Diagnosis and therapy of genetic haemochromatosis (review and 2017 update)

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Summary

Genetic haemochromatosis (GH) is one of the most frequent genetic disorders found in Northern Europeans. GH 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 disorder is inherited in autosomal recessive fashion. The gene involved lies close to the HLA-A region on chromosome 6. This updated guideline follows on from the previously published guideline commissioned by the British Committee for Standards in Haematology in February 2000 (Dooley & Worwood, 2000). This review and updated guidance coincides with the development of a separate guideline on the investigation and management of a raised serum ferritin, also commissioned by the BSH guidelines committee.

Guideline writing methodology

The original literature review (Dooley & Worwood, 2000) was based on a total 40 years of experience in GH 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. The guideline has now been updated to incorporate new evidence up to and including 2017, to coincide with the development of a

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Guideline for the diagnosis and management of a raised serum ferritin (Cullis et al, 2018).

Evidence and strength of recommendations

The BSH Guidelines Committee uses the GRADE nomenclature for evaluating levels of evidence and assessing the strength of recommendations in all Guidance. Details are available at http://www.gradeworkinggroup.org/index.htm

Randomised controlled trials

In 1976 the results of a study of venesection therapy for the removal of excess iron from patients with GH were published (Bomford & Williams, 1976). The authors compared the survival of treated patients with that of historical controls (patients presenting with GH 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 (Adams et al, 1991; Niederau et al, 1996). 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'.

For similar reasons, there have been no randomised controlled trials of the effect of treatment in genetically predisposed family members of probands with GH. Phlebotomy is undertaken if there is biochemical 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 these reasons, there are no recommendations graded A.

Summary of recommendations

1. **Unselected population screening for *HFE* gene mutation is not recommended. (1B)**

2. **Genetic haemochromatosis (GH) patients who present with serum ferritin (SF) >1000 μg/l and any with raised transaminases should be referred to a hepatologist for fibrosis assessment and exclusion of cirrhosis. (1B)**

3. **Liver biopsy is no longer required for diagnosis of *HFE* GH but may be required to assess the severity of fibrosis in GH patients with SF >1000 μg/l and/or elevated transaminases. Transient elastography could be used to select which patients from this group require liver biopsy. (2C)**

4. **Patients of north European ancestry with clinical features suggestive of GH should have the following laboratory investigations; full blood count (FBC), liver function tests (LFTs), SF and transferrin saturation (Tsat). Molecular testing for *HFE* GH should follow if results fulfil the criteria of recommendation 5 (see below). (1B)**

5. **All adult patients of north European ancestry with unexplained raised SF and random Tsat (>300 μg/l and >50% males; >200 μg/l and >40% females) and normal FBC should have molecular testing for *HFE* GH. (1B)**

6. **Laboratory screening to include FBC, LFTs, SF, Tsat and HFE should be offered to family members after the diagnosis of *HFE* GH. Family screening should include parents (if available), siblings, partner and children (over the age of consent). Extended family screening is not recommended if an individual is identified as a C282Y/H63D compound heterozygote. (1B)**

7. **Investigation of all confirmed C282Y homozygotes should include FBC, LFTs, SF and Tsat. Thereafter further investigation may be required as follows:**

   a. **SF <1000 μg/l, normal LFTs, normal clinical examination; no further investigation required. Follow recommendation 8. (1C)**

   b. **SF >1000 μg/l and or abnormal LFTs. All such patients require referral to Hepatology for fibrosis assessment to exclude the presence of cirrhosis. A minimum would be elastography. For patients with confirmed cirrhosis monitor with α-fetoprotein (AFP) and hepatic ultrasound every 6 months (recommendation 11). (2C)**

8. **Non C282Y homozygotes with significant iron loading as confirmed by magnetic resonance imaging and or liver biopsy should be investigated for rare iron loading genotypes or digenic inheritance. (1C)**

9. **At diagnosis, all fit GH patients with biochemical iron loading should undergo weekly venesection until SF ∼ 20–30 μg/l and Tsat <50%. During this phase of venesection FBC should be monitored weekly and SF ± Tsat monitored monthly. Homozygotes with normal iron indices and compound heterozygotes with minimal elevation of iron indices may be suitable for blood donation and annual monitoring of SF and Tsat. (1B)**

10. **During maintenance, venesect as required, preferably at a blood donation centre to maintain normal FBC, SF <50 μg/l and Tsat <50%. (1C)**

11. **HFE GH with cirrhosis; Treat as per recommendations 9 and 10 but not suitable for blood donation. Monitor AFP and hepatic ultrasonography every 6 months. (2C)**

Iron balance

Body iron content is in the order of 4 g and is controlled by the complex regulation of dietary iron absorption from the upper gastrointestinal (GI) tract. There is no regulatory pathway for the excretion of excess iron and daily involuntary losses of ~1 mg occur via desquamation of cells in the GI tract. In females, additional iron loss occurs due to menstrual loss (15–40 mg iron per period) and pregnancy (net iron loss from placental transfer to fetus and blood loss at delivery is ~500 mg/pregnancy). As a result of these minor losses, the adult male/female will only require to absorb 1–2 mg/day of dietary iron to maintain normal iron balance.

Most iron (3 g) is present in haemoglobin with some 300 mg in myoglobin. There are many iron containing enzymes involved in respiration in all tissues (100 mg), and there is a small amount of iron (4 mg) bound to transferrin (Tf) at any one time in the plasma and extravascular circulation.

Transferrin is a glycoprotein produced in the liver and regulated by body iron stores. In iron deficiency, transferrin production increases and in iron overload decreases. Each Tf molecule has two iron binding domains. Over the course of 24 h however Tf will transport some 20–40 mg of iron from the body stores to the tissues. In health, Tf is approximately 30% saturated with iron with only some 10% in the diferric state although diferric Tf will appear more frequently as the transferrin saturation (Tsat) increases. All cells accept iron from Tf via interaction with their cell surface transferrin receptors (TfR) and will accept iron more readily from diferric rather than monoferric Tf (Young *et al*, 1984; Heubers *et al*, 1985). Macrophages of the reticuloendothelial system (RES) have an additional and far greater supply of iron from the ingestion and degradation of effete RBCs.

Ferritin is present in all cells and provides safe iron storage in a readily accessible soluble form. In the event of intracellular iron excess, ferritin molecules may aggregate to form insoluble haemosiderin. The largest body stores of iron exist within the macrophages of the RES, which in health may store up to 1 g of iron as either ferritin or haemosiderin.

A tiny amount of ferritin (serum ferritin, SF) can be detected in the serum. The source of this SF is unknown but it contains little iron and plays no role in iron metabolism. In the vast majority of patients without either tissue damage or inflammation, the SF value is directly proportional to the

amount of RES macrophage storage iron (Worwood, 1982). It is reduced in states of iron deficiency and raised in states of iron overload. Control of iron metabolism is orchestrated by the hormone hepcidin, which, together with the iron transporter ferroportin, acts to regulate both the absorption of dietary iron and RES macrophage iron release (Nemeth et al, 2004).

Mechanism of iron toxicity

As serum iron levels rise, Tf levels fall, Tsat% rises and highly toxic non transferrin bound iron (NTBI) may appear in the plasma. Parenchymal cells will accept more Tf-iron as the Tsat% and diferric transferrin levels rise and have no defence against the entry of NTBI (Porter et al, 2014). Iron toxicity is thought to occur within the cell via pathways such as the Fenton reaction, free radical formation and lipid peroxidation.

Iron loading genotypes

HFE GH (Type 1 GH)

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. Sheldon (1935) reviewed more than 300 published cases and suggested that the disorder resulted from an inherited defect. A human leucocyte antigen (HLA)-class-I-like gene (HFE) was described by Feder et al (1996), in which there were mutations in most patients satisfying the diagnostic criteria for GH. Over 90% of patients with GH carry a mutation of the HFE gene at amino acid 282, which results in the replacement of cysteine by a tyrosine residue (C282Y). They also described a second variant at amino acid 63, in which aspartic acid replaced histidine (H63D). This second mutation is more common in the general population but is not usually associated with iron accumulation.

Genetic haemochromatosis is one of the most frequent genetic disorders found in populations of northern European descent. It seldom causes end organ damage before the age of 30 years, is inherited in an autosomal recessive fashion and is characterised by excessive iron absorption from the GI tract, excessive iron release from RES macrophages, excessive iron saturation of plasma transferrin and, consequently, parenchymal iron overload and toxicity.

The HFE gene product is a 343 residue type 1 transmembrane glycoprotein. This protein has a complex and as yet imperfectly understood interaction between transferrin receptor 2 (TfR2), haemojuvelin (HJV), bone morphogenic protein (BMP) receptor Alk3 and ligands that control hepcidin expression in hepatocytes (Wu et al, 2014). Hepcidin inhibits the efflux of iron from both enterocytes and RES macrophages by inducing the degradation of the iron exporter ferroportin. Hepcidin levels are suppressed in HFE GH, leading to reduced ferroportin degradation and increased iron efflux from enterocytes and macrophages (Fig 1).
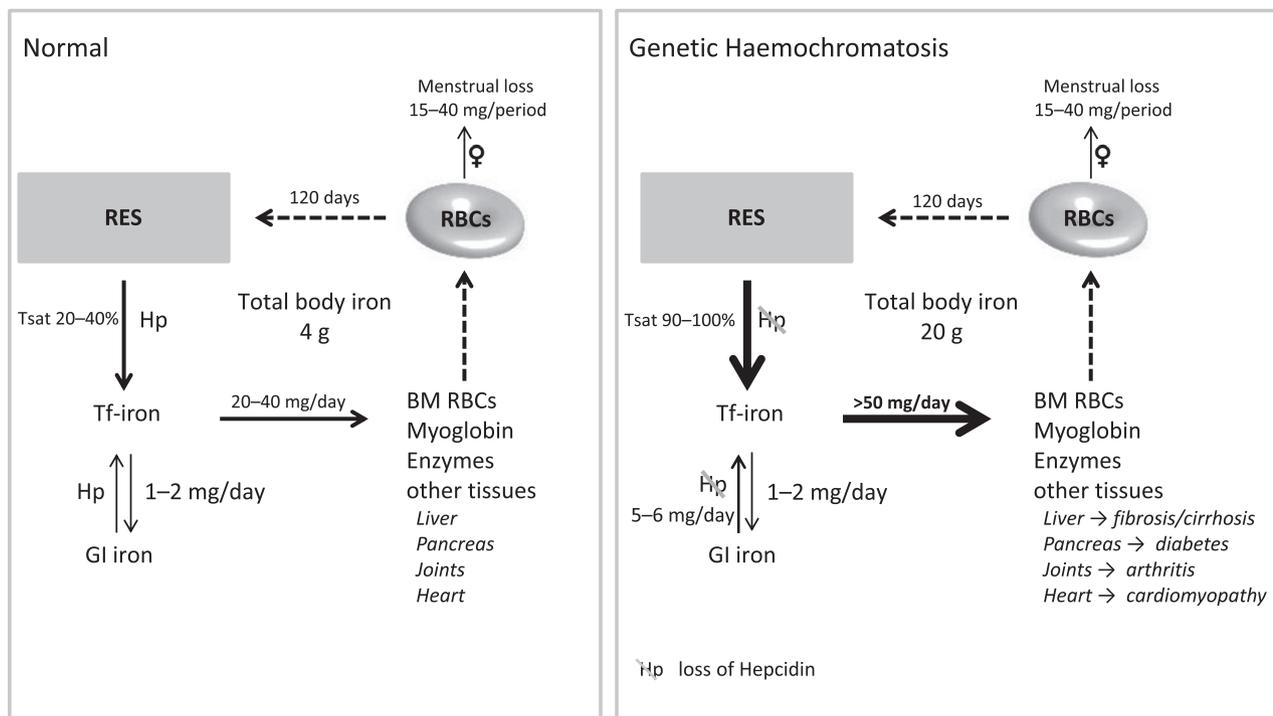

Fig 1. Iron homeostatis; normal and genetic haemochromatosis. BM, bone marrow; GI, gastrointestinal; Hp, hepcidin; RBCs, red blood cells; RES, reticuloendothelial system; Tf, transferrin; Tsat, transferrin saturation.

Variants C282Y and H63D of the *HFE* gene (chromosome 6p21.3) are the two polymorphisms most frequently associated with *HFE* GH. Studies of allele frequencies in northern European populations have reported 10–15% C282Y heterozygotes, 0·25–1·0% C282Y homozygotes, 20–30% H63D heterozygotes and about 2% C282Y/H63D compound heterozygotes (Worwood, 2005). Of the various possible genotype combinations, C282Y homozygotes are most at risk of iron overload and such homozygotes are responsible for some 90% of clinical cases. However, many young genetically predisposed homozygotes will have normal SF and/or Tsat. Over time, some 80% and 50% of these male and female homozygotes will show biochemical penetrance with raised Tsat and SF values. Clinical penetrance is considerably lower and the risk profile for iron loading for the possible genotypes can be summarised as follows: C282Y/C282Y >>> C282Y/H63D > C282Y/-, H63D/H63D, H63D/-.

Compound heterozygotes (C282Y/H63D) will seldom develop clinically significant iron overload; additional factors, such as alcohol excess or metabolic syndrome, may contribute more to any hyperferritinaemia than the molecular abnormality itself (Cullis *et al*, 2018). Indeed, some recommend that molecular testing for GH should be confined to the C282Y mutation alone (Porto *et al*, 2016). In the UK, only C282Y heterozygotes are tested for the H63D mutation and in France laboratories are not reimbursed for H63D genotyping.

Genetic haemochromatosis patients who lack C282Y mutations of the *HFE* gene may require sample referral to specialist centres for detection of very rare iron loading genotypes. Similarly, simple C282Y heterozygotes and C282Y/H63D compound heterozygotes with unexpected severe iron loading should also be investigated for possible digenic inheritance of other contributing iron loading genotypes (Merryweather-Clarke *et al*, 2003). In the UK this service is available at The Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DS (https://www.imm.ox.ac.uk) and through the clinical service provided by the UK Genetic Testing Network via Molecular Genetics at Cambridge University Hospitals (https://ukgtn.nhs.uk).

Rare genotypes and rare forms of GH (reviewed by Camaschella & Poggiali, 2009)

Type 2 Juvenile GH. This is a rare autosomal recessive disorder affecting either the *HFE2* (also termed *HJV*) gene, encoding hemojuvelin (type 2A), or, more rarely, the *HAMP* gene, encoding hepcidin (type 2B). Iron overload is severe. It develops in younger patients and commonly causes cardiac failure from iron overload and panhypopituitarism (Porto *et al*, 2016).

Type 3 GH, Transferrin receptor 2 deficiency. This is a rare autosomal recessive disorder caused by mutations in the *TFR2* gene, encoding transferrin receptor 2. This type has been found in European and Japanese families, leads to high Tsat and a clinical phenotype similar, but intermediate in severity, to Type 1 and Type 2 GH (Olynyk *et al*, 2008).

Type 4 GH, Ferroportin disease. Type 4a is a rare autosomal dominant disorder with a broad geographical distribution including Asia. Mutations in the gene encoding ferroportin (*SLC40A1*) lead to 'loss of ferroportin function', reduced RES macrophage iron release and consequent RES iron overload. Serum ferritin is raised, with Tsat values either low or normal. Significant parenchymal iron overload and organ damage does not occur but iron deficiency anaemia can develop with venesection.

Type 4b is also a rare disorder in which the mutations disrupt the ferroportin-hepcidin axis with 'gain of ferroportin function' causing a clinical phenotype similar to Type 1 GH (Olynyk *et al*, 2008).

Type 5 GH. Iron overload, inherited in an autosomal dominant manner from a mutation in *FTH1,* affecting the iron responsive element of *FTH1* mRNA has been described in a Japanese family (Kato *et al*, 2001).

Early diagnosis of GH and life expectancy

Early diagnosis is not easy, as the symptoms with which many patients present are relatively common and non-specific. Unfortunately, many patients in primary care in the UK with raised SF values are not screened for GH (Ogilvie *et al*, 2010). If patients are however diagnosed in the pre-cirrhotic, pre-diabetic stage and treated by venesection to remove the excess iron, then life expectancy is normal (Niederau *et al*, 1996). 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 and multiple studies, as described below, have been performed to examine possible approaches to improve the early diagnosis.

Biochemical penetrance, population studies and screening for GH

A study of 65 000 volunteers in Norway found that <10% of individuals with raised non-fasting Tsat had *HFE* GH (Asberg *et al*, 2001). As such, it is now recognised that the biological variability of serum iron levels and hence Tsat, limits the use of Tsat alone in screening for GH (Adams & Barton, 2007).

Serum ferritin values are raised in about 80% of male and 50% of female homozygotes (Adams & Barton, 2007). Although measurement of SF might then be considered an effective approach for screening for GH, the vast majority of patients with raised SF do not have GH. The additional finding of a raised Tsat, however, does increase the likelihood of GH

being the cause of a raised SF. The measurement of fasting morning Tsat values has long been advocated to assist the diagnosis of GH by avoiding the effects of diurnal variation and dietary intake on serum iron levels. However repeated venous sampling of patients is often impractical and although a raised Tsat in non-homozygotes may normalise when measured in the fasting state, they do not improve the predictive value of random Tsat for diagnosing *HFE* GH (Adams *et al*, 2005a).

Four major studies of volunteer screening for GH have been reported: the Haemochromatosis and Iron-overload Screening (HEIRS) study (Adams *et al*, 2005b), the Scripps/Kaiser haemochromatosis screening study (Waalen *et al*, 2008), the Melbourne Collaborative Cohort study-Health Iron (Allen *et al*, 2008) and a study of 10 500 blood donors from South Wales (Jackson *et al*, 2001). A non-volunteer study of patients with raised SF values, taken at the initiative of Primary Care physicians, without influence from the laboratories or secondary care has also recently reported (Ogilvie *et al*, 2015). The results of these studies are summarised in Table I. It is noted however that a limitation of these approaches to improve early diagnosis is that females predominate in both volunteer studies and General Practitioner requests for SF (Adams *et al*, 2005b), while clinically significant GH predominates in males.

The different mean/median SF and Tsat values for newly diagnosed GH reflect the different age groups included in the four studies. The lowest values were found in the Welsh blood donor study which included subjects as young as 17 years with a mean age of 36 years (Jackson *et al*, 2001). The highest values were found in the Melbourne study which only accepted volunteers aged 40–70 years (Allen *et al*, 2008). The apparent predominance of new female homozygotes in the HEIRS, Melbourne and West of Scotland publications (Adams *et al*, 2005b; Allen *et al*, 2008; Ogilvie *et al*, 2015) simply reflects the predominance of female volunteers and patients. From the West of Scotland study (Ogilvie *et al*, 2015) the following predictive algorithm was developed to assist early diagnosis for patients >30 years in primary care;

Males with SF >300 µg/l and Tsat >50% and females with SF >200 µg/l and Tsat >40% will have a 19% and 16% likelihood of being C282Y homozygotes.

Taken together, these studies show that GH is almost exclusively a disease of white races, that >70% of patients/volunteers at the time of diagnosis have SF values <1000 µg/l, that male homozygotes have higher SF and Tsat values than female homozygotes and are 3–5 times more likely to have SF values >1000 µg/l.

GH and serum ferritin values ≥1000 µg/l

Serum ferritin values >1000 µg/l in homozygotes at presentation are significant as studies have shown that these patients are at greatest risk of clinical disease, cirrhosis and hepatocellular carcinoma (Powell *et al*, 2006; Allen *et al*, 2008). However, most individuals with SF >1000 µg/l do not have GH

(see Table I). The HEIRS study of almost 100 000 multi-ethnic volunteers found 364 patients (of whom only 29 were C282Yhomozygotes) with SF values >1000 µg/l. Such high values were 2–3 times more prevalent in Blacks and Asians than in whites and were not related to *HFE* genotype.

The prevalence of SF values >1000 µg/l appears to be more common in primary care patients (Ogilvie *et al*, 2010) than in volunteer studies but even in an area of high prevalence only 29 of 180 such patients were found to be C282Y homozygotes (Ogilvie *et al*, 2015).

Non-progressive GH

These and other studies have shown that SF values in adults with GH do not inevitably show a progressive rise and may remain relatively stable over many years (Olynyk *et al*, 2004; Waalen *et al*, 2008). Powell *et al* (2006) observed a subgroup of untreated homozygotes with normal SF values for up to 24 years. Presumably, a steady (but as yet unexplained) state develops in homozygotes when a certain level of iron is reached but that this level will differ between patients.

Clinical penetrance of C282Y homozygous GH: effect of venesection on hepatic fibrosis

Clinical penetrance is very much less common than biochemical penetrance and for this reason population screening for *HFE* genotype is not recommended.

Recommendation 1

Unselected population screening for *HFE* gene mutation is not recommended. (1B)

Different studies report different estimates of clinical penetrance that range from 0% (Jackson *et al*, 2001) and <1% (Beutler *et al*, 2002; Waalen *et al*, 2002) to 28–30% for male homozygotes and 1–11·5% for female homozygotes (Powell *et al*, 2005, 2006; Allen *et al*, 2008). These differences relate to the different study populations and their varying degrees of iron loading.

Hepatic pathology

Liver biopsy performed on 350 asymptomatic homozygotes (SF values usually >500 µg/l) found stage 2–4 hepatic fibrosis in 29·7% and 13·3% of male and female patients respectively. The presence of hepatic fibrosis and cirrhosis correlated significantly with hepatic iron concentration and SF in both men and women. No case of cirrhosis was found in patients with SF values <1000 µg/l (Powell *et al*, 2006).

Role of liver biopsy and imaging techniques

Liver biopsy is no longer required for GH diagnosis but remains important for both fibrosis assessment and exclusion

Table I. Biochemical expression C282Y homozygous genetic haemochromatosis in five major population studies.

Study	Age (years)	C282 homozygotes (n)	Mean SF (µg/l)		Mean Tsat (%)		C282Y homozygotes with SF >1000 µg/l at diagnosis		Total volunteers/ patients with SF >1000 µg/l
			M	F	M	F	n	%	
HEIRS* (Adams et al, 2005b) 43 453 White 26 847 Black 12 672 Asian 12 337 Hispanic	>25	299 (281 white) 72 previously diagnosed	698 (Previously diagnosed homozygotes excluded)	212	76%	61%	29	10	364 87 White 130 Black 72 Asian 36 Hispanic
Scripps/Kaiser* (Waalen et al, 2008) 29 699 White 1462 Black 1774 Asian 4049 Hispanic	>20	152 (140 white) 45 previously diagnosed	395	159	64%	46%	20 (15 males)	13	59 White (47 male) (Data on other racial groups N/A)
Melbourne* (Allen et al, 2008) Health-Iron (31 192 north European extraction)	40–70	203	1195 (data obtained from 120 homozygotes at least 12 previously diagnosed)	374	73%	53%	40 (33 males)	33 (45% male 8% female)	N/A
West of Scotland (Ogilvie et al, 2015) (1745 patients (non-volunteers) from primary care with SF > 200 µg/l and Tsat > 30%)	>30	132 Six previously diagnosed	725 (median values)	413	85%	70%	29 (21 males)	22 (36% male 11% female)	180 (110 male) (Data on racial groups N/A)
South Wales* (Jackson et al, 2001) (10 500 blood donors, 5005 men and 5374 women). They had given mean of 2·5 units blood in previous 3 years	Mean age 36	72 (none previously diagnosed)	154	65	64%	50%	0	0	0 0%

F, female; HEIRS, hemochromatosis and iron-overload screening; M, male; N/A, not available; SF, serum ferritin; Tsat, transferrin saturation.
*Volunteer studies.

of additional liver diseases, such as alcoholic and non-alcoholic fatty liver. The importance of fibrosis assessment is to select those who require surveillance for hepatocellular carcinoma, essentially those with cirrhosis. Significant fibrosis is excluded in C282Y homozygotes with normal transaminases and SF values <1000 μg/l (Guyader et al, 1998). In addition, transient elastography (TE, often referred to as FibroScan) can further refine the cohort that require biopsy. This non-invasive method of assessing liver fibrosis has been validated in most forms of chronic liver disease. The shear wave velocity correlates with liver stiffness, most accurately at the lower and higher ends of the range. In homozygotes with raised transaminases or SF values >1000 μg/l a TE <6·4 kPa excluded significant fibrosis whereas TE >13·9 kPa was predictive for severe fibrosis (Legros et al, 2015). Serum hyaluronic acid measurement has also shown excellent sensitivity and specificity for cirrhosis when values >46·5 ng/ml are obtained but this assay is not in routine clinical use (Crawford et al, 2009).

Recommendation 2

GH patients who present with SF >1000 μg/l and any with raised transaminases should be referred to a hepatologist for fibrosis assessment and exclusion of cirrhosis. (1B)

Magnetic resonance imaging (MRI) can be used for iron quantification with reasonable accuracy using the T2*method (St Pierre et al, 2005). The latter is helpful in patients negative for *HFE* mutations where SF may not correlate directly with the degree of body iron loading. MRI has the advantage over biopsy of being able to identify heterogeneous iron distribution within the liver. It may also obviate the immediate need for liver biopsy to determine the necessity for venesection in non-*HFE* cases. However, biopsy remains the gold standard for iron quantification, by which iron is typically graded 1–4 or, if needed, more accurately by measurement of the historical hepatic iron index (HII). Patients with unexplained significant iron overload should be considered for extended genetic testing.

Recommendation 3

Liver biopsy is no longer required for diagnosis of *HFE* GH but may be required to assess the severity of fibrosis in GH patients with SF >1000 μg/l and or elevated transaminases. Transient elastography could be used to select which patients from this group require liver biopsy. (2C)

Phlebotomy programmes deliver significant (7·5-fold) reduction in mean hepatic fibrosis scores. They have also been used to provide quantitative estimates of iron depletion leading to estimates that 1 μg/l SF in homozygotes represents approximately 3·3 mg of storage iron (Powell et al, 2006) and that SF values >900 μg/l correlate with >4 g of storage iron in men and >2 g in women (Gordeuk et al, 2008).

Bronzed diabetes and other endocrine disorders

Although 'Bronzed Diabetes' remains the historical moniker for GH, studies of *HFE* mutation frequencies in diabetes mellitus have not shown any increase in frequency compared with control groups (Frayling et al, 1998). However, these results more likely reflect the now overwhelming contribution of obesity to the development of diabetes rather than any lack of iron toxicity on the pancreas in GH.

Hypogonadotropic hypogonadism can develop in cases with severe iron loading as the result of iron deposition in pituitary.

GH arthropathy

Genetic haemochromatosis arthropathy typically affects the second and third metacarpophalangeal joints (painful handshake sign). It is unrelated to SF values and indeed seldom responds to venesection and iron depletion. It does however appear to develop more frequently in known homozygotes who fail to maintain Tsat <50% (Bardou-Jacquet et al, 2017).

GH cardiac toxicity

Cardiac toxicity in the form of cardiomyopathy and conduction defects can occur in GH but are more frequently seen in patients with transfusional iron overload.

Diagnosis of GH; patient selection and laboratory evaluation

As clinical penetrance is low and highly variable, unselected population screening for *HFE*-GH is not recommended. As such, the appropriate investigation of patients and individuals for GH is currently restricted to;

1. *Patients with symptoms in keeping with iron overload*

Symptoms are, however, often vague and longstanding. Nonetheless, suspicion should be raised for male subjects of north European ancestry presenting with unexplained weakness or fatigue, abnormal liver function tests, arthralgia/arthritis, impotence, diabetes of late onset, cirrhosis or bronze pigmentation.

It is recommended that initial screening requests for GH in these patients should include full blood count (FBC), SF, serum iron and Tsat. There is no place for the single measurement of either SF or Tsat in the preliminary detection of GH. Both measurements are essential. Thereafter, for patients aged >30 years from Primary care in areas of high prevalence if SF is beyond the normal range (200 and 300 μg/l female:male and Tsat >40% and 50% (female:male) *HFE* genotyping should be performed with an expected diagnostic yield of almost 20% for *HFE*-GH (Ogilvie et al, 2015). There is no such evidence for patients aged <30 years but similar values might be expected, particularly in males.

Recommendation 4

Patients of north European ancestry with clinical features suggestive of GH should have the following laboratory investigations; full blood count (FBC), liver function tests (LFTs), SF and transferrin saturation (Tsat). Molecular testing for *HFE* GH should follow if results fulfil the criteria of recommendation 5 (see below). (1B)

2. *Patients with unexplained raised SF (a frequent incidental finding)*

Raised SF values are found frequently in secondary care where they often occur in the context of alcohol abuse, established liver disease, malignant disease, renal dialysis, inflammatory disease and transfusional or secondary iron overload (see Cullis *et al*, 2018).

There are already a high and increasing number of SF requests being made from primary care. These samples can be exploited to detect undiagnosed homozygotes (Ogilvie *et al*, 2015). In primary care, SF values above the upper limits of normal (300 µg/l for adult males and 200 µg/l for adult females) can be found in almost 20% of males aged >30 years and in 10% of females aged 50–70 years and 17% of females >70 years of age. These raised values frequently appear to be unexplained (Ogilvie *et al*, 2010). For such patients, FBC and Tsat values as described above should guide requests for *HFE* genotype.

Recommendation 5

All adult patients of north European ancestry with unexplained raised SF and random Tsat (>300 µg/l and >50% males; >200 µg/l and >40% females) and normal FBC should have molecular testing for HFE GH. (1B)

3. *Targeted screening of family members of an index case of C282Y homozygous GH.*

Siblings, parents and children (over the age of consent) of a patient should be offered testing. Testing of partners can assist in determining the risk for children. It is not recommended that family screening be performed after identification of a heterozygote carrier or, indeed, a compound heterozygote. Initial family screening should be as above but also include *HFE* genotype with expected frequencies of 25% and 50% for GH when parents are either both heterozygotes or one heterozygous and the other homozygous.

Recommendation 6

Laboratory screening to include FBC, LFTs, SF, Tsat and HFE should be offered to family members after the diagnosis of *HFE* GH. Family screening should include parents (if available), siblings, partner and children (over the age of consent). Extended family screening is not recommended if an individual is identified as a C282Y/H63D compound heterozygote. (1B)

Recommendation 7

Investigation of all confirmed C282Y homozygotes should include FBC, LFTs, SF and Tsat. Thereafter further investigation may be required as follows;

a. **SF <1000 µg/l, normal LFTs, normal clinical examination; no further investigation required. Follow recommendation 8. (1C)**
b. **SF >1000 µg/l and or abnormal LFTs. All such patients require referral to Hepatology for fibrosis assessment to exclude the presence of cirrhosis. A minimum would be elastography. For patients with confirmed cirrhosis monitor with α-fetoprotein (AFP) AFP and hepatic ultrasound every 6 months (recommendation 11). (2C)**

Recommendation 8

Non C282Y homozygotes with significant iron loading as confirmed by magnetic resonance imaging and or liver biopsy should be investigated for rare iron loading genotypes or digenic inheritance. (1C)

Treatment: at diagnosis

Following confirmation of GH, the decision to start venesection should be made on the basis of both clinical and laboratory findings. The frequency of venesections may need to be individualised, taking into account the presenting severity of iron overload, organ damage, age, comorbidities and tolerability of the procedure. The risks of venesection *per se* in a frail newly diagnosed patient with only minor iron loading may well outweigh any potential benefit.

Venesection is indicated for all fit patients with biochemical iron overload with or without clinical features. In line with European Association for the Study of the Liver (EASL) guidelines (European Association for the Study of the Liver, 2010) and previous British Committee for Standards in Haematology (BCSH) recommendations (Dooley & Worwood, 2000) this should initially be performed once weekly (450–500 ml, ~200–250 mg iron). Monitor Hb levels weekly, SF monthly and reduce rate of venesection if anaemia develops. Tsat should be measured every 1–3 months as a disproportionate fall in Tsat and Hb would occur if other factors (such as alcohol or metabolic syndrome) together with GH were contributing to the raised SF. Continue with venesection until SF concentration is 20–30 µg/l and Tsat is <50%.

Recommendation 9

At diagnosis, all fit GH patients with biochemical iron loading should undergo weekly venesection until SF ~20–

30 μg/l and Tsat <50%. During this phase of venesection FBC should be monitored weekly and SF ± Tsat monitored monthly. Homozygotes with normal iron indices and compound heterozygotes with minimal elevation of iron indices may be suitable for blood donation and annual monitoring of SF and Tsat. (1B)

Iron depletion for patients who cannot tolerate venesection

In rare circumstances, such as juvenile haemochromatosis or delayed diagnosis, it may be necessary to use iron 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 great expertise in dealing with heart failure and should be consulted.

There is limited experience of using the oral iron chelator deferasirox (Exjade) in patients with C282Y homozygous GH and modest iron loading (median SF 645 μg/l). SF values were modestly reduced but significant patient withdrawal from GI, renal and hepatic toxicities was observed (Phatak et al, 2010).

Homozygotes without iron accumulation

For those subjects identified by family screening as C282Y homozygous but without iron accumulation, it is reasonable to monitor SF and iron status at yearly intervals in order to detect the onset of tissue iron accumulation. These patients may be encouraged to become blood donors (see below).

Treatment: in maintenance phase

Once excess iron has been removed and treatment by phlebotomy has ceased, iron will begin to re-accumulate. The actual rate of re-accumulation cannot be predicted but will occur more rapidly in those with marked iron loading at presentation. Many patients appear to lose some or all iron loading tendency over time and maintain normal iron balance with decreasing need for venesection. Patients usually return to the outpatient clinic every 3–6 months, and further phlebotomy is carried out when necessary.

The SF should be maintained at <50 μg/l. The question as to whether C282Y homozygotes with a raised Tsat despite SF < 50 μg/l should be treated has not been fully resolved. This situation is frequently found during maintenance. Previous BCSH guidelines (Dooley & Worwood, 2000) recommended that maintenance venesection programmes should maintain SF <50 μg/l and Tsat <50%. However, guidelines prepared by the American Association for the study of liver diseases (AASLD; Bacon et al, 2011)

and EASL (European Association for the Study of the Liver, 2010) only recommend keeping SF values between 50 and 100 μg/l and do not recommend Tsat monitoring. For the reasons given below, we have chosen to follow previous BCSH guidance (Dooley & Worwood, 2000) and recommend monitoring Tsat values during maintenance. Firstly, it would seem unwise for a patient with high SF and iron loading at diagnosis not to restart venesection as Tsat starts to rise once again. Furthermore, as cellular iron uptake and the risk of NTBI increase as Tsat values rise and as GH arthropathy has recently been shown to develop more often in patients who fail to maintain Tsat <50% (Bardou-Jacquet et al, 2017), we would recommend maintenance venesection targets for both SF <50 μg/l and Tsat <50% in all fit patients.

Patients frequently ask about dietary restriction of iron rich foods or the use of proton pump inhibitors (Hutchison et al, 2007) to reduce iron absorption. Such measures may offer some benefit, particularly for patients who do not tolerate or comply with venesections, but venesection remains the mainstay of maintenance treatment.

Recommendation 10

During maintenance, venesect as required, preferably at a blood donation centre to maintain normal FBC, SF <50 μg/l and Tsat <50%. (1C)

Management of patients with liver cirrhosis

As these patients have a 100-fold increased risk of developing primary liver cancer, α-fetoprotein (AFP) levels and hepatic ultrasonography should be carried out every 6 months.

Recommendation 11

HFE GH with cirrhosis; Treat as per recommendations 9 and 10 but not suitable for blood donation. Monitor AFP and hepatic ultrasonography every 6 months. (2C)

Homozygotes and blood donation

Some countries encourage homozygotes to donate blood to the transfusion service either after initial venesections have restored normal iron stores or to prevent iron loading in asymptomatic homozygotes with normal SF values. This has now been accepted by UK Transfusion services (National Blood Service, 2013; www.transfusionguidelines.org.uk) although the responsibility to monitor SF and Tsat remains with the referring clinician. It would seem reasonable to suggest that such patients with GH be allowed to donate blood three or four times a year and so provide mutual benefit to both patient and society.

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Author contributions

All authors contributed to writing the Guideline and approved the final draft.

References

Adams, P.C. & Barton, J.C. (2007) Haemochromatosis. *Lancet*, **370**, 1855–1860.

Adams, P.C., Speechley, M. & Kertesz, A.E. (1991) Longterm survival analysis in hereditary haemochromatosis. *Gastroenterology*, **101**, 368–372.

Adams, P.C., Reboussin, D.M., Leiendecker-Foster, C., Moses, G.C., McLaren, G.D., McLaren, C.E., Dawkins, F.W., Kasvosve, I., Acton, R.T., Barton, J.C., Zaccato, D., Harris, E.L., Press, R., Chang, H. & Eckfeldt, J.H. (2005a) Comparison of the unsaturated iron binding capacity with transferrin saturation as a screening test to detect C282Y homozygotes for haemochromatosis in 101,168 participants in the HEIRS study. *Clinical Chemistry*, **51**, 1048–1051.

Adams, P.C., Reboussin, D.M., Barton, J.C., McLaren, C.E., Eckfeldt, J.H., McLaren, G.D., Dawkins, F.W., Acton, R.T., Harris, E.L., Gordeuk, V.R., Leiendecker-Foster, C., Speechley, M., Snively, B.M., Holup, J.L., Thomson, E. & Sholinsky, P. (2005b) Hemochromatosis and iron-overload screening in a racially diverse population. *The New England Journal of Medicine*, **352**, 1769–1778.

Allen, K.J., Gurrin, L.C., Constantine, C.C., Osborne, N.J., Delatycki, M.B., Nicoll, A.J., McLaren, C.E., Bahlo, M., Nisselle, A.E., Vulpe, C.D., Anderson, G.J., Southey, M.C., Giles, G.G., English, D.R., Hopper, J.L., Olynyk, J.K., Powell, L.W. & Gertig, D.M. (2008) Iron-overload-related disease in HFE hereditary hemochromatosis. *The New England Journal of Medicine*, **358**, 221–230.

Asberg, A., Hveem, K., Thorstensen, K., Ellekjær, E., Kannelønning, K., Fjøsne, U., Halvorsen, T.B., Smethurst, H.-B.G., Sagen, E. & Bjerve, K.S. (2001) Screening for hemochromatosis: high prevalence and low morbidity in an unselected population of 65,238 persons. *Scandinavian Journal of Gastroenterology*, **10**, 1108–1115.

Bacon, B.R., Adams, P.C., Kowdley, K.V., Powell, L.W. & Tavill, A.S. (2011) Diagnosis and management of hemochromatosis: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology*, **54**, 328–343.

Bardou-Jacquet, E., Laine, F., Guggenbuhl, P., Morcet, J., Jezequel, C., Guyader, D., Moirand, R. & Deugnier, Y. (2017) Worse outcomes of patients with HFE haemochromatosis with persistent increases in transferrin saturation during maintenance therapy. *Clinical Gastroenterology and Hepatology*, **15**, 1620–1627.

Beutler, E., Felitti, V.J., Koziol, J.A., Ho, N.J. & Gelbart, T. (2002) Penetrance of 845G→A (C282Y) HFE hereditary haemochromatosis mutation in the USA. *Lancet*, **359**, 211–218.

Bomford, A. & Williams, R. (1976) Longterm results of venesection therapy in idiopathic haemochromatosis. *Quarterly Journal of Medicine*, **Vol XLV**, 611–623.

Camaschella, C. & Poggiali, E. (2009) Rare types of genetic haemochromatosis. *Acta Haematologica*, **122**, 140–145.

Crawford, D.H., Murphy, T.L., Ramm, L.E., Fletcher, L.M., Clouston, A.D., Anderson, G.J., Subramaniam, V.N., Powell, L.W. & Ramm, G.A. (2009) Serum hyaluronic acid with serum ferritin accurately predicts cirrhosis and reduces the need for liver biopsy in C282Y hemochromatosis. *Hepatology*, **49**, 418–425.

Cullis, J., Fitzsimons, E.J., Tsochatzis, E., Griffiths, W.J.H. & Thomas, W. (2018) Investigation and management of a raised serum ferritin. *British Journal of Haematology*, **181**, 331–340.

Dooley, J. & Worwood, M. (2000) Guidelines on Diagnosis and Therapy: Genetic haemochromatosis. British Committee for Standards in Haematology. Darwin Medical Communications Ltd., Abingdon, Oxford. http://www.bcshguidelines.com

European Association for the Study of the Liver. (2010) EASL clinical practice guidelines for HFE hemochromatosis. *Journal of Hepatology*, **53**, 3–33.

Feder, J.N., Gnirke, A., Thomas, W., Tsuchihashi, Z., Ruddy, D.A., Basava, A., Dormishian, F., Domingo, R., Ellis, M.C., Fullan, A., Hinton, L.M., Jones, N.L., Kimmel, B.E., Kronmal, G.S., Lauer, P., Lee, V.K., Loeb, D.B., Mapa, F.A., McClelland, E., Meyer, N.C., Mintier, G.A., Moeller, N., Moore, T., Morikang, E., Prass, C.E., Quintana, L., Starnes, S.M., Schatzman, R.C., Brunke, K.J., Drayna, D.T., Risch, N.J., Bacon, B.R. & Wolff, R.K. (1996) A novel MHC class 1-like gene is mutated in patients with hereditary haemochromatosis. *Nature Genetics*, **13**, 399–408.

Frayling, T., Ellard, S., Grove, J., Walker, M. & Hattersley, A.T. (1998) C282Y mutation in HFE (haemochromatosis) gene and type 2 diabetes. *Lancet*, **351**, 1933–1934.

Gordeuk, V.R., Reboussin, D.M., McLaren, C.E., Barton, J.C., Acton, R.T., McLaren, G.D., Harris, E.L., Reiss, J.A., Adams, P.C., Speechley, M., Phatak, P.D., Sholinsky, P., Eckfeldt, J.H., Chen, W.-P., Passmore, L. & Dawkins, F.W. (2008) HFE mutations in Caucasian participants of the Hemochromatosis and Iron Overload Screening Study with serum ferritin level <1000 µg/L. *American Journal of Haematology*, **83**, 618–626.

Guyader, D., Jazquelinet, C., Moirand, R., Turlin, B., Mendler, M.H., Chaperon, J., David, V., Brissot, P., Adams, P. & Deugnier, Y. (1998) Non-invasive prediction of fibrosis in C282Y homozygous hemochromatosis. *Gastroenterology*, **115**, 929–936.

Heubers, H.A., Csiba, E., Heubers, E. & Finch, C.A. (1985) Molecular advantage of diferric transferrin in delivering iron to reticulocytes: a comparative study. *Proceedings of the Society for Experimental Biology and Medicine*, **179**, 222–226.

Hutchison, C., Geissler, C.A., Powell, J.J. & Bomford, A. (2007) Proton pump inhibitors suppress the absorption of dietary non-haem iron in hereditary haemochromatosis. *Gut*, **56**, 1291–1295.

Jackson, H.A., Carter, K., Darke, C., Guttridge, M.G., Ravine, D., Hutton, R.D., Napier, J.A. & Worwood, M. (2001) HFE mutations, iron deficiency and overload in 10 500 blood donors. *British Journal of Haematology*, **114**, 474–484.

Kato, J., Fujikawa, K., Kanda, M., Fukuda, M., Sasakik, K., Takayama, T., Kobune, M., Takada, K., Takimoto, R., Hamada, H., Ikeda, T. & Niitsu, Y.A. (2001) A mutation in the element, iron-responsive of ferritin, H mRNA causing autosomal dominant iron overload. *The American Journal of Human Genetics*, **69**, 191–197.

Legros, L., Bardou-Jacquet, E., Latournerie, M., Guillygomarch, A., Turlin, B., Le Lan, C., Desille, Y., Laine, F., Moirand, R., Brissot, P., Deugnier, Y. & Guyader, D. (2015) Non invasive assessment of liver fibrosis in C282Y homozygous HFE hemochromatosis. *Liver International*, **35**, 1731–1738.

Merryweather-Clarke, A.T., Cadet, E., Bomford, A., Capron, D., Viprakasit, V., Miller, A., McHugh, P.J., Chapman, R.W., Pointon, J.J., Wimhurst, V.L.C., Livesey, K.J., Tanphaichitr, V., Rochette, J. & Robson, K.J.H. (2003) Digenic inheritance of mutations in HAMP and HFE results in different types of haemochromatosis. *Human Molecular Genetics*, **12**, 2241–2247.

National Blood Service. (2013) Genetically determined conditions. In: Guidelines for the Blood Transfusion Services UK. The Red Book, 8th edn (ed. by S MacLennan), pp. 31. The Stationery Office, UK.

Nemeth, E., Tuttle, M.S., Powelson, J., Vaughn, M.B., Donovan, A., Ward, D.M., Ganz, T. & Kaplan, J. (2004) Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalisation. *Science*, **306**, 2090–2093.

Niederau, C., Fischer, R., Purschel, A., Stremmel, W., Haussinger, D. & Strohmeyer, G. (1996)

Longterm survival of patients with hereditary haemochromatosis. *Gastroenterology*, 110, 1107–1119.

Ogilvie, C., Fitzsimons, K. & Fitzsimons, E.J. (2010) Serum ferritin values in primary care: are high values overlooked. *Journal of Clinical Pathology*, 63, 1124–1126.

Ogilvie, C., Gaffney, D., Murray, H., Kerry, A., Haig, C., Spooner, R. & Fitzsimons, E.J. (2015) Improved detection of hereditary haemochromatosis. *Journal of Clinical Pathology*, 68, 218–221.

Olynyk, J.K., Hagan, S.E., Cullen, D.J., Beilby, J. & Whittall, D.E. (2004) Evolution of untreated hereditary haemochromatosis in the Busselton population. A 17 year study. *Mayo Clinic Proceedings*, 79, 309–313.

Olynyk, J.K., Trinder, D., Ramm, G.A., Britton, R.S. & Bacon, B.R. (2008) Hereditary haemochromatosis in the post HFE era. *Hepatology*, 48, 991–1001.

Phatak, P., Brissot, P., Wurster, M., Adams, P.C., Bonkovsky, H.L., Gross, J., Malfertheimer, P., McLaren, G.D., Niederau, C., Piperno, A., Powell, L.W., Russo, M.W., Stoelzel, U., Stremmel, W., Griffel, L., Lynch, N., Zhang, Y. & Pietrangelo, A. (2010) A phase " dose escalation trial of desferasirox for the treatment of iron overload in HFE-related hereditary hemochromatosis. *Hepatology*, 52, 1671–1779.

Porter, J.B., Walter, P.B., Neumayr, L.D., Evans, P., Bansal, S., Garbowski, M., Weyhmiller, M.G., Harmatz, P.R., Wood, J.C., Miller, J.L., Brynes, C., Weiss, G., Seifert, M., Grosse, R., Grabowski, D., Schmidt, A., Fischer, R., Neilsen, P., Neimeyer, C. & Vichinsky, E. (2014) Mechanisms of plasma non transferrin bound iron generation: insights from comparing transfused diamond blackfan anaemia with sickle cell & thalassaemia patients. *British Journal of Haematology*, 167, 692–696.

Porto, G., Brissot, P., Swinkels, D.W., Zoller, H., Kamarainen, O., Patton, S., Alonso, I., Morris, M. & Keeney, S. (2016) EMQN best practice guidelines for the molecular genetic diagnosis of hereditary haemochromatosis. *European Journal of Human Genetics*, 24, 479–495.

Powell, E.E., Ali, A., Clouston, A.D., Dixon, J.L., Lincoln, D.J., Purdie, D.M., Fletcher, L.M., Powell, L.W. & Jonsson, J.R. (2005) Steatosis is a cofactor in liver injury in hemochromatosis. *Gastroenterology*, 129, 1937–1943.

Powell, L.W., Dixon, J.L., Ramm, G.A., Purdie, D.M., Lincoln, D.J., Anderson, G.J., Subramaniam, N., Hewett, D.G., Searle, J.W., Fletcher, L.M., Crawford, D.H., Rodgers, H., Allen, K.J., Cavanaugh, J.A. & Bassett, M.L. (2006) Screening for hemochromatosis in asymptomatic subjects with or without a family history. *Archives of Internal Medicine*, 166, 294–301.

Sheldon, J.H. (1935) Haemochromatosis. Oxford University Press, London.

St Pierre, T.G., Clark, P.R., Chua-anusorn, W., Fleming, A.J., Olynyk, J.K., Pootrakul, P., Robins, E. & Lindeman, R. (2005) Non-invasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood*, 105, 855–861.

Waalen, J., Felliti, V., Gelbart, T. & Beutler, E. (2002) Prevalence of hemochomatosis-related symptoms among individuals with mutations in the HFE gene. *Mayo Clinic Proceedings*, 77, 522–530.

Waalen, J., Felitti, V.J., Gelbart, T. & Beutler, E. (2008) Screening for haemochromatosis by measuring ferritin levels: a more effective approach. *Blood*, 111, 3373–3376.

Worwood, M. (1982) Ferritin in human tissues and serum. *Clinics in Haematology*, 11, 275–307.

Worwood, M. (2005) Inherited iron loading: genetic testing in diagnosis and management. *Blood Reviews*, 19, 69–88.

Wu, X., Wang, Y., Chen, W., Liu, W., Zhao, Y., Mayeur, C., Schmidt, P.J., Yu, P.B., Wang, F. & Xia, Y. (2014) HFE interacts with the BMP type 1 receptor ALK3 to regulate hepcidin expression. *Blood*, 124, 1335–1343.

Young, S., Bomford, A. & William, R. (1984) Effect of iron saturation of transferrin on its binding and uptake by rabbit reticulocytes. *Biochemical Journal*, 219, 505–510.