HFE* (see section 3) confirms the diagnosis at any age and however much iron has accumulated. If this genotype is not found, confirmation of the presence of iron overload requires the determination of liver iron concentration. In the C282Y homozygous, however, liver biopsy may be done to define the degree of fibrosis and whether cirrhosis has developed. HC is a treatable condition. The excess iron can be removed by phlebotomy – weekly venesection of 500 ml of blood. This may take up to 2 years.

The syndrome now recognised as *HFE*-related haemochromatosis was first described in 1865 by Trousseau, and the name was first used by von Recklinghausen in 1889 (see ref. 13). Sheldon[13] provided a full description of the disorder and also suggested that it was inherited. However, until the 1970s this proposition was not universally accepted and McDonald[14] believed that it was the co-occurrence of two conditions: liver disease and a high dietary iron intake, both resulting from a high intake of iron-containing alcoholic drinks. In 1969 Saddi and Feingold (see ref. 15) made a firm proposal for a recessive mode of transmission. The introduction of the serum ferritin assay[16] in Cardiff in 1972 provided a considerable impetus for the study of haemochromatosis, but the ferritin assay has not proved to be as valuable in detecting the early stages of iron accumulation as was first hoped. It was the discovery of the association between HC and certain HLA antigens (in particular HLA-A3) by Simon *et al.*[17] in 1975 which made possible the genetic investigation of the disorder. This discovery made possible the testing of families for HC. Any sibling sharing the same haplotypes (not necessarily involving HLA-A3) as the proband is at risk from iron overload. Siblings sharing one haplotype are carriers, and those not sharing haplotypes are unaffected.

Table 1 Distribution of iron in the body (70 kg man)

Protein	Location	Fe content (mg)
Haemoglobin	Erythrocytes	3000
Myoglobin	Muscle	400
Cytochromes, other haem and Fe-S proteins	All tissues	50
Transferrin	Plasma and extravascular fluid	5
Ferritin and haemosiderin	Liver, spleen and bone marrow	0–1000

Iron balance and iron overload

Table 1 shows the distribution of iron in the body of a normal 70 kg man. Most of the iron is present in haemoglobin, and some as myoglobin in muscle. There are many iron-containing proteins involved in respiration in all tissues, and there is a small amount of iron bound to transferrin in the plasma and extravascular circulation. Ferritin and haemosiderin iron is found in all cells and in a normal man may account for up to 1 g Fe. This so-called 'storage iron' is available for haem synthesis if required. In haemochromatosis there is an increase (perhaps a doubling) in the amount of transport iron and a large increase in the storage iron compartment. This may exceed 40 g. Treatment by phlebotomy (bleeding) works because each 500 ml of blood taken from the body removes about 250 mg of iron in the form of haemoglobin. Synthesis of new haemoglobin removes iron from the stores, which are gradually depleted.

The pathogenetic mechanism leading to iron overload has been the focus of research for over forty years. Despite an increasing understanding of the proteins involved in iron metabolism, no defect or abnormality had been found in any of the iron transport or storage proteins or their receptors, including ferritin, transferrin and the transferrin receptor. Moreover, none of the genes for these proteins was on chromosome 6, the locus for the HC defect derived from the genetic linkage to the HLA class I serotype. Because of the lack of a candidate protein, the focus of research turned to positional cloning, particularly as resources such as Yeast Artificial Chromosome (YAC) libraries became available.

3 The *HFE* gene and its mutations

In 1996 Feder *et al.*[18] described an HLA-class-I-like gene (*HFE*) in which there were mutations in most patients satisfying the diagnostic criteria for haemochromatosis. Ninety percent of patients with HC carried a mutation at amino acid 282 of *HFE* which resulted in the replacement of cysteine by a tyrosine residue. Feder *et al.*[18] also described a second mutation at amino acid 63, in which aspartic acid replaces histidine. This was common in the general population and was not usually associated with iron accumulation. Structural and functional implications are described in Appendix 1.

Prevalence of genetic haemochromatosis and *HFE* mutations

HC is common, at least in some countries where the population is largely of northern European origin. A number of population surveys of more than one thousand subjects were carried out before the discovery of the *HFE* gene. Suspected iron overload was confirmed by liver biopsy in most cases. The prevalence of iron overload ranged from 0.05% (1 case per 2000) in parts of Finland to nearly 0.5% (1 case per 200) in Utah. An incidence of about 1 in 300 has been reported from Brisbane, Denmark, Germany, Iceland and parts of Sweden[12].

Since the description of the *HFE* gene mutations by Feder *et al.*[18] genotypes from patients with HC have been determined in many countries. This has proved to be relatively straightforward because restriction sites are created or abolished by the C282Y and H63D mutations, allowing a simple analysis by digestion with restriction enzymes after the PCR. Many methods of analysis have now been described[19].

From 60% to 100% of patients satisfying the criteria for HC have been found to be homozygous for the C282Y mutation. The highest value was reported for Queensland, Australia (100%) and the lowest for Italy (64%), with intermediate values reported for other countries. These results are summarised in Table 2. In all the groups of patients with HC a few percent appear to be compound heterozygotes (C282Y, H63D), a few patients are apparently heterozygous for the C282Y mutation, and a variable proportion lack detectable mutations in this gene. So far, no case has been found where the C282Y and H63D mutations are on the same chromosome.

It has been pointed out that the frequency of the H63D mutation on chromosomes lacking the C282Y mutation in HC patients is significantly greater than in the general population[19, 57]. A study from Montpelier[58] showed that about three quarters of the subjects with this genotype investigated as family members of a patient with HC or for possible iron overload had some evidence of iron overload and some had clinical haemochromatosis. The significance of homozygosity for *HFE* H63D in terms of iron overload is not known.

Table 2 **Genotype frequencies (%) for mutations in the *HFE* gene in patients with haemochromatosis**

Country/ Region	No of Subjects	Genotypes (C282Y/H63D) ++/--	+-/+-	+-/--	--/--	--/+-	--/++	Reference
Australia	112	100*	0	0	0	0	0	(24)
Brittany	132	92	2.3	2.3	0	1.5	1.5	(25)
UK	115	91	2.6	0.9	4.3	0	0.9	(26)
Germany	57	90	3.5	1.8	0	5.2	0	(27)
USA	178	83	4.5	0.6	12	0	0.5	(18)
USA	147	82	5.4	1.4	6.8	2.7	1.4	(28)
France	94	72	4.3	4.3	8.5	8.5	2.1	(29)
Italy	75	64	6.7	2.7	21	4.0	1.3	(30)
USA (Alabama)	74	60	5.4	15	8.1	8.1	4.0	(31)

The selected studies include more than 50 subjects. Three systems of mutation nomenclature are in use for the *HFE* genes: amino acid (3 letter abbrev), amino acid (single letter) or cDNA based[28]. The mutations are therefore described as Cys282Tyr, C282Y or 845A; His63Asp, H63D or 187G.

** Includes more than one family member in some families*

Other mutations associated with haemochromatosis

A number of groups have sequenced the cDNA for HFE, β_2-microglobulin and the transferrin receptor in patients with HC who lack either the C282Y or H63D mutation, in order to discover other causative mutations[20–22]. None has been discovered for β_2-microglobulin and the transferrin receptor. To date, other mutations have been found in the *HFE* gene in 5 patients. Pointon *et al.*[23] found a heterozygous deletion of a single nucleotide in exon 3 (478delC) in a patient negative for C282Y and H63D but with classic haemochromatosis. They suggested that this mutation has a dominant effect as it causes a premature stop codon downstream of the mutation. Wallace *et al.*[32] described a splice site mutation (IVS3+1G→T) in a patient with classic HC who was heterozygous for the C282Y mutation. The mutation would lead to the formation of a protein lacking the extracellular α_2 domain. The mutation S65C is found on about 2% of chromosomes in the general population[33, 34] and on about 8% of chromosomes from patients with HC lacking the C282Y and H63D mutations. This mutation appears to be associated with a mild form of HC[33]. In the UK the S65C mutation may be implicated in about 1% of cases of HC. Barton *et al.*[34] have described a family in which a G93R mutation is associated with haemochromatosis and another family in which the mutation I105T is associated with iron overload. In the first case patients with iron overload also carried the C282Y mutation, and in the second family H63D was present.

A PCR artifact?

There have been recent reports that a polymorphism in intron 4 (IVS4+48G/A), originally described by Totaro *et al.*[30] may cause false results on genotyping for the C282Y mutation. This polymorphism is in the binding region of a reverse PCR primer[18]

widely used in the diagnosis of HC and may prevent amplification of the wild-type allele in subjects heterozygous for C282Y. This may result in the incorrect assignment of homozygosity for C282Y[35, 36]. However, extensive investigation has shown that under suitable PCR conditions this does not occur and that the validity of previous publications was not compromised by this polymorphism [37]. It is clearly desirable to avoid this potential cause of mistyping by selecting alternative primers.

HFE gene mutations in various countries

Of considerable interest is the frequency for the C282Y mutation throughout the world. The C282Y mutation is confined to populations of European origin and is, furthermore, more common in northern than in southern Europe (see Figure 1). For further details see Appendix 2.

Interaction of *HFE* mutations with other iron-loading conditions

It has long been thought that there may be a high frequency of heterozygosity for HC in patients with porphyria cutanea tarda, sideroblastic anaemia, and hereditary spherocytosis with iron overload, amongst other conditions[38]. Until recently this was a matter for debate, but investigation of *HFE* mutations has enabled these questions to be answered (Appendix 3).

Selective advantage or pathological condition?

See Appendix 4.

Early diagnosis and life expectancy

If patients are diagnosed in the pre-cirrhotic, pre-diabetic stage and treated by venesection to remove the excess iron then life expectancy is normal[5]. However, once cirrhosis and diabetes mellitus have developed, patients have a shortened life expectancy and, if cirrhosis is present, a high risk of liver cancer even when iron depletion has been achieved. It is therefore important to diagnose the condition as early as possible.

Clinical penetrance

Within families

Relatively few studies of the clinical penetrance of HC within families have been published. These were reviewed by Bradley *et al.*[39]. A total of 197 homozygous relatives (sharing two HLA haplotypes) of symptomatic probands were assessed. On first investigation almost 90% of male relatives had a transferrin saturation of > 60%, and 80% of female relatives had a saturation of > 50%. Of the males, 67% (95% CI, 56–75%) showed at least one of the following clinical manifestations: hepatomegaly, abdominal pain, skin

Figure 1 Frequency (%) of the C282Y mutation in various countries or regions in Europe and Algeria. There are more than 90 subjects in each sample. Sources of data: Iceland, Norway, Italy and Greece[41], Norwich[42], Oxford[43], Belfast[44], S. Wales[45], France[29], Brittany[25], Finistère Sud[46], Brest[47], Austria[48], Germany[27], Hungary (Budapest)[49], Eastern Hungary/Romany[50], Denmark[51], Sweden and N. Finland[52], Spain[53], Algeria[54] and N.E. Scotland[121]. Note the surprising variations between adjacent regions, probably reflecting variation due to sample size as well as population differences. Allele frequencies for the H63D mutation are about 12%[41].

pigmentation, weight loss, fatigue, arthropathy, hypogonadism, impotence, liver disease and cirrhosis. The corresponding figure for females was 41% (95% CI, 29–54%). For men there was an increased likelihood of clinical manifestations with age. More recently, Adams *et al.*[40] reported on 133 patients detected as a result of family screening. The mean transferrin saturation was 71%, and 53% of those with a raised transferrin saturation displayed at least one clinical manifestation of haemochromatosis. However, some people who are homozygous for the C282Y mutation never develop iron overload[55, 56].

In C282Y homozygotes detected by genetic testing

Olynyk *et al.*[59] conducted a population-based study of 3011 unrelated, white adults in Busselton, Australia and found that 0.5% were homozygous for *HFE* C282Y. The serum transferrin saturation was 55% or more in a fasting sample in 15 of these 16 subjects, but only half of these had clinical features of haemochromatosis, and in one quarter serum ferritin levels remained normal over a 4-year period. The subjects homozygous for C282Y were from 26 to 70 years old at the start of the study. There is no information about the proportion of relatives who will show clinical manifestations of haemochromatosis.

In heterozygotes

Very few heterozygous family members display clinical manifestations of haemochromatosis. Bulaj *et al.*[60] assigned heterozygosity to 1058 members from 202 pedigrees. Four percent of males showed an initial transferrin saturation of > 62%, and 8% of females had an initial transferrin saturation of > 50%, but in most of these subjects the transferrin saturation in a fasting sample did not exceed these thresholds. The geometric mean serum ferritin concentration was higher in heterozygotes than in those lacking the gene and increased with age. Twenty percent of males and 8% of female heterozygotes had concentrations in excess of the 95 percentile value for age-matched controls. Clinical manifestations were rare, and liver disease was usually associated with alcoholism, hepatitis or porphyria cutanea tarda. The extent to which heterozygosity for C282Y increases the risk of developing other conditions is controversial.

Population screening

See Appendix 6 for a discussion of the benefits and disadvantages.

4 Diagnosis of HC

Recommendation 1: Clinical features which justify investigation for HC

Subjects of European ancestry presenting with unexplained weakness or fatigue, abnormal liver function tests, arthralgia/arthritis, impotence, diabetes of late onset, cirrhosis, or bronze pigmentation should be investigated as in Recommendation 2.

Evidence IIb–IV; Grade B, C

The difficulties of early diagnosis

Unfortunately, early diagnosis is not easy. The symptoms with which patients present are relatively common and non-specific (Table 3). Raised ferritin concentrations are common in hospital patients, and serum iron concentrations are very labile, but most adults with HC have an elevated, fasting transferrin saturation[39]. A genetic test offers the best approach to early detection, but the lack of information on clinical penetrance is delaying its use for population screening.

Table 3 Presenting symptoms in patients with haemochromatosis

Symptom or physical finding	% of patients	
	1	2
Weakness or fatigue	52	82
Pigmentation	47	72
Arthralgia	32	44
Impotence (% of males)	40*	36
Cirrhosis	27	57
Diabetes mellitus	15	48
Cardiac disease	10	12†

1 277 patients presenting in Rennes (Brittany) and London (Ontario) between 1962 and 1995[40]. The incidence of symptoms was lower in family members tested after the diagnosis was made in the proband. *All patients – including family members

2 251 patients presenting in Düsseldorf and Bad Kissinger (Germany) from 1947–1991[5]. 8% of these were identified through family screening. †Dyspnoea on exertion.

Laboratory evaluation of an iron-loading tendency

Transferrin saturation

The most specific and sensitive test for iron accumulation is the transferrin saturation. This is calculated from the serum iron concentration and the total iron-binding capacity (TIBC), which is a measure of transferrin concentration as each transferrin molecule can bind two atoms of iron. The percentage saturation is then calculated (100 x serum iron / TIBC) and a value of greater than 55% (men) or 50% (women) suggests iron accumulation due to HC. The measurement of transferrin saturation should be repeated on a fasting sample to confirm its elevation. (See later discussion about diagnostic limits for transferrin saturation.)

Although a raised transferrin saturation provides an early indication of iron accumulation[61], the transferrin saturation is not necessarily raised in young people who are homozygous for HC. Furthermore there are many other causes of a raised transferrin saturation. Unfortunately, serum iron concentrations are highly variable, and it is necessary to measure the transferrin saturation on a morning, fasting sample in order to obtain a result which is not influenced by recent dietary intake or by diurnal variation. Over 90% of blood donors from Utah who had a transferrin saturation of > 50% on first testing did not have iron overload. On testing a fasting sample, only 9% of this initial cohort had a transferrin saturation of > 62% (men) or > 55% (women). However, 42% of this 9% had a raised liver iron concentration. In a more recent study of 16,031 primary care patients 932 had a transferrin saturation of > 45%, 42% of these had a fasting saturation of > 45%, and 18% of these had biopsy or clinically proven haemochromatosis. Another 9% were described as having 'probable haemochromatosis'. Again a diagnosis of haemochromatosis was made in under 10% of those with a raised, non-fasting transferrin saturation[62].

Recommendation 2: Detecting iron accumulation

1 Measure serum iron concentration and total iron-binding capacity and calculate transferrin saturation.

2 If transferrin saturation is greater than 50% repeat the measurement on a fasting sample.

A fasting transferrin saturation of greater than 55% (men and post-menopausal women) or 50% (pre-menopausal women) indicates iron accumulation.

3 Measure serum ferritin concentration.

Evidence IIb–IV: Grade B,C

The definition of an elevated transferrin saturation has ranged from > 45% to > 62%[63]. The lower the value selected the greater will be the sensitivity and the lower the specificity. A lower threshold may be more appropriate for fasting samples[64].

Standardisation of the assay for total iron-binding capacity remains an aim rather than a reality. Both the ICSH (International Committee for Standardization in Haematology)[65] and the NCCLS (National Committee for Clinical Laboratory Standards)[66] have proposed reference methods based on the removal of excess iron, added to saturate the transferrin, with magnesium carbonate, but this has not yet been accepted as an international reference method. Furthermore it is not easily automated. Although the alternative of measuring transferrin concentration immunologically is intrinsically better and widely used, standardisation has not been achieved[67]. It is clear that the selection of a suitable threshold for an abnormal transferrin saturation depends on the population, the methodology and the acceptable efficacy. The figures of 55% (male) and 50% (female)

represent a compromise but should provide useful diagnostic information until an appropriate threshold has been determined for the UK.

Assays of unsaturated iron-binding capacity (UIBC) are more easily automated but have not been shown to be as reliable as the assay of total iron-binding capacity and have not been standardised[68].

Serum ferritin concentrations

These reflect the level of storage iron in the body but do not exceed the upper limit of normality until liver iron concentrations are elevated; they then rise disproportionately with the degree of liver damage. Serum ferritin concentrations are not usually abnormal in the early stages of iron accumulation. False positives include acute and chronic inflammatory conditions and hepatic steatosis. In normal subjects, concentrations of > 300 μg/l for men and post-menopausal women and > 200 μg/l for pre-menopausal women indicate elevated iron stores[69].

Genotypic testing

This has the advantage of providing a result which is the same at any stage of iron accumulation and is not influenced by dietary intake or tissue damage. However, it is not certain that the majority of people homozygous for the C282Y mutation will eventually develop the clinical condition. People heterozygous for both the C282Y and H63D mutations may also accumulate iron, but the risk of clinical haemochromatosis is much less. Some recommend that testing should be confined to the C282Y mutation, but heterozygotes should also be tested for the H63D mutation. In the UK, some 5% of patients lack these mutations of the *HFE* gene, and at the moment only biochemical assays can detect iron overload in this group.

Role of liver biopsy

Before the identification of the *HFE* gene, liver biopsy was favoured, particularly by gastroenterologists, to demonstrate increased hepatic iron and its location and to measure the liver iron concentration. However, this is no longer necessary when the patient is homozygous for the *HFE* C282Y mutation. There is, however, a place for liver biopsy to determine the degree of hepatic fibrosis and specifically to show whether or not cirrhosis has developed. This information determines the plan of management during follow-up (see below).

The approach now being suggested is as follows: a liver biopsy should be carried out for any patient with a raised transferrin saturation, a serum ferritin concentration of > 1000 μg/l and/or evidence of liver damage (hepatomegaly or raised AST activity)[70]. For patients with a raised transferrin saturation, a ferritin concentration of < 1000 μg/l, no hepatomegaly

Recommendation 3: Confirming the diagnosis of HC in a patient with evidence of iron overload but no evidence of liver damage

No hepatomegaly, AST activity normal, serum ferritin concentration is > 300 µg/l (200 µg/l pre-menopausal women) and < 1000 µg/l:

1 In most cases genotyping will confirm the diagnosis of genetic haemochromatosis. 90% of patients have the genotype C282Y +/+, 5% have C282Y +/-, H63D +/-. However, in the UK about 5% of patients do not have these genotypes (see Recommendation 6).

2 Commence quantitative phlebotomy. Removal of more than 4 g iron (about 20 phlebotomies of 450 ml) demonstrates that body iron stores are compatible with genetic haemochromatosis.

Evidence IIb–IV; Grade B, C

and normal AST activity, no biopsy is necessary, because the risk of hepatic fibrosis or cirrhosis being present is low[70]. Venesection therapy may be used not only to remove iron but to calculate the degree of iron overload (see below). The question as to whether C282Y homozygotes with a raised transferrin saturation and a normal serum ferritin should be treated has not been resolved, but treatment is not usually given at this stage of iron accumulation. No treatment is necessary for those with normal values for transferrin saturation and serum ferritin concentration[70].

Recommendation 4: Confirming the diagnosis of HC in a patient with evidence of liver damage

Serum ferritin concentration is > 300 µg/l (200 µg/l in pre-menopausal women), AST activity is above normal or there is hepatomegaly:

1 Genotyping (see Recommendation 3)

2 Carry out liver biopsy to show hepatic architecture (normal/fibrosis/cirrhosis). The presence of cirrhosis has significant prognostic implications and will affect management (see Recommendation 8).

3 Carry out histological grading of iron concentration (Perl's stain). Increased stainable iron in hepatic parenchymal cells confirms iron loading.

Evidence IIb–IV; Grade B, C

For those subjects homozygous for the C282Y mutation but without iron accumulation, it is reasonable to monitor iron status at yearly intervals in order to detect the onset of tissue iron accumulation, as indicated by raised transferrin saturation. It will be advisable to provide the subject and his/her GP with a card giving the necessary information to ensure that regular monitoring takes place.

Recommendation 5: Confirming the diagnosis of HC in a patient with only a raised transferrin saturation

If the fasting transferrin saturation is raised (see Recommendation 2) but serum ferritin and AST levels are normal:

1 In most cases genotyping will confirm genetic haemochromatosis (see Recommendation 3).

2 If the genotype is that of homozygous haemochromatosis, transferrin saturation and serum ferritin should be monitored at yearly intervals. If serum ferritin becomes elevated, phlebotomy should be started (see Recommendation 6).

3 If the genotype is normal the serum ferritin concentration should be monitored at yearly intervals.

Measurement of hepatic iron concentration

Liver iron concentration may be assessed both histochemically and chemically. Characteristically, iron is found in parenchymal cells in the early stages of iron accumulation. A value greater than 80 μmol/g dry weight is diagnostic for iron overload in haemochromatosis. This may be expressed as greater than 1000 μg/g wet weight or 6 μg/mg protein. The **Hepatic Iron Index**[71] (HII) may be calculated from the hepatic iron concentration in μmol/g dry weight divided by age. A value of 1.9 or more differentiates patients with increased hepatic iron due to HC from heterozygotes and patients with iron excess on staining due to alcoholic liver disease. With the availability of genetic testing, such a differentiation can usually be made by mutation analysis, and in these cases calculation of HII adds nothing further to the diagnosis.

Recommendation 6: Investigation of patients with evidence of iron accumulation but negative for *HFE* mutations

1 Search for other causes of elevated transferrin saturation or serum ferritin concentration, e.g. fatty liver, alcoholic liver disease, haematological disease.

2 Consider referral to specialist centre.

3 Measure liver iron concentration and calculate the hepatic iron index: μmol/g dry weight of liver divided by age (years) at time of biopsy (> 1.9 in homozygous genetic haemochromatosis with iron overload). In the absence of the common *HFE* genotypes, this allows a diagnosis of parenchymal iron overload compatible with genetic haemochromatosis.

Quantitative phlebotomy

The degree of iron overload can be evaluated retrospectively by quantitative phlebotomy in which the amount of iron removed by weekly venesection is calculated and an allowance of 3 mg Fe/day is made for iron absorption during treatment[72]. Removal of more than 4 g of iron used to be one of the criteria to define genetic haemochromatosis, but with genotyping now available it has lost much of its diagnostic usefulness.

Recommendation 5: Confirming the diagnosis of HC in a patient with only a raised transferrin saturation

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1 Search for other causes of elevated transferrin saturation or serum ferritin concentration, e.g. fatty liver, alcoholic liver disease, haematological disease.

2 Consider referral to specialist centre.

3 Measure liver iron concentration and calculate the hepatic iron index: μmol/g dry weight of liver divided by age (years) at time of biopsy (> 1.9 in homozygous genetic haemochromatosis with iron overload). In the absence of the common *HFE* genotypes, this allows a diagnosis of parenchymal iron overload compatible with genetic haemochromatosis.

Quantitative phlebotomy

The degree of iron overload can be evaluated retrospectively by quantitative phlebotomy in which the amount of iron removed by weekly venesection is calculated and an allowance of 3 mg Fe/day is made for iron absorption during treatment[72]. Removal of more than 4 g of iron used to be one of the criteria to define genetic haemochromatosis, but with genotyping now available it has lost much of its diagnostic usefulness.

The role of imaging techniques

When there is sufficient iron overload, the attenuation value of the liver on computed tomography increases. However, the sensitivity is insufficient using standard settings to detect lower levels of iron accumulation, and therefore this technique is not useful for screening or follow-up. Magnetic resonance imaging[73] is used to assess liver iron concentration in some hospitals, but again a special interest appears to be necessary to use this approach clinically for evaluation of iron overload or follow-up. Magnetic susceptibility[74] is a very powerful technique allowing quantitation of hepatic iron levels from low to very high, but there are only two instruments in the world – neither of them in the UK.

The introduction of genetic testing

There are four ways in which genetic testing may be employed to detect homozygosity for *HFE* mutations and thus to prevent morbidity and premature mortality:

(a) confirmation of the diagnosis in cases of suspected iron overload

(b) investigation of the families of patients with haemochromatosis

(c) supporting biochemical testing for iron overload in population screening

(d) as the primary test for HC in population screening.

For (a) the genetic test will probably reduce costs. For about £20 the diagnostic process will be accelerated so that effective treatment can be started earlier.

In case (b) replacement of HLA typing by testing for *HFE* mutations will yield considerable savings in most cases (Class I HLA testing is about £100 per sample compared with about £20 for *HFE* testing). Approach (c) may enhance the specificity of phenotypic screening for HC without making a significant increase in the overall cost of testing (see Figure 2). (d) In the context of the published cost–benefit analyses[68, 75–78] the replacement of transferrin saturation or UIBC measurement by a genetic test is likely to increase testing costs significantly with current technology, but this may not always be the case. However, at the present time, genetic testing cannot be justified at the first level of screening. It would not be possible to provide those genetically at risk with a reliable estimate of the likelihood of developing clinical features of haemochromatosis. If subjects heterozygous for both the C282Y and H63D were included, about 3% of the population would need to be offered regular testing to detect iron overload as well as counselling and family studies.

5 Treatment of HC

Initial therapy

For those subjects who have already accumulated iron the usual treatment is phlebotomy. This should be carried out at weekly intervals with a proper record being kept of the volume (or weight) of blood removed, so that the amount of iron removed during the course of treatment can be calculated. It is necessary to allow for the daily absorption of iron (approximately 3 mg/day) during venesection therapy. A diagnosis of iron overload may be made when more than 4 g Fe is removed.

Recommendation 7: Treatment – for all patients with HC

Venesection once weekly (450–500 ml) until the serum ferritin concentration is < 20 μg/l and transferrin saturation is < 16%. Monitor Hb levels weekly and reduce rate of venesection if anaemia develops. Monitor serum ferritin monthly. Measure transferrin saturation as the ferritin concentration drops below 50 μg/l.

Calculate the amount of iron removed, by weighing the blood bag before and after venesection (density of blood is 1.05 g/ml) and assuming that 450 ml blood (Hb concentration = 13.5 g/dl) contains 200 mg Fe. Allow for iron absorption at a rate of 3 mg/day (20 mg/week). With these assumptions 25 weekly venesections will remove 4.5 g Fe.

Evidence IIb–IV; Grade B, C

Complications of iron overload – response to therapy and further management

Appendix 7 summarises the response of the various complications of haemochromatosis to phlebotomy. Management generally follows that in patients without iron overload.

If a diagnosis is made at a late stage it may be necessary to use chelation therapy to reverse cardiac damage. Physicians dealing with the treatment of iron overload due to blood transfusion in patients with homozygous beta-thalassaemia have much expertise in dealing with heart failure[79, 80]. Such problems are recognised more frequently in juvenile hemachromatosis than in HC but do require effective treatment.

Recommendation 8: Management of patients with liver cirrhosis

As such patients have a high risk of developing primary liver cancer, α-fetoprotein levels should be determined every 6 months and hepatic ultrasonography carried out every 6 months. (Clinical value of such testing not formally established.)

Evidence IIb–IV; Grade D

Maintenance of normal iron levels

Once excess iron has been removed and treatment by phlebotomy has ceased, iron will begin to re-accumulate. Usually patients return to the outpatient clinic every 3 months, and further phlebotomy is carried out when necessary. The transferrin saturation

should not be allowed to exceed 50%, and the serum ferritin concentration should be maintained at less than 50 μg/l. In some countries such people are encouraged to give blood regularly in order to maintain body iron levels. In Britain the view is that giving blood for any reason other than to benefit society must not be encouraged. However, it would seem reasonable to suggest that patients with haemochromatosis who have been properly treated should be allowed to consider giving blood three or four times a year if the blood otherwise meets the criteria for donation. Clearly this will be beneficial for the donor, but will also benefit society and provide extra donations[81].

Recommendation 9: Maintaining normal iron levels

The transferrin saturation should be kept below 50% and the serum ferritin concentration below 50 μg/l. This may require up to 6 venesections per year.

Evidence IIb–IV; Grade D

Consequences for the family

Once a subject has been identified as having HC, it is necessary to explain that other family members are also at risk. Counselling and testing should be offered to siblings and parents of the proband. In addition the proband's partner may wish to be tested, as there is a 1 in 10 chance that the partner will carry the C282Y mutation and a 1 in 5 chance that the H63D mutation will be present and children may also be at risk. Written consent should be obtained after explaining the possible consequences of genetic testing for life assurance and medical insurance proposals. Some, but not all, insurance companies take the view that HC, properly diagnosed and managed, does not justify refusal of cover or the levying of an increase in premium. Family members should be tested by taking a fasting blood sample for measurement of transferrin saturation, serum ferritin concentration and genotyping.

Recommendation 10: Investigating and treating family members

Siblings, parents, partners and children of a patient should be offered testing. This includes *HFE* genotyping and measurement of transferrin saturation and serum ferritin concentration (see Recommendation 2). Further investigation, treatment or monitoring will follow guidelines for patients.

Evidence IIb–IV; Grade B, C

6 Summary

Figure 2 summarises the steps required to diagnose, treat and prevent re-accumulation of iron in patients with HC. The thresholds for transferrin saturation and serum ferritin are given in Recommendations 2 to 4.

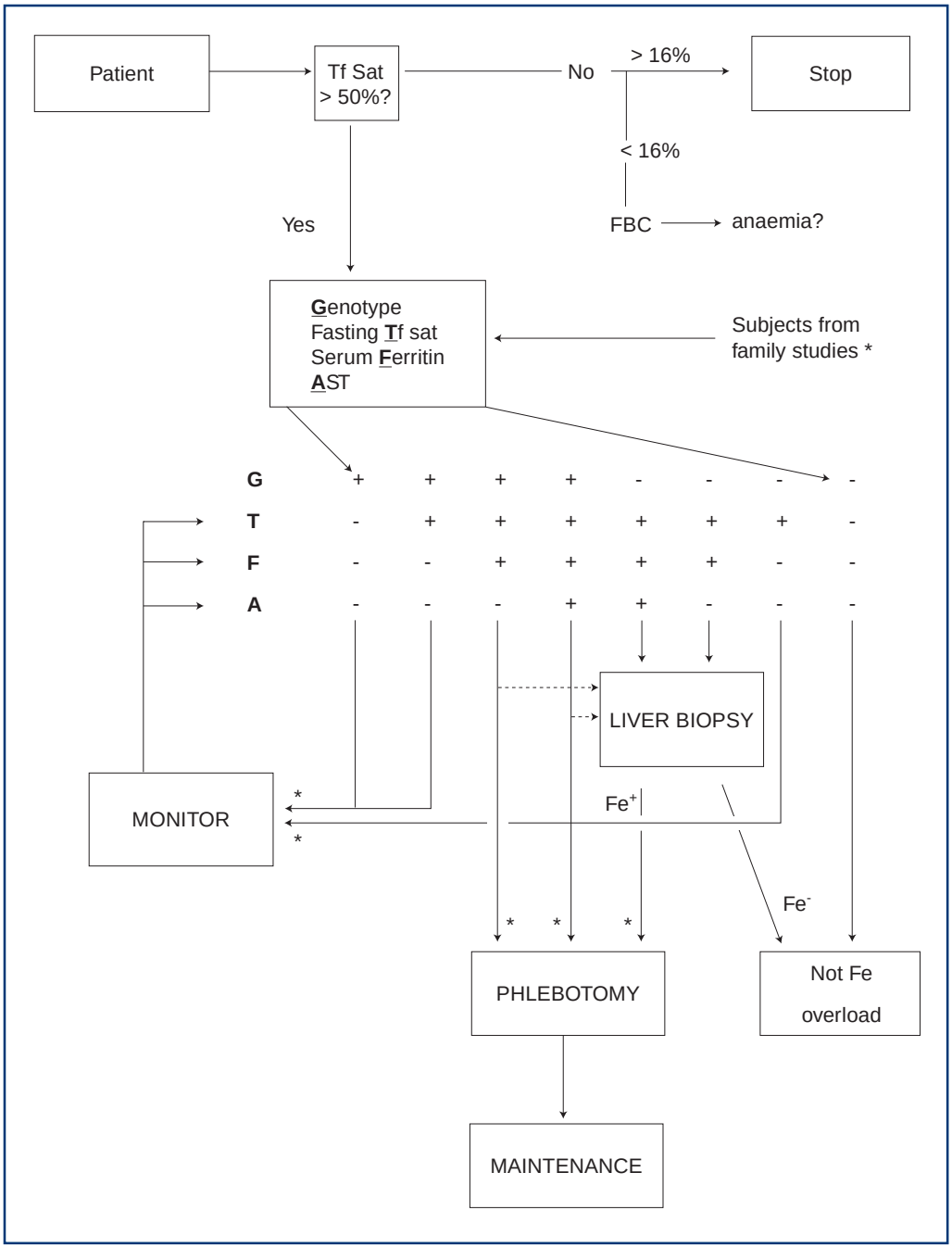

Figure 2 Diagnosis and treatment of haemochromatosis. An asterisk indicates that counselling and discussion of implications of the diagnosis for the family should be offered. The dotted lines indicate that liver biopsy is desirable to determine whether or not there is fibrosis or cirrhosis.

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Appendix 1: The *HFE* gene – discovery and function

Discovery: Feder *et al.*[18] in 1996 described an HLA class-I-like gene in which there were mutations in patients satisfying the criteria for diagnosis of HC. They continued the strategy developed by Raha-Chowdhury *et al.*[82] – physical mapping of the region telomeric to HLA-A, identification of multiple microsatellite markers, high-resolution linkage disequilibrium analysis and haplotype analysis.

Linkage disequilibrium analysis indicated that the likely gene location was close to a microsatellite marker D6S2239, lying about 5 Mb from HLA-A. Feder *et al.* also examined haplotypes from patients using a large number of microsatellite markers. They identified a characteristic haplotype on chromosome 6 of almost all HC patients, giving a region of about 300 kb as the likely location of the gene responsible for iron accumulation. Sequencing identified 3 expressed genes, and one of these was of particular interest. This was a gene which they initially called HLA-H and which was homologous to the classical HLA class I genes (Figure 3). The accepted name for the haemochromatosis gene[83] is now '*HFE*'. Sequencing of this gene revealed that in 90% of patients with HC there was a mutation at amino acid 282 which resulted in the replacement of a cysteine by a tyrosine residue. There was a second mutation at position 63 in which aspartic acid replaced histidine. This mutation was common in the general population, and its significance was not clear. For a description of the various nomenclatures in use see the legend to Table 2. The mutation at position 282 was clearly associated with haemochromatosis, and moreover would cause disruption of the heavy chain structure and failure to bind β_2-microglobulin. Interestingly it had been shown earlier that in β_2-microglobulin knockout mice there was enhanced iron absorption and the development of iron overload. This implicated HLA genes in the control of iron absorption[84, 85] because HLA class I proteins consist of the HLA heavy chain and β_2-microglobulin.

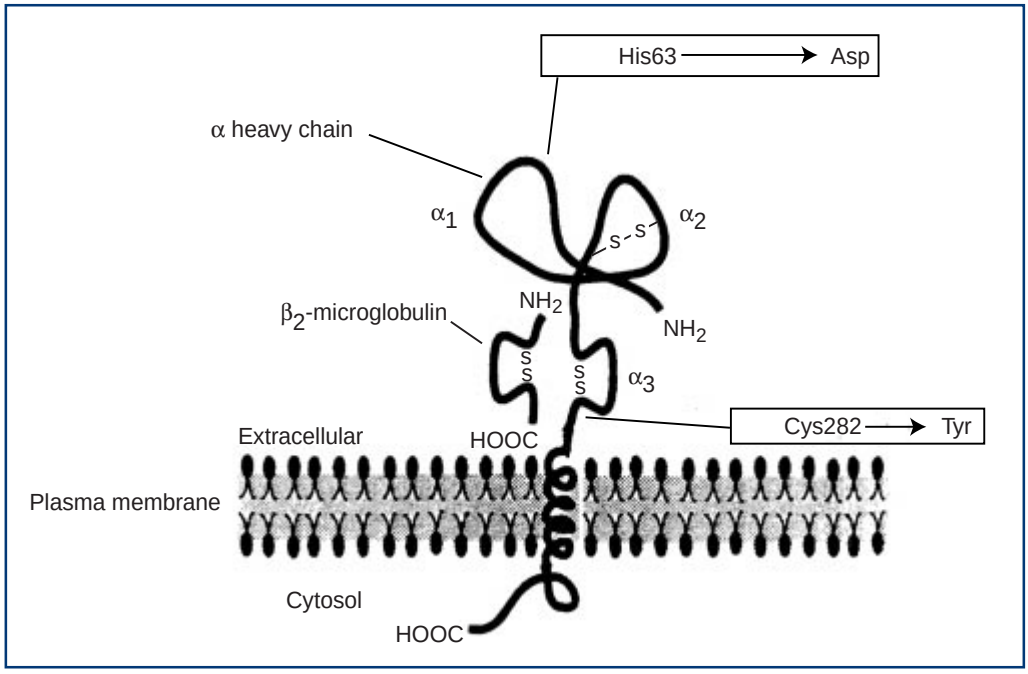

Figure 3 Model of the HFE protein based upon its homology with MHC class I molecules. It is a single polypeptide chain with three extracellular domains analogous to the α_1, α_2 and α_3 domains of other MHC class I molecules. In contrast to other members of the MHC class I family, the α_1 and α_2 domains in the HFE protein are non-polymorphic. β_2-microglobulin is a separate protein which interacts with the HFE protein in a non-covalent manner in the α_3 homologous region. The approximate locations of the Cys282Tyr and His63Asp mutations are indicated. From Feder *et al.*[18] with permission.

Function: The *HFE* gene appears to be widely expressed, as demonstrated by northern blotting[18]. The protein has been detected with polyclonal antibodies to a C-terminal peptide sequence and has been shown to be associated with the plasma membrane of epithelial cells from the upper and lower gastrointestinal tract. However, in epithelial cells from the crypts of the duodenum and jejunum there is a curious, peri-nuclear distribution of the protein. The reason for this distribution is not understood, although Parkkila *et al.*[86] suggest that this indicates a relationship with iron absorption. Both the wild-type protein and the protein with the H63D mutation are expressed on the cell surface and bind β_2-microglobulin, but the protein with the C282Y mutation neither reaches the cell surface nor binds β_2-microglobulin[87]. In studies of wild-type and mutant HFE proteins which were over-expressed in cultured cells, both the wild-type and H63D proteins formed stable complexes with the transferrin receptor but the C282Y protein showed little binding to the transferrin receptor. The wild-type HFE protein decreased the affinity of the transferrin receptor for transferrin but the H63D protein had little effect[88]. Lebron *et al.*[89] have crystallised a soluble form of the HFE protein and confirmed its tight binding to the soluble transferrin receptor at pH 7.5 (but not at pH 6). *HFE* knockout mice develop a very similar form of iron overload to human haemochromatosis[90].

One current hypothesis for the normal role of the HFE protein is that it participates with TfR in the regulation of entry of iron into the duodenal crypt cell, according to the level of body iron stores. The iron content of the crypt cell then determines the activity of the iron transporter (Divalent Metal Transporter-1, DMT-1) in the cells of the villus. In HC, the contribution of HFE protein is lost as a result of the C282Y mutation. Iron does not enter the crypt cell, which is then iron deplete. DMT-1 is upregulated and iron absorbed from the villus, despite total body iron stores being increased[119]. The increased expression of DMT-1 in duodenal biopsies of patients with HC is consistent with this hypothesis[120].

There is thus a clear indication at the molecular level of a possible mode of action of HFE in regulating iron transport. Further studies on the native proteins in cells will reveal how these interactions cause haemochromatosis. Whatever function the normal HFE protein has in the regulation of iron absorption is removed by the presence of the C282Y mutation. Although the H63D mutation, in isolation, does not appear to increase iron absorption, the studies of the over-expressed protein and the fact that compound heterozygotes may accumulate iron suggests that this mutation causes some disturbance of iron metabolism but less than that associated with the C282Y mutation.

Appendix 2: *HFE* gene mutations in various countries

The most comprehensive study is that by Merryweather-Clarke *et al.*[41]. Figure 1 is a map in which the frequencies of the C282Y mutation throughout Europe are shown. The highest frequencies of both haemochromatosis, defined in terms of iron overload[12], and C282Y mutations are found in northern European populations but with a low prevalence in parts of Finland. The H63D mutation is found throughout the world, although frequencies are variable and are very low in Australasia[41]. Assuming a gene frequency for C282Y of 7% implies that 1 in 204 subjects will be homozygous for the C282Y mutation and about 1 in 8 will be carriers. In several parts of the UK the gene frequency for C282Y is over 8% (Figure 1).

Appendix 3: Interaction of *HFE* mutations with other iron-loading conditions

The most studied condition is porphyria cutanea tarda (PCT). Some studies showed a high frequency of *HLA-A3* (suggesting the presence of a haemochromatosis mutation) in patients with PCT while others did not demonstrate a significantly increased frequency[91]. Although patients with PCT demonstrate some iron accumulation and the biochemical and clinical symptoms disappear on venesection therapy (to cause iron depletion), relatively few of these patients have an iron overload which would be considered diagnostic for HC[91]. Roberts *et al.*[92] showed that patients with PCT had a high frequency not only of *HLA-A3* but of D6S265-1, D6S105-8 and D6S1260-4. These are all alleles of markers associated with the ancestral haplotype for haemochromatosis. Furthermore several patients were homozygous for these alleles and appeared to have two copies of the ancestral haplotype. With the cloning of the *HFE* gene it was possible to test directly the hypothesis that this group of patients contained carriers. In the same group of 41 patients 45% carried at least one copy of the C282Y mutation of the *HFE* gene and over 20% were homozygous for this mutation. Surprisingly, patients homozygous for the C282Y mutation did not have iron overload consistent with HC even though their average age of presentation with PCT was 67 years. This study was extended to a further 68 patients. The same frequency of the C282Y mutation was found, but those homozygous for the latter presented at an earlier mean age than other patients[91].

The relationship between *HFE* mutations and PCT is more subtle than just more rapid accumulation of iron due to the presence of an *HFE* (C282Y) mutation. In Italy the C282Y mutation is not common in the general population (frequency = 0.5%). Sampietro *et al.*[93] showed that patients with PCT did not have an increased frequency of this mutation. However, they found a significant increase in the frequency of the H63D mutation in patients with PCT compared with other patients with viral hepatitis and control subjects. They proposed that the H63D mutation causes a subtle disturbance of iron metabolism which allows a form of iron to accumulate which inactivates the enzyme porphobilinogen decarboxylase but does not cause general accumulation of iron in the body. They also suggest that some of their patients may have other iron-loading genes, as they have the ancestral haplotype for HC but lack the C282Y mutation. *HFE* mutations appear to cause susceptibility to the development of sporadic PCT, but it is not necessary for major iron overload to develop for this to happen.

Because *HFE* mutations are so common in some populations it seems likely that they will coexist with other conditions and in some cases exacerbate the clinical course. Of particular concern are those disorders associated with increased iron absorption due to an increased but ineffective production of red cells in the bone marrow. Such conditions include the beta-thalassaemias, congenital/inherited sideroblastic anaemias and congenital dyserythropoietic anaemias.

Beta-thalassaemia intermedia is an anaemia of moderate to mild severity caused usually by the homozygous or doubly heterozygous inheritance of beta-thalassaemia mutations, or more rarely by the heterozygous inheritance of a ‘dominant’ beta-thalassaemia. Iron absorption is increased, apparently exacerbated by splenectomy, and iron overload occurs in adulthood. Iron overload in these patients clearly does not require the coinheritance of HC genes, but the coinheritance of HC heterozygosity or homozygosity might enhance the rate at which iron overload would occur[94]. Detailed studies are awaited.

Inherited/congenital sideroblastic anaemias are rare anaemias of varying severity. The X-linked form, responsive to pyridoxine, is usually an anaemia of moderate severity and is often associated with iron overload. Yaouanq *et al.*[95] have described a family with pyridoxine-responsive sideroblastic anaemia in which one brother was a compound heterozygote for the haemochromatosis mutations. He had a significantly greater degree of iron overload than his older brother lacking both mutations. Another study of 18 X-linked sideroblastic anaemia hemizygotes found a small but significantly higher frequency of C282Y amongst these patients, indicating a role for coinheritance of *HFE* alleles in the expression of this disorder[96].

Appendix 4: Selective advantage or pathological condition?

For many years it has been thought that HC has spread because carriers of the gene were less likely to develop iron deficiency anaemia than those with the normal genotype[97]. Heterozygote family members may accumulate slightly more iron than family members lacking the gene[60, 98, 99] but rarely accumulate sufficient iron to cause tissue damage. Recently, Datz *et al.*[100] have demonstrated increased haemoglobin concentration and serum iron concentration in fasting samples from young women who are heterozygous for the C282Y mutation compared with those lacking this mutation. An alternative explanation for the persistence of such an extended, ancestral haplotype for haemochromatosis is that the haplotype itself conveys a selective advantage unrelated to iron metabolism[101]. Perhaps the situation will be analogous to that of cystic fibrosis where the ΔF508 mutation of the cystic fibrosis transmembrane regulator (CFTR) may provide decreased susceptibility to typhoid fever, as the CFTR is used by *Salmonella typhi* to enter gastrointestinal epithelial cells[102].

Appendix 5: Iron and morbidity (the frequency of the C282Y mutation in other conditions)

The clinical manifestations of iron overload include diabetes mellitus, liver disease, coronary heart disease and endocrine disorders. There has been an expectation that a higher proportion of patients in clinics dealing with these disorders may be homozygous for HC than in the general hospital population. Before the description of the *HFE* gene it was difficult to detect iron overload by measuring transferrin saturation and serum ferritin concentration in hospital patients, since a high proportion showed abnormalities of these indicators of iron status. Since the gene discovery it has been possible to re-evaluate this issue. Two preliminary studies of *HFE* mutation frequencies in diabetes mellitus have not shown any increase in frequency compared with control groups[103, 104]. These results confirm findings based on screening with serum ferritin and transferrin levels in patients in North-East England[105]. Studies of patients with premature atherosclerotic vascular disease[106] and alcoholic liver disease[107] have not shown an increase in frequency of the C282Y mutation compared with the control populations. A review of such studies is by Cogswell and coworkers[108]. Recently two studies have shown a strong association between heterozygosity for C282Y and death from cardiovascular disease[109, 110]. This requires further study.

In recent years there has been much interest in the possibility that iron levels within or just above the upper limit of normal may be associated with diabetes mellitus, heart disease and cancer[111]. However, most studies have related to serum ferritin concentration rather than increased levels of tissue iron, and there is no consensus about such associations. If high iron concentrations do cause susceptibility to common conditions then up to 15% of the population may be at additional risk because of the presence of one copy of the C282Y mutation. Nelson *et al.*[112] examined data from 1950 heterozygotes for HC and found an increased risk for colorectal, gastric and nasal neoplasia, diabetes and haematological malignancy. No increased risk of heart disease, cancers of the lung, breast or cervix or death from cancer was demonstrated.

Appendix 6: Population screening

In the past the diagnosis of HC was often made at a very late stage when the patient was bronzed, had diabetes mellitus and abnormal liver function and a liver biopsy showed cirrhosis. More patients are now diagnosed earlier, sometimes as a result of measurement of serum ferritin or transferrin saturation for another purpose, such as a health check.

The present availability of a genetic test which identifies nearly 95% of patients with HC in the UK (Table 2) permits early identification of those at risk from iron overload, rather than late diagnosis in those who already have tissue damage. If the clinical penetrance is such that the majority of people who are C282Y homozygous eventually accumulate iron, the most logical approach will be to screen all adults by genetic testing and to follow those at risk by measuring transferrin saturation at 3-yearly intervals. However, the absence of the C282Y mutation from non-European populations (see earlier) means that decisions may have to to be made about selective testing.

There are various intermediate stages of screening between the two extremes of investigating manifest disease and population screening. One approach already piloted in the United States[62] is to measure the transferrin saturation in primary care patients. If the saturation is high a fasting sample is requested and both the transferrin saturation and serum ferritin concentration measured. This approach provides many false positive results and does not always identify subjects who have not yet accumulated iron. However, coupled with confirmatory genetic testing, most subjects with genetic haemochromatosis will be identified. Another possibility is to test specific groups of patients who may already have iron overload, by carrying out genetic or phenotypic testing in arthritis, cardiac, diabetic or liver clinics (see Appendix 5) although initial experience of such testing has not indicated that it is likely to be worthwhile.

Appendix 7: Effects of venesection therapy on symptoms

Questions and answers

1 *What symptoms and signs are irreversible?*

- Totally irreversible: cirrhosis, arthritis
- Improve in some patients: diabetes mellitus, hypogonadism, arthralgia
- Usually reversible: fatigue, transaminase elevation

Early cardiac changes on echocardiography usually improve. Several case reports show that severe cardiac failure and arrhythmias may ameliorate with therapy, but the reporting may be selective. Treatment by heart transplantation has also been reported.

2 *Are all early symptoms and signs reversible?*

No. Arthralgia and sexual dysfunction in men may not improve with venesection.

3 *At what stage do signs and symptoms become irreversible?*

There is no measurement of iron status that will define a point at which signs and symptoms become irreversible. Once sufficient tissue damage has occurred, the changes are not reversible. This stage is clear when cirrhosis, diabetes mellitus and destructive arthritis have occurred.

Effect of venesection therapy on:

Asymptomatic haemochromatosis. An increasing number of individuals with *HFE*-related haemochromatosis are being diagnosed before any symptoms or signs have developed. This is through the testing of transferrin saturation or ferritin at a health check, through screening (family or population) or because of a separate clinical problem. If venesection treatment is initiated, the majority will remain without symptoms or signs, but it is recognised that, despite treatment, fatigue, arthralgia and impotence may still develop, affecting about 14% of patients for each feature[5].

Early symptoms. The most frequent presenting symptoms of *HFE*-related haemochromatosis are fatigue, abdominal pain, arthralgia and impotence. They may therefore be regarded as early symptoms. (See also Table 3.) Early signs have not been well defined, but hepatomegaly and mildly abnormal liver function tests (transaminases) may be part of the early picture. Venesection therapy improves these to a differing degree (percentage improvement in brackets[5]): fatigue (55%), abdominal pain (68%), arthralgia (30%) and impotence (19%). Worsening despite venesection is reported for arthralgia (20%) and impotence (12%). Transaminases improve in the majority after venesection (73%), with few patients having worse results (2%).

Many case reports of the improvement of testosterone levels and sexual function after venesection exist, but where several patients have been studied, the majority do not show improvement. This may relate to age[113, 114].

Intermediate features. These are features which have a spectrum depending upon the stage of the disease. Thus cardiac changes range from echocardiographic changes in asymptomatic individuals to severe cardiac failure and life-threatening arrhythmias. Pancreatic endocrine dysfunction ranges from glucose intolerance to insulin-dependent diabetes. Niederau *et al.*[5] reported data on ‘electrocardiographic changes’, with improvement in 34% after venesection, no change in 61%, and deterioration in 5%. ECHO data show minor changes in a proportion of patients with haemochromatosis, and improvement with venesection in the majority[115, 116]. Several case reports describe recovery of patients with severe congestive cardiomyopathy after iron removal (see ref. 117 for a list of references) although such treatment is not always successful.

Cardiac problems occur in both *HFE*-related haemochromatosis and juvenile (non-*HFE*-related) haemochromatosis, but whether the response to treatment differs is not known. Hramiak *et al.*[118] found evidence of impaired insulin secretion and glucose intolerance early in the course of haemochromatosis before cirrhosis had occurred. The acute insulin response to glucose improved after phlebotomy by 35% and glucose tolerance returned to normal. However, in patients with cirrhosis, impaired glucose tolerance due to insulin resistance did not improve after phlebotomy.

Late features. These are skin pigmentation, cirrhosis and diabetes mellitus. Venesection therapy improves pigmentation in 68% (unchanged 32%; worsening 0%). Cirrhosis once present is regarded as irreversible. The presence of cirrhosis also predisposes to the development of hepatocellular carcinoma. All patients with cirrhosis should be monitored by measuring α-fetoprotein levels in serum and carrying out hepatic ultrasonography every 6 months, although the clinical value of such testing is not formally established. Patients with diabetes mellitus who are insulin dependent remain insulin dependent[5], although the daily dose of insulin could be reduced in 41% of patients. Non-insulin-dependent diabetes mellitus or impaired glucose tolerance may be improved in around 40% of patients by venesection therapy.

Conclusion

Features of *HFE*-related haemochromatosis vary in their response to iron reduction by venesection. This will depend upon the degree of iron deposition and tissue damage in different organs. Thus cirrhosis cannot be reversed, while degrees of fibrosis prior to the development of cirrhosis may resolve. There is no clear relationship between the degree of iron overload and the tissue/organ damage produced. The best data come from studies of hepatic damage, where Bassett *et al.*[71] reported hepatic fibrosis or cirrhosis only with hepatic iron concentrations above 400 μmol/g dry weight. Guyader *et al.*[70] found that severe fibrosis or cirrhosis were unlikely in patients with a serum ferritin less than 1000 μg/l. Above this concentration, although theoretically the degree of damage could be directly related to the degree of iron overload, other influences may play a role: for example alcohol intake.

Abbreviations

ALL	acute lymphoblastic leukaemia
AST	aspartate aminotransferase
AML	acute myelogenous leukaemia
BM	bone marrow
CAP	cyclophosphamide + doxorubicin + prednisolone
2-CDA	2-chlorodeoxyadenosine (cladribine)
CHOP	cyclophosphamide + doxorubicin + vincristine + prednisolone
CLL	chronic lymphocytic leukaemia
CML	chronic myelogenous leukaemia
COP	see CVP
CR	complete remission
CT	computerised tomography
CVP	cyclophosphamide + vincristine + prednisolone
2´-DCF	2´-deoxycoformycin (pentostatin)
DFI	disease-free interval
EFS	event-free survival
G-CSF	granulocyte colony stimulating factor
GM-CSF	granulocyte–macrophage colony stimulating factor
Hb	haemoglobin
HC	haemochromatosis
HCL	hairy cell leukaemia
IFN	interferon
LPL	lymphoplasmacytic lymphoma
McAb	monoclonal antibody
MGUS	monoclonal gammopathy of uncertain significance
MRD	median remission duration / minimal residual disease
MS	median survival
NHL	non-Hodgkin’s lymphoma
PB	peripheral blood
PLL	prolymphocytic leukaemia
PR	partial remission
SLVL	splenic lymphoma with villous lymphocytes
SMZL	splenic marginal zone lymphoma
TRAP	tartrate resistant acid phosphatase
UIBC	unsaturated iron-binding capacity
WBC	white blood cell (count)
WM	Waldenström’s macroglobulinaemia

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