Liver disorder and the \textit{HFE} locus

Hereditary haemochromatosis (HH) is the most common inherited disease in Northern Europeans, with a prevalence of around 1 in 300.\textsuperscript{1} When Feder \textit{et al.} identified a mutation in a novel MHC-class-I-like gene, \textit{HFE}, that was present in over 80\% of these patients, it was evident that there was potential to improve not only patient diagnosis, but also the understanding of iron absorption and transport, and the mechanisms of iron toxicity.\textsuperscript{2} HH is characterized by an increase in total body iron stores due to abnormally high intestinal absorption. The classical features of cirrhosis, diabetes, cardiac failure, arthritis and bronze skin pigmentation have been recognized since 1889, and, prior to the development of biochemical markers of iron overload, diagnosis was entirely clinical. The subsequent addition of testing of iron indices helped the diagnosis in suspected cases, with increased transferrin saturation being the most sensitive marker,\textsuperscript{3} and serum ferritin providing the best measure of total body iron stores. Treatment is with regular phlebotomy until the ferritin level is low normal, and in the non-cirrhotic patient this results in a normal life expectancy.\textsuperscript{4,5} Practice guidelines for the diagnosis and management of HH have recently been revised by the American Association for the Study of Liver Diseases, based on the evidence to date.\textsuperscript{6} While iron deposition is a feature of many liver disorders, only HH has been associated with genetic variants at the \textit{HFE} locus. Most cases are associated with homozygosity for a G$\rightarrow$A transition in nucleotide 845 of the open reading frame that results in a cysteine to tyrosine substitution at amino acid 282 (C282Y). In addition, there is a second, C$\rightarrow$G, mutation in exon 2 resulting in a histidine to aspartic acid substitution at position 63 (H63D) that may be associated with HH if inherited together with C282Y—the so-called compound heterozygous state. We describe the discovery of these mutations, and how they have improved our understanding of iron metabolism and influenced population screening.

The clear hereditary basis of the disease encouraged the search for a genetic explanation for increased iron absorption, and close HLA associations initially localized the abnormal gene to the short arm of chromosome 6.\textsuperscript{7} Using multiple microsatellite markers and high-resolution linkage disequilibrium analysis, a 300 kb region was identified that was likely to harbour the mutated gene in patients with HH. Subsequent sequencing of this region revealed the presence of three genes. One of these genes, now called \textit{HFE}, was homologous to HLA class I genes and was found to contain two frequent missense mutations.\textsuperscript{2} The first, C282Y, was found to be homozygous in 83\% of 178 patients and subsequently figures of between 60\% and 100\% homozygosity in HH patients have been reported worldwide.\textsuperscript{8,9} This would imply either a direct causative effect of the mutation(s) in disease pathogenesis, or very tight linkage disequilibrium with the disease-causing mutation. A role for \textit{HFE} in iron metabolism, discussed below, implies the former explanation.

The close, but not complete, association of C282Y homozygosity with HH encouraged Feder \textit{et al.} to search for additional mutations. Evidence of disease association for the second, H63D, mutation is less straightforward, but equally compelling. The fact that C282Y is in complete linkage disequilibrium with H63D means that the gene frequency of the second mutation in HH patients is low, due to the high prevalence of C282Y. Nonetheless, the H63D mutation was found in 89\% of C282Y heterozygotes with HH compared to a control gene frequency of 17\%. Although there are undoubtedly a small percentage of patients with HH that is not linked to the HFE gene, the vast majority are either C282Y homozygotes or compound heterozygotes (C282Y/H63D). The penetrance of the compound heterozygous state appears to be very low, with only around 1\% developing clinical disease. Another adult mutation, tentatively termed \textit{HFE3}, in the transferrin receptor 2 (TFR2) gene,\textsuperscript{10} has recently been reported by an Italian group, and the gene mutated in juvenile haemochromatosis, termed \textit{HFE2}, has recently been mapped to chromosome 1q.\textsuperscript{11} To date these
mutations have not been confirmed in other populations (P.C. Adams, personal communication).

C282Y heterozygotes make up 10% of the Caucasianoid population. Although they have mean transferrin saturation and ferritin levels higher than the normal population, complications due to iron overload are extremely rare and usually associated with co-morbid disease. Increased levels of liver iron are frequently observed in patients with viral hepatitis and non-alcoholic steatohepatitis (NASH), and may play some role in disease progression. It was initially thought that heterozygosity for C282Y may explain the iron overload in these patients. This has largely been refuted by a recent study that examined the prevalence of the mutation in patients with a variety of different liver diseases. Only in autoimmune disease was the mutation more common than expected, and it was associated with only minor increases in iron indices and no increase in fibrosis. Moreover, another study observed that the proportion of C282Y heterozygotes in liver transplant recipients for end-stage liver diseases other than HH was no different from that in the general population. These data accord with those from previous studies showing no association between C282Y heterozygosity and advanced alcoholic liver disease or hepatitis C. Evidence from small, poorly-controlled studies initially suggested that the HFE mutations were increased in North American patients with NASH, and were associated with increased fibrosis. In a larger study, however, the hepatic iron index (HII) was not significantly raised in these patients and was not associated with inflammation or fibrosis. Controversy also exists over whether heterozygosity for HFE mutations increases susceptibility to porphyria cutanea tarda (PCT). In this condition, the mild increase in hepatic iron seen in heterozygotes may accelerate the inactivation of uroporphorinogen decarboxylase, leading to the classical skin lesions. One study suggested a role for the H63D mutation, but not C282Y, while another reported that both increase risk. There are geographical differences between the two populations, and this may partly explain the discrepant results. These issues are obviously of importance when it comes to population screening; however, there is as yet no convincing evidence that simple heterozygotes should be followed up or treated with venesection.

The HFE protein binds to the transferrin receptor, reducing its avidity for iron-loaded transferrin. The extracellular portion is very similar to MHC I, with three domains, termed $\alpha 1$, $\alpha 2$ and $\alpha 3$. The $\alpha 3$ domain binds to $\beta 2$ microglobulin. The C282Y mutation disrupts the MHC I like configuration, interferes with $\beta 2$ microglobulin binding and prevents HFE expression on the cell surface. The importance of this has been highlighted by the fact that $\beta 2$ microglobulin knockout mice develop iron overload. Although this observation provides clues towards the function of HFE and the significance of the mutation in disease development, the full complexity of iron transport and control, discussed below, is far from fully resolved.

Redox coupling of free iron with oxygen results in the formation of superoxide, hydrogen peroxide and the hydroxyl radical, all of which are capable of inducing oxidative damage. For this reason, higher organisms have developed mechanisms for transporting and storing iron in protein-bound states that minimize these deleterious reactions. In plasma, iron is transported bound to transferrin, and in cells it is stored bound to ferritin. Accordingly, at any time only a small proportion (<1%) is present as free iron in the intracellular labile iron pool. This labile iron pool appears to be crucial in regulating the coordinate synthesis of proteins involved in the regulation of iron uptake and storage, and in protection against iron-mediated toxicity. The mechanisms behind this control vary between cell types, depending on whether they are iron-receiving (e.g. erythrocyte precursors, duplicating cells) or iron-donating (e.g. macrophages, gut epithelial cells). Hepatocytes seem to fall between these two categories. Ferritin and transferrin receptor levels are tightly controlled at the post-transcriptional stage. Intracellular iron regulatory proteins (IRP 1 and IRP 2) are capable of binding free iron, and this in turn alters their conformation, reducing their ability to bind to iron-responsive elements (IREs) on the ferritin and transferrin receptor mRNA. For the transferrin receptor RNA, this IRP/IRE binding augments translation, and for the ferritin mRNA it favours degradation by ribonucleases. These variable effects are due to the different sites of the IREs on the two mRNAs. The result is an increase in iron import if the labile iron pool is low, and increased iron storage capacity if there is an excess of free iron. It is possible that mutations in these proteins may account for some of the cases of non-classical (non-HFE-linked) haemochromatosis, however none have yet been identified.

Unfortunately, the mechanisms through which the HFE gene product influences iron absorption are less clear. Although HFE is similar in structure to MHC class I, and associates with $\beta 2$ microglobulin on the cell surface, it does not present antigen to T cells as classical MHC I does. Instead, it complexes with the transferrin receptor and reduces its avidity for iron-loaded transferrin. This effect of HFE facilitates the release of iron and transferrin from the receptor into the cell after
endocytosis. This effect is lost when the C282Y mutation is present. It has been proposed that in the duodenal crypt, enterocytes act as ‘sensors’ of body iron stores, such that iron depletion results in an increase in gut absorption.26 This occurs through non-transferrin dependent iron transport mechanisms. The primary, and best recognized, method is via the divalent metallic transporter 1 (DMT1) mechanism. This protein is found on the luminal side of mature villi enterocytes in the proximal duodenum, the primary site of iron absorption.27 The protein is up-regulated in iron deficiency, and down-regulated when there is excess iron. This is controlled by an IRE on the mRNA, similar to the transferrin receptor mRNA. In HH, enterocytes have reduced free iron, and DMT1 is up-regulated, explaining the increased luminal absorption. Further research is focusing on how enterocytes with HFE and the transferrin receptor on their basolateral membrane can be iron-depleted in HH, while other iron-receiving cells with these proteins can succumb to iron overload. The complexity of the role of iron in metabolism and disease is evidently matched by the complexity of its cellular control systems. These will need to be better elucidated before the link between mutation and disease is fully understood.

HH would appear to be an ideal candidate for genetic screening. Life-threatening complications occur in a significant proportion of affected individuals,28 and there is an effective and simple treatment, which, if started early, improves survival and reduces morbidity. Whether screening should be universal, rather than solely for family members of an affected proband, however, is a subject of debate. This is because penetrance and phenotypic expression are still relatively poorly understood. Family screening has uncovered many C282Y homozygotes with normal iron indices, and, even in overt HH, the liver iron stores do not correlate with patient age. Concomitant disease undoubtedly plays a role. Excessive alcohol intake and chronic hepatitis C can both lead to iron overload, and will increase the likelihood of a C282Y homozygote developing end organ damage. Supplementary iron tablets or vitamin C may have the same effect, while physiological or pathological blood loss will have the opposite effect.

There is also debate over the screening tool to use. Genotyping is expensive and will identify many homozygotes with no evidence of iron overload. Biochemical iron indices will detect iron-overloaded patients, but not C282Y homozygotes that have the potential to develop iron overload, but have yet to do so. An algorithm using a fasting transferrin saturation > 55% was suggested based on a pilot scheme screening 1064 randomly selected subjects,29 while another using unbound iron-binding capacity as the initial tool has also been suggested as cost-efficient.30 While these are cheap, easy and sensitive, the age at which these tests should be done is still unclear. At this point in time it seems appropriate to suggest that genetic screening is reserved for the families of affected probands. Universal population screening with either biochemical or genetic tests should be withheld until there is more evidence of a favourable benefit.31 A large-scale study screening 100 000 Americans is planned which should answer this question.

The discovery of the HFE gene and its association with HH has dramatically changed how we look at iron transport and homeostasis, as well as improving disease diagnosis. Homozygosity for C282Y and compound heterozygosity (C282Y/H63D) are associated with overt iron overload; however, penetrance is variable, and depends on other factors. Some of these are understood, but there are probably other genetic or environmental factors that influence phenotype which are yet to be discovered. A better understanding of iron metabolism may highlight these. Simple heterozygosity has been linked with moderate iron overload, but not convincingly to pathology, either directly, or associated with other liver diseases. At the present time, transferrin saturation is probably a more appropriate first tool for population screening, but this is a subject of considerable debate. Molecular screening is an appropriate method of screening first-degree family members of an affected proband.

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References


