Genetic haemochromatosis

A B Bomford, I W Dymock, E B D Hamilton

Early years

By the time of his arrival at King's College Hospital in 1966 Roger Williams had already published extensively in the field of haemochromatosis. During the years 1959-1965 while working at the Royal Free Hospital with Sheila Sherlock he focused on two areas. The first was the study of families of patients with haemochromatosis to determine whether there was a genetic component to the disease and, if so, identify the mode of inheritance. The second area was the use of radioactive tracer techniques to investigate iron absorption, in particular the changes in response to treatment and its measurement in the relatives of index patients.

A detailed family study of patients with haemochromatosis culminated in two papers published in 1962.^{1,2} This type of study was timely because the view that haemochromatosis was an inborn error of iron metabolism, based on the early work of Sheldon,³ had come under attack by an American physician, R A Macdonald. Sheldon's hypothesis which was formulated before the classical theories of iron balance had been published,⁴ was that haemochromatosis was an iron storage disease in which tissue damage was the result of excess iron deposition; the iron accumulated because of an inherited, albeit unknown metabolic defect. He considered that the excess iron would eventually lead to tissue damage manifesting as the clinical features of hepatic cirrhosis, diabetes mellitus and hypogonadism.³ Macdonald's opposing view that iron overload and cirrhosis were not pathogenetically linked was based on clinical, pathological and experimental evidence.⁵ It is worthwhile briefly considering how this evidence was marshalled because it is here that the controversy over whether hepatic iron overload occurs in alcoholic cirrhosis has its origins. Macdonald suggested that iron overload in cirrhosis was always the result of exposure to excess dietary iron, the major source of which he considered to be alcoholic drinks, iron in food from cooking utensils and pharmacological iron.^{5,6} It is difficult to provide quantitative data for dietary iron levels, however, and it is also necessary to postulate a separate cause for the portal cirrhosis, the major aetiological groups in the 1960's being alcoholic, malnutrition, postviral and cryptogenic. The experimental evidence against iron deposition causing tissue injury was stronger; all the attempts to produce liver damage in animals by dosing with enteral or parenteral iron⁵ had failed. Tissue damage could only be produced by the concomitant administration of a diet deficient in choline.

Because of these difficulties the approach taken was to search for evidence that there was a heritable component to iron overload with the inference that haemochromatosis was a distinct pathological entity. A detailed analysis of hepatic

pathology was undertaken and a method developed with Peter Scheuer and A R Muir to assess hepatic iron overload in relatives with early iron loading.¹ This was based on the characteristic electron microscopic appearances of the iron core of ferritin which is distributed freely within the cytoplasm of hepatocytes. The counting of diffusely scattered electron dense particles provided a means of quantifying tissue iron concentrations as compared with liver sections from control subjects. If iron stores are only minimally increased the excess hepatic iron, being in a soluble form, does not produce a marked reaction when cytochemical methods such as Pearls stain are used. Application of this method to a group of 46 relatives, mainly siblings and children, showed that 74% had increased concentrations of hepatic iron.² A strong case was made on the basis of these findings for an autosomal recessive mode of inheritance with heterozygotes showing a minimal increase in hepatic iron stores and partial biochemical abnormalities such as raised concentrations of serum iron.

The second abnormality that was found to unequivocally characterise haemochromatosis was the increased absorption of dietary iron. Even this simple assertion, however, was controversial in the 1960s because patients with greatly increased iron stores had been observed to absorb normal amounts of iron at the time of presentation.⁵ Working with Chris Pitcher it was soon established that iron absorption was indeed within the normal range in most patients at presentation.⁷ With reduction of iron stores by venesection, absorption rose to high levels and only became suppressed as iron stores reaccumulated. The group of index patients and relatives was investigated by the use of double isotope techniques and whole body counting, good agreement being found for the results obtained using the two methods in patients with a wide range of iron loading and serum iron concentrations.⁸ Over half of the relatives examined had increased absorption of dietary iron, a mean of 26% of the dose as compared with 5% in the controls, when this was taken as a radiolabelled iron salt with a standard meal.⁹ It is clear in retrospect that many of the relatives examined were heterozygotes who, it is now known, do not accumulate significant amounts of iron in the liver or elsewhere and it is difficult to sustain the argument that iron absorption continues at this rate in carriers of a single gene.

TREATMENT AND NATURAL HISTORY

Although venesection therapy is now commonplace and the accepted form of treatment of iron loaded patients with haemochromatosis, the pervasive controversy over whether the disease was a distinct pathological entity led to the claim in 1966 that this form of treatment did not improve

The Institute of Liver Studies and Department of Rheumatology, King's College Hospital and School of Medicine and Dentistry, London
A B Bomford
E B D Hamilton

Stepping Hill Hospital, Southport, Cheshire
I W Dymock

Correspondence to:
Dr A B Bomford, Institute of Liver Studies, King's College School of Medicine and Dentistry, Denmark Hill, London SE5 9PJ.

prognosis or clinical features.⁶ At the time, reports of the effectiveness of venesection in removing excess tissue iron and producing clinical improvement were limited to isolated case studies or small series.^{10,11} In 1969 an analysis of patients who had been venesected to iron deficiency by the removal of 15–20 g iron, was published jointly with the Department of Medicine, Royal Free Hospital.¹⁰ This compared survival in 40 treated patients with a group of 18 untreated patients whose records were available at King's College Hospital and who had presented before the idea had taken hold that depletion of excess tissue iron could be beneficial. Although treatment was associated with a decrease in the calculated five year mortality from 67% to 11% the groups were not strictly comparable with respect to age of presentation and clinical complications of iron overload. A subsequent analysis of a larger patient series in which the age of presentation of treated and untreated groups was the same, survival was again found to be significantly improved by treatment.¹¹ In this latter study a correction was made for clinical differences between the two groups, such as the presence of diabetes mellitus and cirrhosis, which could have weighted survival in favour of the treated patients. Both these studies differentiated clearly between those clinical features that could be expected to improve with treatment – that is, pigmentation, hepatomegaly, cardiac failure, and diabetes in about a third of patients, from those which were irreversible – arthritis, hypogonadism and portal hypertension. In spite of reports of reversal of cirrhosis after iron depletion¹² we consider it a rare event and in the majority of patients cirrhosis remains many years after the complete removal of iron with the attendant increased risk of hepatocellular carcinoma.¹³

Nearly 80% of the patients in our series had developed cirrhosis by the time of presentation. More recently, increased numbers of patients have been diagnosed at the precirrhotic stage and analysis of a large group of German patients has shown that these patients have a normal life expectancy whereas cirrhotic, diabetic, and the more heavily iron loaded patients have a reduced life expectancy.¹⁴ The high risk of hepatocellular cancer, over 200-fold increase, was noted in this study.

ARTHRITIS AND HAEMOCHROMATOSIS

The recognition that arthritis frequently occurs in patients with genetic haemochromatosis is one of the important clinical additions to our knowledge in recent years and this arthritis can be the cause of much pain and disability. Since the first case reports by Schumacher in 1964,¹⁵ we have, over the past 20 years, made a detailed study of the specific features, the pattern of joint involvement and how it develops.^{16–19}

Arthritis was found in more than half the patients, and the presence of widespread chondrocalcinosis as a result of the deposition of calcium pyrophosphate was a distinguishing feature. One of the earliest recognisable signs was often bony swelling of the second and third metacarpophalangeal joints more marked in the

right hand. Flexion of these joints was limited and radiographs showed cystic changes in the metacarpal heads, sometimes with infracturing of the cysts on to the joint surface which could be irregular. There was narrowing of the joint space but not erosions or ulnar deviation of the phalanges. We agree with Resnick that this pattern and radiological features of arthritis in the hands frequently differs from that seen in other cases of pyrophosphate arthropathy.²⁰

The chondrocalcinosis was most frequently seen in the knees both in the fibro and hyaline cartilage, but it was also found in the wrists, symphysis pubis and other large joints. Together with Eric Bywaters we have studied the spines at autopsy from six patients and observed calcium pyrophosphate in the outer part of the intervertebral discs and in the ligamentum flavum.²¹ This was not associated with back pain but the radiographs gave superficial resemblance to ankylosing spondylitis.

Arthritis starting in the hands has been reported as one of the presenting features of genetic haemochromatosis in some patients^{22,23} but as the disease progresses, arthritis of the hips can be a major problem causing severe disability, which in many cases can be relieved by arthroplasty. In our 20 year study of 112 patients, 10 had hip surgery; also noted were characteristic radiological and histological features in which there was unusual lifting of the hyaline cartilage off the underlying bone in the hip joint.²⁴

In addition to this destructive metabolic arthritis which can lead to damaged joints, it is important to stress that sometimes an acute crystal synovitis or pseudogout caused by deposits of calcium pyrophosphate in the cartilage can occur. These inflammatory episodes have been known to lead to the mistaken diagnosis of rheumatoid arthritis.

The presence of iron in the synovium was first recorded by Sheldon.³ We have examined synovium obtained by needle biopsy in 22 patients and at necropsy in five and related the findings to the clinical and radiological evidence of arthropathy and to the measurement of liver and total body iron stores.²⁵ At the time of the biopsy the iron did not relate directly with the joint changes, but correlated well with liver iron and the iron burden removed by venesection therapy.

The incidence, severity and radiological progression of the arthritis and chondrocalcinosis was recorded over 10 years in 18 patients. Chondrocalcinosis did not get less or disappear, despite adequate venesection therapy but spread to involve other joints and became more severe, being found in seven patients initially and in 13 at the end of the study. The knees and wrists were the joints most frequently involved.²⁶

Iron salts act as nucleating agents for crystal formation and it has been shown that low concentrations of ferric salts promote crystal growth. The amount of pyrophosphate needed for crystal formation was reduced to 25% of that required in the absence of iron.²⁷ McCarty has also attempted to show that the removal of such crystals may be inhibited by iron.²⁸ He induced haemosiderosis in rabbit knees by injecting autologous blood and measured the rate of clearance of ⁸⁵Sr and ⁴⁵Ca from the joints. This was significantly reduced in

haemosiderotic joints suggesting inhibition of intracellular pyrophosphatase and confirming earlier work that the erythrocyte form of the enzyme was inhibited by ferrous ions.²⁹

Additional evidence supporting the relationship between the arthritis of haemochromatosis with iron overload is that a similar type of joint damage has been reported in patients with secondary spherocytosis.³⁰ Age is also an important factor; we found that the arthritis was significantly more frequent in those patients whose first symptom of haemochromatosis appeared after the age of 50 years. This age factor is also seen in the pyrophosphatase arthropathy that occurs in patients with primary hyperparathyroidism.

HAEMOCHROMATOSIS GENE

The most important advance in understanding the pathogenesis of genetic haemochromatosis came in 1975 with the report by Simon and colleagues that the frequency of the HLA antigen A3 was increased in a group of unrelated patients.^{31, 32} This association was confirmed by an analysis of our own patients³³ and by reports from several other centres.³⁴ Pedigree analysis substantiated the conclusion of earlier studies of the iron loading phenotype that the disease was recessively transmitted.² The discovery of linkage of the haemochromatosis allele to the HLA locus has had an enormous impact on the management of the condition and in clarifying the problem of iron loaded alcoholic patients, as well as localising the disease gene to the short arm of chromosome 6. The haemochromatosis gene being tightly linked to the HLA locus is inherited with the particular HLA haplotype on which it is carried. In family studies, subjects who have the same two HLA haplotypes as the index patient will have homozygous genetic haemochromatosis, those with one shared haplotype are heterozygotes, while subjects carrying neither of the affected haplotypes are normal homozygotes.^{34, 35} The only exceptions are offspring of heterozygous-homozygous matings and patients in whom recombination of genetic material has taken place. While there is strong evidence that the disease gene is closely linked to the region encoding the HLA class I genes, the location of the disease allele relative to the A and B loci is not known. One approach to identifying the gene is to examine restriction fragment length polymorphism of DNA using probes to HLA class I genes, other genes close to this region and to overlapping sequences of adjacent DNA.^{36, 37}

The prevalence of genetic haemochromatosis has recently been estimated from the results of a study in the United States of over 11 000 blood donors.³⁸ A transferrin saturation of 62% and above was used to recognise the homozygous genotype, because this has previously been shown to recognise 92% of such cases.³⁹ Serum ferritin was found to indicate only those with heavy iron loading. The frequency of homozygosity was calculated to be 0.0045, corresponding to a gene frequency of 0.067. These figures are similar to those derived from pedigree analyses from several centres using a combination of HLA typing and biochemical assays for

transferrin saturation and serum ferritin concentration.³⁴ The demonstration of tight linkage between the HLA locus and the haemochromatosis gene has also been used to show that iron loaded alcoholic patients are homozygous for the gene and do not differ significantly from non-drinking homozygotes.⁴⁰

FERRITIN AND TRANSFERRIN RECEPTOR: EXPRESSION AND REGULATION

Transferrin, its receptor and ferritin provide an essential sequence for making iron available to the cell.⁴¹ Disturbances in the structure, function and regulation of this sequence have been repeatedly sought in genetic haemochromatosis. An early report claiming that the ferritin phenotype was abnormal in genetic haemochromatosis⁴² was not confirmed in studies from this laboratory.⁴³ Serum ferritin, however, has been found to be a reliable means of assessing tissue iron that can be mobilised by venesection⁴⁴ and is widely used in detecting affected relatives in family screening studies.⁴⁵ Functional studies have used peripheral blood monocytes because of their accessibility and because of the observation that the cells of the reticuloendothelial system are spared in the iron loading process in genetic haemochromatosis and may be unable to retain stored iron.⁴⁶ Ferritin synthesis and transferrin receptor function have been shown to be normal.⁵⁴ The only site at which an abnormality has been consistently observed is the epithelial cells of the duodenum. This was first noted 25 years ago by Crosby who observed paucity of collections of ferritin iron cores in duodenal biopsies studied by electron microscopy.⁴⁷

During the last 10 years the genes encoding the ferritin H and L chains, transferrin and its receptor have been cloned and sequenced and their chromosomal locations determined. This has important implications for our understanding of genetic haemochromatosis. First, apart from a ferritin pseudogene, none of the genes localise to the short arm of chromosome 6 indicating that genetic haemochromatosis is not caused by structural abnormalities of these genes. Second, examination of the ferritin subunit mRNAs revealed a highly conserved sequence of 28 nucleotides in the 5' untranslated region in amphibia, birds, and man.⁴⁸ Structural analysis of this region predicted that it would assume a stem loop configuration in the form of a paired stem supporting a single stranded loop comprised of the bases CAGUGU.⁴⁸ Analysis of the transferrin receptor mRNA revealed a similarly conserved region, although in this case there were important differences in that five stem loop structures were found in the 3' untranslated region.⁴⁹ The importance of these structures which have been termed iron responsive elements,⁵⁰ is that they provide the cisacting component of a translational control mechanism that allows for the coordinate regulation by cells of the transferrin receptor (iron uptake) and ferritin (iron storage). The transacting element of this homeostatic mechanism is a cytosolic protein which in the absence of iron binds to iron responsive elements to suppress ferritin mRNA translation⁵¹ and increase translation of transfer-

rin receptor mRNA by increasing its stability.⁵² Conversely, when iron is abundant, the iron responsive element binding protein dissociates from iron responsive elements so that ferritin mRNA translation proceeds and iron is stored in newly synthesised ferritin; transferrin receptor mRNA rapidly decays, the protein becomes less abundant and iron uptake by the cell is decreased. How the iron responsive element binding protein senses iron concentrations or what form the iron takes is not yet known; *in vitro* experiments suggest that the redox state of the protein is important in determining its binding to mRNA.⁵⁰

The expression of ferritin and the transferrin receptor can be used as an indicator of the iron status of the cell and their study provides a means of investigating homeostatic mechanisms. In an investigation of cellular iron homeostasis in genetic haemochromatosis we found that both proteins were appropriately regulated in the liver.³⁷ This is illustrated by the finding that in untreated patients with increased concentrations of tissue iron, receptor protein was undetectable, using an antireceptor monoclonal antibody. In venesected patients with normal iron stores, the receptor was re-expressed, being found in parenchymal and non-parenchymal cells in a cytoplasmic and membranous distribution.⁵³ In contrast, the transferrin receptor was inappropriately regulated in the villus enterocytes of the duodenal mucosa.⁵⁴ At this site, in spite of greatly increased iron stores the receptor was expressed with the same intensity as in controls. In patients with β thalassaemia and transfusional iron overload, however, the enterocyte receptor was clearly down regulated.⁵⁴ This observation is significant because iron absorption is increased at this site in both conditions as a result of a primary genetic disorder of iron metabolism in genetic haemochromatosis and because of increased erythroid drive in β thalassaemia. Both groups showed down regulation of the receptor in the gastric mucosa which is an appropriate response considering the greatly increased iron stores. These findings are supported by recent reports in which ferritin expression, determined by radioimmunoassay⁵⁵ and immunocytochemical⁵⁶ methods, was reduced in duodenal enterocytes in genetic haemochromatosis. It appears that although coordinate regulation of the two proteins is maintained in the enterocyte in genetic haemochromatosis their pattern of expression indicates that this cell type is iron deficient. This paradox returns us to Crosby's original observation of nearly 30 years ago when he found a paucity of ferritin iron cores in enterocytes.⁴⁷ It is not clear at present at which point(s) in the regulatory pathway an abnormality lies nor how this abnormality relates to the haemochromatosis gene on chromosome 6; it is possible to rule out a structural defect of the iron responsive element binding protein as the gene encoding this protein localises to chromosome 9.⁵⁰

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