Iron, haemochromatosis and thalassaemia as risk factors for fibrosis in hepatitis C virus infection

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Iron depletion is required in patients with HCV and iron overload to retard liver fibrosis

Excess iron is an important cause of liver damage. Aerobic metabolism generates oxygen species as a by-product of the reduction of O₂ but these are generally of low reactivity. The presence of transition metal ions such as iron (Fe²⁺) catalyses the generation of highly reactive oxygen species, the most important of which is the hydroxyl radical (•OH).

The hydroxyl radical causes oxidation of lipids, lipoproteins, proteins, DNA and carbohydrates leading to injury to the cell membranes and genomic damage. Mitochondrial cell membranes are particularly susceptible to iron-catalysed, hydroxyl-mediated damage.¹ Reactive oxygen species may also activate hepatic stellate cells and stimulate transformation into the collagen-producing myofibroblast phenotype associated with hepatic fibrosis.²

The presence of excess iron in the liver in the absence of other aetiologies of liver damage may not be sufficient to cause liver disease. However, excess liver iron is now clearly recognised as a cofactor for the development of advanced fibrosis and cirrhosis in patients with hepatitis C virus (HCV) infection and alcohol-related liver disease.^{3–8}

There is enormous variation in the rate at which fibrosis develops and the time taken for cirrhosis or decompensated liver disease to occur among patients with chronic HCV infection.^{9–10} Certain factors influencing the progression of disease have been identified through cross-sectional and cohort studies so that some of this variation in the rate of fibrosis may be explained by sex of the patient, age at the time of infection and, in some studies, alcohol abuse.^{9–11} However, these factors only account for 20–30% of the variability in the progression rates of liver fibrosis leading many authors to conclude that

much of the unexplained variability may be accounted for by patients' genetic background. Although a number of groups are currently searching for genes that influence liver fibrosis in patients with chronic viral hepatitis, only a small number has been identified so far. Among these, a limited number has shown reproducibility and/or biological relevance. These include complement factor V, coagulation factor V, carnitine palmitoyltransferase 1A and DEAD box polypeptide 5.^{12–14}

It might be predicted that mutations in the HFE gene that cause iron overload and haemochromatosis would meet the requirements of biological plausibility and reproducibility as a cause for accelerated fibrosis. However, the association of HFE mutations and advanced fibrosis in patients with HCV infection is controversial. An initial report by Smith found that patients with HCV infection, who were heterozygous for the HFE-C282Y mutation, had higher ferritin levels and were more likely to have stainable liver iron than patients who were homozygous for the wild-type HFE gene.¹⁵ Furthermore, in Smith's study, carriers of the HFE-C282Y mutation had more advanced fibrosis and 4 of 10 had cirrhosis. Although these findings have been replicated in other populations,^{16–17} some studies, including the new one published in this issue of *Gut*, have not been able to show a clear association between HFE-C282Y and the degree of fibrosis.^{8, 18–19} Indeed, in our own population of over 900 European patients with chronic HCV infection, heterozygous carriage of HFE-C282Y was not associated with advanced or rapid liver fibrosis.

There are two possible explanations why the HFE-C282Y mutation is not consistently found to be a cause of advanced fibrosis in patients with chronic HCV infection. Firstly, most studies have effectively sought an association of advanced fibrosis with the heterozygous HFE-C282Y state as homozygotes for the HFE-C282Y

mutation and compound heterozygotes, carrying one abnormal allele of both the HFE-H63D and HFE-C282Y, are relatively rare among the population with HCV infection. It is generally accepted that people who are heterozygous for the HFE-C282Y mutation or homozygous for the HFE-H63D mutation do not develop iron overload. Secondly, iron overload is not an inevitable consequence of being homozygous for the HFE-C282Y mutation. Among patients who carry the disease genotype, HFE-282YY, the proportion who develop either biochemical abnormalities or liver disease is clearly <100%. In a population-based study, biochemical expression of disease, as determined by transferrin saturation of >50%, was found in 50% of male homozygotes and 40% of female homozygotes.²⁰ Three large population-based studies found low rates of penetrance for clinical liver disease, as determined by abnormal liver function tests or clinical symptoms, in HFE-282YY homozygotes.^{20–22} In contrast, two smaller, less well controlled, studies found higher rates of clinical disease penetrance.^{23–24} As penetrance of the HFE mutations is low and the heterozygous state is not associated with iron overload then it should not be surprising that HFE does not consistently influence the severity of HCV infection.

Mutations in the haemoglobin genes that cause thalassaemia are strongly associated with heavy iron overload and the penetrance of these mutations is high. The strong erythropoietic drive produced by the haemoglobinopathies suppresses hepcidin expression in the liver leading to high rates of iron absorption from the gastrointestinal tract.²⁵ Thalassaemia major has previously been reported as a risk factor for advanced liver disease in patients with HCV infection.²⁶ In this issue of *Gut*, Sartori *et al*¹⁹ (see page 693) publish a study in which they have explored the importance of haemoglobin β mutations in the development of hepatic iron overload and advanced fibrosis in patients with chronic HCV infection in Italy. As mentioned earlier, HFE mutations were not found to correlate with iron loading or with fibrosis stage but heterozygous carriers of β-globin mutations were found to have higher hepatic iron concentrations, stainable hepatic iron and more advanced fibrosis than patients with HCV-infection who had wild-type haemoglobin genes and HFE heterozygotes. The prevalence of β-globin mutations was much higher than what would normally be expected in the Italian population, which may be the result of a bias introduced by using a clinic-based population and the risk of bloodborne virus infection in patients with thalassaemia.²⁷

The difference in the effect of HFE and β -globin mutations emphasise the central importance of iron in the development of advanced liver disease associated with HCV infection. It has been reported that iron increases the replication of HCV, possibly through the induction of translation initiation factor 3.^{28–29} However, levels of viraemia have not been correlated with histological severity and an alternative explanation must be proposed to explain the advanced liver disease associated with iron excess. HCV itself seems to be a cause of oxidative stress.³⁰ Histological examination of HCV-infected liver frequently shows steatosis and mitochondrial swelling: hallmarks of oxidative damage. Transgenic mice expressing either the HCV nucleocapsid or the complete polyprotein also show mitochondrial damage and excess oxidative damage. It therefore seems likely that HCV and iron act synergistically to increase oxidative damage, and to accelerate liver fibrosis and the progression of disease. In support of this hypothesis, mice transgenic for the HCV core protein have high levels of hydroperoxides, lysosomal and mitochondrial membrane damage and deletion of mitochondrial DNA.³¹ These mice go on to develop hepatocellular carcinoma.³¹ Mice transgenic for the complete polyprotein have high levels of lipid peroxidation and develop hepatocellular carcinoma when iron overload is induced.³²

Although HCV infection cannot always be eliminated iron overload is a treatable condition. Dietary iron restriction and phlebotomy reduce serum iron indices and reduce transaminase levels in patients with HCV infection.^{33–34} Furthermore, levels of hepatic lipid peroxidation and histological severity may be improved by phlebotomy in patients without β thalassaemia. In patients with thalassaemia, phlebotomy may not be feasible but iron chelation therapy with desferrioximine or the oral chelator deferiprone does seem to prevent the progression of liver fibrosis in patients with HCV infection, and is therefore indicated in patients where the HCV infection cannot be eliminated.^{35–36}

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