12.7.1 Hereditary haemochromatosis 2098 William J.

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Wilson’s disease and Menkes’ disease 2115 Michael L. Schilsky and Pramod K. Mistry 12.7.1 Hereditary haemochromatosis William J.H. Griffiths and Timothy M. Cox ESSENTIALS Hereditary haemochromatosis syndromes are inherited disorders whereby inappropriate absorption of iron by the small intestine leads to iron deposition in the viscera, endocrine organs, and other sites, causing structural injury and impaired function. The most common form is classical adult (HFE-related) haemochromatosis, but other forms are recognized. Extended genetic platforms are increasingly used for specific diagnosis and noninvasive methods are increas- ingly used to evaluate hepatic damage. The mainstay of treatment is venesection although iron chelation therapy is an emerging oral alternative. Unravelling the molecular genetics of haemochroma- tosis is underpinning promising new therapies for disorders of iron homeostasis. Classical adult (HFE-related) haemochromatosis Aetiology and pathogenesis—inherited as a recessive trait and due to mutations in the major histocompatibility complex class I-related HFE gene that appear to reduce liver production of hepcidin. The principal mutant allele of HFE, designated C282Y, is carried by ap- proximately 1 in 10 individuals of European ancestry, hence around 1 in 200 are homozygotes, usually with biochemical abnormalities of iron storage that may lead to full-blown clinical

haemochromatosis. Clinical features—expression of disease may range from slight abnormalities of blood parameters that reflect iron metabolism to the established clinical syndrome of cutaneous pigmentation, car- diomyopathy, endocrine failure (especially diabetes mellitus and hypogonadism), arthritis, and pigment cirrhosis. Typical symptoms include fatigue, arthralgia, abdominal pain, and loss of libido. Diagnosis—usually established by demonstrating abnormalities of iron metabolism, with fasting serum transferrin iron saturation above 55% in males and 45% in females along with elevated serum ferritin concentration. Molecular analysis of the HFE gene, in particular for homozygosity for the C282Y allele, is confirmatory. Management and prognosis—this is directed to the removal of iron by phlebotomy, typically 500 ml of blood each week, until the serum ferritin concentration is reduced to within the low normal range, after which the frequency of phlebotomy is reduced. The oral iron che- lator deferasirox has shown promise as an alternative to venesection in phase II trials. Specific treatment may be required for estab- lished end-organ failure. Patients with advanced fibrosis or cirrhosis should undergo 6-monthly surveillance by ultrasonography and serum α-fetoprotein estimation for early detection of hepatocellular carcinoma. The main causes of death in untreated patients are hepatocellular failure and carcinoma of the liver. Intervention prior to the onset of cirrhosis or diabetes avoids the deterioration in sur- vival associated with late presentation. Family members—first-degree relatives should be offered screening. Asymptomatic subjects in whom molecular analysis of the HFE or non-HFE iron overload genes indicates a genetic predispos- ition to the disease require re-evaluation by clinical and biochemical testing at intervals of between 2 and 5 years. Symptomatic subjects or those with significant iron indices (i.e. ferritin >750 µg/litre) should be considered for immediate treatment. Introduction Pathological storage of iron The body contains about 4 g of iron, 3 g of which is complexed with haem to form haemoglobin, myoglobin, and the cytochromes. The nonhaem storage compartment, which consists of ferritin and its proteolytic degradation product haemosiderin, represents up to 0.5 g of elemental iron in adult women and slightly more than 1 g in adult men. Excess storage of body iron (iron overload) is asso- ciated with an increase in hepatic iron concentrations and of the surrogate biomarker, serum ferritin. Minimal iron storage occurs when more than 1.5 g of total body iron is present. This is reflected in a hepatic iron concentration of approximately 30 µmol/g of tissue 12.7 Trace metal disorders

12.7.1 Hereditary haemochromatosis 2099 with a serum ferritin level of usually less than 250 µg/litre. Moderate iron storage disease is reflected by a serum ferritin of approximately 500 µg/litre. Under these circumstances, the hepatic iron concentra- tion rises to 100 µmol/g. Severe iron storage disease (>5 g of storage iron) is shown by a hepatic iron concentration of over 200 µmol/g liver tissue, with a serum ferritin level of at least 750 µg/litre. Under these circumstances, tissue injury with impaired function is almost invariably present. Clinical subtypes of haemochromatosis Adult haemochromatosis The familiar form of haemochromatosis is the classical adult type, which typically presents in middle age and is usually expressed in men. The disorder is inherited as a recessive trait and is due to mu- tations in a gene, HFE, that maps to the short arm of chromosome 6 in close apposition to the HLA class I loci of the human major histo-compatibility complex (MHC). Expression of iron storage disease in individuals carrying mutations in the HFE gene is very variable and is influenced by several environmental and sexual factors, as well as emerging genetic modifiers. Mutant alleles of the HFE gene that predispose to adult-type haemochromatosis are widespread and fre- quent in populations of northern European origin. There is evidence from haplotype analysis that a single mutation arose on an ances- tral chromosome 6 and spread throughout this population, prob- ably as a result of the migration of the

Vikings from Scandinavia. The disease occurs throughout the world as a result of intermarriage but is at its highest frequency in France, Germany, Great Britain, Ireland, Northern Italy, Scandinavia, Spain, and Eastern Europe as far as European Russia. Colonization has led to its appearance in all populations of the United States of America and in Australasia, and for the same reason hereditary adult-type haemochromatosis also occurs in South America. Classical adult-type haemochromatosis (HFE-related haemo- chromatosis) is a slowly progressive disease affecting the liver, endocrine system, heart, and joints; it is often only diagnosed when irreversible tissue injury has occurred. The condition predisposes to the development of primary carcinomas of the liver. A rare genetic form of adult haemochromatosis occurs in patients homozygous for mutations in the transferrin receptor 2 (TFR2). This form has been described mainly in southern Europeans and is termed type 3 haemochromatosis using the Online Mendelian Inheritance in Man (OMIM) classification (Table 12.7.1.1). The phenotype resem- bles HFE-related haemochromatosis (type 1) although it is gener- ally more severe and presents at a younger age. The TFR2 protein is mainly expressed in the liver and has a lower affinity for iron uptake than the ubiquitous transferrin receptor. Latterly, a role for TFR2 in erythrocyte production has been suggested whereby it forms a component of the erythropoietin receptor complex and may act as an iron sensor. Identification of the protein responsible for iron transport across the basolateral surface of enterocytes provided a candidate for a re- cently recognized atypical form of haemochromatosis. The iron ex- porter in question has been termed ferroportin and appears to also control iron release from hepatocytes and, importantly, macrophages. Single missense mutations in the SLC40A1 mutations which en- code ferroportin are associated with a specific dominantly inherited phenotype. Haemochromatosis due to heterozygous SLC40A1 muta- tions has been coined ferroportin disease and also referred to as type 4 haemochromatosis. The disorder is typified by a raised ferritin level with normal or low transferrin saturation and a tendency for anaemia with poor venesection tolerance. Not restricted to white people, the condition is recognized in Asians and a unique and common poly- morphism (p.Q248H) in Southern African populations may con- tribute to the indigenous iron overload observed. Iron loading in type 4 haemochromatosis occurs predominantly within the reticuloendothelial system with splenic uptake visible on MRI (Fig. 12.7.1.1). On liver microscopy, Kupffer cells are iron- laden with relative sparing of hepatocytes. SLC40A1 mutations re- sult in iron trapping within macrophages and it has been proposed that the reduced availability of plasma iron either directly or via an ensuing anaemia drives increased intestinal absorption. As well as the phenotype described earlier, where spillover of iron into hepato- cytes is minimal and disease course benign, a second ‘nonclassical’ phenotype, less commonly observed, is characterized by an elevated transferrin saturation, hepatic parenchymal iron deposition, and liver disease.

Juvenile haemochromatosis Since the identification of adult iron storage disease by several European physicians during the 19th century, a similar disease has Table 12.7.1.1 Inherited disorders of iron storage Disorder OMIM number Locus Gene/protein Atransferrinaemia 209300 3q21 Transferrin Acaeruloplasminaemia 604290 3q23–q25 Caeruloplasmin Haemochromatosis (type 1) (adult) 235200 6p21.3 HFE Haemochromatosis (type 2A) (juvenile) 608374 1q HJV/haemojuvelin Haemochromatosis (type 2B) (juvenile) 606464 19q13.12 HAMP/hepcidin Haemochromatosis (type 3) (adult) 604250 7q22 Transferrin receptor 2 Haemochromatosis (type 4) (adult, dominant) 606069 2q32 SLC40A1/ferroportin Haemochromatosis (type 5) (adult, dominant) 615517 11q12.3 FTH1 Haemochromatosis (neonatal) 231100 Unknown Unknowna a Autosomal recessively inherited in only a few families.

section 12 Metabolic disorders 2100 been recognized in children and young adults who may develop iron storage disease of a more severe character, now designated juvenile haemochromatosis (Fig. 12.7.1.2). This is defined as iron storage disease occurring before the age of 35 years. It evolves rapidly, typ- ically affects the heart and endocrine system, and causes infantilism and hypogonadism, as well as life-threatening cardiac arrhythmias. Juvenile haemochromatosis is inherited as a very rare recessive trait in which there is an increased frequency of consanguinity among the parents of affected subjects. Juvenile haemochromatosis resem- bles the severe iron storage disease associated with the iron-loading anaemias, such as β-thalassaemia. Juvenile haemochromatosis af- fects males and females equally—an observation that reflects the overwhelming nature of the iron homeostatic defect. Iron overload develops before the modifying effects of menstruation and dietary factors supervene. The genetic basis of juvenile haemochromatosis has been elu- cidated and revealed key proteins involved in iron metabolism. Most cases have been associated with mutations in the HJV gene on chromosome 1q, encoding the protein haemojuvelin; the homozygous mutation G320V accounts for approximately 50% of HJV-associated or type 2A haemochromatosis. Haemojuvelin is ex- pressed predominantly by hepatocytes but also in cardiac and endo- crine tissues. The common mutations abrogate expression at the cell surface of hepatocytes where haemojuvelin may act as a coreceptor for bone morphogenetic protein as part of an intracellular signalling mechanism for synthesis of the peptide hepcidin. A smaller number of cases (type 2B haemochromatosis) are explained by mutations in the HAMP gene on chromosome 19 which codes directly for hepcidin. In mice with disruption of murine HAMP, or its promoter sequence, hepatic iron loading occurs. Conversely, overexpression of murine HAMP results in anaemia in keeping with hepcidin sup- pressing intestinal iron absorption. Indeed, HAMP overexpression overrides the effect of the C282Y mutation on dietary iron up- take and prevents haemochromatosis in HFE-deficient mice. This finding supports the more severe phenotype observed in this form of juvenile disease compared with HFE-related haemochromatosis. Hepcidin is thought to play a central role in iron homeostasis and current models are premised on hepcidin acting as a putative iron- regulatory hormone with effects on end organs, including the intes- tine and the monocyte/macrophage system. Neonatal haemochromatosis Neonatal haemochromatosis is a newly identified syndrome of un- certain cause, characterized by congenital cirrhosis or fulminant hepatitis associated with the widespread deposition of iron in hep- atic and extrahepatic tissues. Approximately 100 cases of neonatal haemochromatosis have been reported. Neonatal haemochroma- tosis occurs in the context of maternal disease (including viral in- fection) and in the presence of maternal antinuclear factor, as a complication of metabolic disease in the fetus, and sporadically or recurrently, without overt cause, in siblings, including maternal half-siblings. This latter observation indicates that conception by Fig. 12.7.1.1 Magnetic resonance imaging (T2 weighted) demonstrating iron overload in the liver and spleen of a patient with classical ferroportin (FPN) disease (top panel). This 48-year-old male presented with a serum ferritin of 3000 μg/litre, transferrin saturation of 21%, and was heterozygous for the W158C mutation of the SLC40A1 gene. For comparison is a normal control film (bottom left panel) and a patient with HFE-related haemochromatosis (bottom right panel) demonstrating low signal in the liver only as iron loading here is predominantly in hepatocytes rather than the reticuloendothelial system.

 the use of sperm donors in women who have had a previously af- fected infant should not be recommended. Although infants with neonatal haemochromatosis die of liver disease shortly after birth, there are many instances where survival

is associated with a com- plete recovery and thereafter normal growth and development with no signs of abnormal iron metabolism. Recently, it has been shown that the outcome of pregnancies at risk for neonatal haemo- chromatosis is improved by treatment of the mother with high-dose intravenous infusions of pooled human immunoglobulin, thereby suggesting the operation of a humoral factor and a significant overlap with gestational alloimmune liver disease. However, the in- volvement of genetic determinants, possibly of paternal origin, in an alloimmune response has not been excluded. In other pedigrees, although neonatal haemochromatosis appears to have a clear her- editary basis, no predictive genetic test is yet available to inform the outcome of at-risk pregnancies for this devastating disease. Prevalence and epidemiology Juvenile and neonatal haemochromatosis are rare disorders that occur sporadically, but hereditary adult haemochromatosis is widely disseminated and of global importance. Removal of toxic iron by re- peated venesection improves the outcome for adult haemochroma- tosis. If this treatment is instituted before irreversible tissue injury occurs, venesection may restore health and a normal life expectancy. (a) (b) (c) (d) Fig. 12.7.1.2 Juvenile cardiac haemochromatosis. Histological appearances of explanted heart from a 27-year-old woman (patient 1) who underwent orthotopic cardiac transplantation for refractory heart failure; hypogonadotrophic hypogonadism had been present for 8 years. Whole-mount sections of left ventricular wall stained with haematoxylin and eosin (a) or (b), Perls’s ferrocyanide reagent, show iron deposits principally affecting sub-epicardium and peripheral one-third of myocardium. High-powered microscopic views (×400: haemotoxylin and eosin, (c); Perls’s reagent, (d)) show widespread punctate aggregates of stored iron in cardiac myocytes and interstitial cells. Note the absence of inflammatory changes. Although iron was most abundant in the sub-epicardial zone, microscopic views show that degenerating myocytes containing iron deposits, some with hyperchromatic nuclei, were distributed throughout the myocardium. Reproduced from Kelly AL et al. (1998). Hereditary juvenile haemochromatosis: a genetically heterogeneous life-threatening iron-storage disease. QJM, 91, 607–618 with permission from Oxford University Press.

section 12 Metabolic disorders 2102 For these reasons, there has been much discussion about the early recognition of iron storage disease by the introduction of population-based screening programmes, using genetic testing or phenotypic biochemical screening methods, that can be applied to communities at risk. In European populations, around 1 in 10 individuals carries one copy of an allele of the HFE gene that predisposes to iron storage disease, and between 1 in 100 and 1 in 400 people in these popula- tions are homozygotes or compound heterozygotes with biochem- ical abnormalities of iron storage that may lead to full-blown clinical haemochromatosis. Thus, the mutant allele, designated C282Y of HFE, which is the principal determinant of iron storage disease, occurs at polymorphic frequency and is one of the most common genetic abnormalities leading to an autosomal recessive disease in populations of northern European origin. In European patients with iron storage disease due to hereditary haemochromatosis, the fre- quency of homozygosity for the C282Y HFE allele ranges from about 35% in southern Italy to more than 90% in the British Isles, including Ireland. In Australia, homozygosity for C282Y occurs in almost 100% of patients with hereditary haemochromatosis. However, as discussed later, although useful for diagnosis, homozygosity for the C282Y mu- tation of HFE is not tantamount to a diagnosis of established iron storage disease nor, therefore, of clinical haemochromatosis. Clinical expression of haemochromatosis is highly dependent on age and it is very rare for there to be detectable disease in adults below the age of 20 years. As clinical disease is much more common in men than women, it is likely to reflect environmental factors and the modification of disease expression due to blood

loss associated with menstruation and the investment in pregnancies, as well as the comparatively reduced dietary complement of iron in women. Other environmental factors, particularly the consumption of alcohol, ap- pear to interact with predisposing genetic factors to induce the clin- ical expression of iron storage disease in C282Y homozygotes. Most patients with the disease develop symptoms at, or above, the age of 40 years. However, studies of iron metabolism by biochemical meas- urements or tissue biopsy may reveal early evidence of iron storage in the long presymptomatic phase of this condition. With greater awareness of the diverse clinical manifestations of adult type heredi- tary haemochromatosis, detection on the basis of early symptoms, for example arthralgia and fatigue, may be possible. Thus, there is a marked disparity in populations in which C282Y homozygosity is prevalent and the frequency with which symptomatic haemo- chromatosis is diagnosed. Phenotypic expression of disease For epidemiological purposes, since there is no internationally agreed case definition of haemochromatosis, caution is needed in interpreting claims that haemochromatosis is the most common inherited disorder affecting European peoples. Phenotypic expres- sion of the disease may range from the established clinical syn- drome (which includes cutaneous pigmentation, cardiomyopathy, endocrine failure—especially diabetes mellitus and hypogonadism, arthritis, and pigment cirrhosis) to a slight abnormality of blood parameters that reflect iron loading—elevated serum transferrin iron saturation and serum ferritin measurements. Such studies that are available to determine the penetrance and expressivity of the haemochromatosis gene have provided widely varying re- sults in different populations. In Australia, where the mean intake of iron in the diet appears to be much greater than in the average European population today, most middle-aged male C282Y homo- zygotes appear to express at least one clinical manifestation of iron storage disease. Similarly, a study of homozygous relatives (princi- pally siblings) within pedigrees known to have haemochromatosis suggest that about one-half of the men over 40 years of age, and about one in six of the women over 50 years of age, have at least one haemochromatosis-related clinical disorder. This latter survey, conducted in the United States of America, suggests that an im- portant proportion of homozygous relatives of patients with estab- lished haemochromatosis, especially men, have conditions such as cirrhosis and arthropathy, as well as abnormalities of serum liver- related tests that are not detected by spontaneous clinical referral. Many reports of disease expression in haemochromatosis may, however, be questioned because of the prevalence of cosegregating genes within affected pedigrees, as well as early household environ- mental factors common to siblings that may predispose to disease expression. Studies in mice support this explanation, since it has been shown that several independent genetic determinants control the extent of iron loading observed in mouse models of iron storage disease generated by targeted disruption of the murine homologue of the HFE gene. In contrast, surveys conducted in outbred popu- lations, for example, in Jersey, show a great disparity between the predicted frequency of homozygosity for C282Y and the number of recorded cases with the disease attending local hospitals. These latter studies may reflect the underdiagnosis of haemochromatosis, and an inability to bring together the unitary clinical manifestations of the disease into a unifying diagnostic category. However, widely differing degrees of disease penetrance almost certainly account for the apparent shortfall of diagnosed cases in populations at risk. At present, no clear data in large unbiased population surveys are available to assess disease penetrance and the modifying effects of lifestyle factors such as alcohol, nutrition, and diet, as well as preg- nancy and menstruation, that are likely to influence the effects and rate of iron storage in human C282Y homozygotes. Mortality figures show that death is rarely attributed to hereditary haemochroma- tosis in populations at risk. This fact contrasts starkly with the well- established known

complications of the full clinical syndrome, in which early death results from cirrhosis of the liver, hepatocellular carcinoma, endocrine failure, or cardiac complications. In a North American study of more than 41 000 individuals at- tending a health appraisal clinic, no evidence of an increased fre- quency of symptoms was identified in those genetically predisposed to iron storage disease. The only significant clinical history iden- tified in the at-risk group was that of hepatitis or prior liver com- plaints. Only one of the 152 identified C282Y homozygotes had signs and symptoms of adult haemochromatosis. This provoca- tive report, indicating a very low clinical penetrance (<1%) of the haemochromatosis genotype in an unusual group of adults over the age of 26 years, raises important questions about the introduc- tion of mass population screening for this potentially treatable iron storage disease by genetic or even biochemical methods. However, the high prevalence of impotence, joint symptoms, chronic fatigue, and other complaints such as cardiac arrhythmias in the study group as a whole, raises disturbing questions about the valid application of this report to other populations. It is perhaps not surprising that in a group where, on average, more than 40% complained of a general limitation of their health and/or joint symptoms, and in which more

 than 35% of the male participants scored positively on symptom en- quiry about impotence, a significant contribution from predisposing haemochromatosis alleles could not be identified. Nonetheless, this large study raises key questions about the utility of screening for adult haemochromatosis as a genetic disease. To provide evidence for screening in haemochromatosis, other population surveys which address the morbidity and mortality of individuals harbouring disease alleles, have been attempted. For ex- ample, the effects of iron storage in C282Y homozygotes were re- ported in a comprehensive study of about 30 000 individuals aged between 40 and 69 years from Melbourne, Australia. Of 203 subjects found to be homozygous for the C282Y allele, ‘iron overload- related disease’ occurred in 28% of the men and 1.2% of the women. Longitudinal studies have shown that iron overload in C282Y homo- zygotes is not always progressive and indeed may recede in some cases. In the Melbourne study, follow-up for an average of 11.4 years showed that the hazard ratio for death from any cause was 1.04 (con- fidence limits 0.67–1.62) in C282Y homozygotes compared with subjects who did not harbour any copy of this mutant HFE allele. Not all individuals with mild iron loading require treatment and this clearly has implications for the introduction of mass population screening programmes for HFE-related haemochromatosis. Aetiology, pathophysiology, and pathology Young patients with haemochromatosis absorb an increased amount of dietary iron in their upper intestine compared with normal con- trol subjects. In established iron storage disease, iron absorption continues at a rate that is inappropriate for the level of iron stores, as reflected by serum ferritin and tissue iron determinations. In the absence of an effective excretory pathway, the increased ab- sorption of iron by the intestine leads to a progressive accumulation of the metal in the parenchymal cells of the liver, heart, endocrine glands, and specialized type B synoviocytes. Excess iron accumu- lates in the pancreas where it is found in both acinar and endocrine cells of the islet, although there is a particular predisposition in the early phases of iron loading to the islet β-cell. Iron also accumulates to toxic levels in the gonadotrophs of the anterior pituitary gland, leading to hypogonadotropic hypogonadism. Iron may accumulate in the adrenal gland, where it is concentrated particularly in those cells that secrete aldosterone, in the zona glomerulosa. Iron accumu- lates in the cardiac myocytes and conducting tissue of the heart, in the chief cells of the parathyroid, and in parenchymal cells throughout the body. The consequences of toxic iron storage include diabetes mellitus, cirrhosis of the liver, cardiomyopathy with or without

con- duction defects, hypogonadism, arthritis with chondrocalcinosis, adrenocortical deficiency, and, rarely, hypoparathyroidism. Evidence for the intrinsic toxicity of iron in haemochromatosis is provided by the regression of the pathological changes following measures taken to reduce iron, for example, the use of iron chelators and removal of body iron by venesection. Venesection stimulates the mobilization and removal of iron from the storage compartment by increasing the demand for red cell production in the bone marrow. Mechanism of iron toxicity High concentrations of iron salts are toxic to cultured cells. The ad- ministration of iron chelates to experimental animals has induced diabetes with iron loading in the liver and pancreas, as well as the generation of (renal) carcinomas. Injections of iron salts induce local sarcomas in experimental animals, with evidence of species sus- ceptibility. In humans, sarcomas or carcinomas have arisen, albeit rarely, at sites of therapeutic injections of iron, and it is possible that the complications of silicosis and asbestos exposure result from the complement of iron associated with these particulates. A wealth of indirect but corroborative evidence indicates that the primary effect of excess free iron is to promote the formation of oxygen free rad- icals, which mediate the damage to cells and tissues that is observed in iron storage disease. In established haemochromatosis, the iron- binding capacity of plasma transferrin may be exceeded, so that a proportion of the iron present in the blood remains reactive as a low molecular weight species only loosely attached to plasma proteins. Nontransferrin iron in human plasma stimulates the peroxidation of unsaturated lipids and can form reactive complexes that react with DNA, thus suggesting a mechanism for genome toxicity and car- cinogenesis related to iron overload. Iron is highly electroreactive, and coupling of the Fenton and Haber–Weiss reactions leads to the formation of hydroxyl radicals as a result of the catalytic interactions between superoxide and ferric ions. Tissues with significant iron storage show peroxidative injury in membrane lipid fractions. The lysosomal compartment appears to be particularly suscep- tible to iron-mediated damage, since iron in the form of ferritin and its degradation product haemosiderin accumulates within lysosomes to form the particulate ferrugineous granules known as siderosomes. In haemochromatosis, there is an increased activity of lysosomal enzymes with biochemical evidence of increased lyso- somal fragility indicating disruption of the integrity of the lysosomal membrane by iron. These changes revert to normal when the tissue iron is removed by venesection or by the use of specific iron chela- tors. It seems likely that the electrochemical reactivity of iron, and its particular propensity to accelerate the formation of oxygen free radicals, mediate its injurious effects on cell membranes, and on the nuclear genome, leading to cancerous change. However, des- pite great advances in the understanding of free-radical chemistry, the cause-and-effect relationship between iron storage and tissue injury is difficult to prove unequivocally. Nonetheless, much ex- perimental evidence points to the development of iron-mediated peroxidative injury of cellular membranes including the lysosome, as well as iron-mediated genotoxicity. Whatever their physiochem- ical basis might be, common mechanisms of iron toxicity clearly exist, since the pathological and clinical manifestations of all iron storage syndromes, including secondary haemochromatosis associ- ated with blood transfusion and the iron-loading anaemias, are al- most identical. Iron absorption In established haemochromatosis, where the burden of iron may increase body iron stores by at least 10-fold, measurements usu- ally show that iron absorption is within the normal range. Studies in young patients with rapidly progressive disease show a markedly increased absorption of iron. After depletion therapy, the rate of re- covery of iron stores is greatly enhanced for many years in patients with haemochromatosis, reflecting a persistent homeostatic abnor- mality in the retention of dietary iron. The daily absorption of be- tween 2 and 4 mg of iron over a period of 30 to 40 years accounts for the degree of iron loading that occurs at presentation in patients

section 12 Metabolic disorders 2104 with haemochromatosis, and compares with the normal absorption of 0.8 to 1.0 mg in men and in women, up to 2 mg daily. In effect, the abnormal absorption of iron represents a disturbed regulation of the final common pathway for the acquisition of iron from the environ- ment by the small intestinal mucosa. A report, describing the transplantation of intestine and liver from an HFE C282Y homozygote into a recipient without haemochroma- tosis, supports the small intestine as a key site of expression of the hereditary defect in adult haemochromatosis. The transplantation was associated with early iron overloading in the recipient, together with raised serum transferrin iron saturations—a phenomenon not observed in recipients of hepatic allografts obtained from donors later found to be homozygous for C282Y. In addition to recent evi- dence for a principal role of the key hepatic iron regulator hepcidin, prior studies in vitro and in vivo have suggested that there is a quali- tative abnormality of the uptake and transfer of iron from the intes- tinal lumen in patients with hereditary haemochromatosis that may represent a local HFE-dependent mechanism. Previous studies of mutant strains of mice with abnormalities of iron metabolism shed light on the iron-absorption mechanism. The identification of a single gene encoding the divalent metal transporter protein, DMT1, which is expressed in the upper small intestine and cells of the erythron, provided a molecular understanding of the iron deficiency and the microcytic anaemia that occurs in the mk/mk mouse strain. A single point mutation in the DMT1 gene interferes with the uptake of ferrous iron, since it disrupts the cognate transmembrane carrier protein mainly expressed in the mucosa of the proximal small in- testine, at the site of iron absorption, and in the erythroid precursor cells. Since in vitro studies of the expressed protein DMT1 show that it serves only as a carrier of divalent cations, and that interference with this pathway is sufficient to induce iron deficiency in a mam- malian species, ferrous iron uptake is probably the main pathway by which inorganic iron is acquired by the intestine. A variable, but often substantial, component of dietary iron is pre- sent in the organic form as haem. A full molecular understanding of the uptake and transfer pathways for the absorption of iron com- plexes to the porphyrias is also needed. Whole-body studies show that the absorption of the radiolabelled iron moiety of haemoglobin is enhanced in patients with adult-type haemochromatosis. Early studies in dogs have shown that, in the presence of proteolytic di- gestion products of globin, the haem complex is taken up intact by mucosal epithelial cells; free iron is then released by the action of intracellular haem oxygenases. The contribution of haemoglobin, myoglobin, and cytochromes to the iron overload in patients with haemochromatosis has not been quantified, but iron complexed to haem may well represent an important component of the total burden of body iron in symptomatic haemochromatosis. Recent identification of a putative transporter of haem iron on the brush border of mammalian duodenum is a key advance. Haem carrier protein 1 (HCP1) is up-regulated in response to iron deficiency and hypoxia, but its contribution to the dysregulated absorption of iron in hereditary haemochromatosis is unclear. The discovery of DMT1 immediately indicated a possible role for this important protein in human haemochromatosis. Overexpression of DMT1 mRNA had been identified in the intestinal mucosa of patients homozygous for the C282Y mutation with her- editary haemochromatosis, as well as in mice with iron storage dis- ease due to targeted disruption of the HFE gene. Contemporaneous studies in experimental animals identified a cytochrome-containing ferrireductase that is also localized to the intestinal brush-border membrane; this reductase was cloned from murine intestine and its human homologue subsequently identified. Expression of mu- cosal ferrireductase is specific to the apical microvillous membrane of mammalian intestinal mucosa and appears to be induced in re- sponse to nutritional iron deficiency. Mucosal ferrireductase re- duces ferric irons derived from the diet in the lumen for delivery to the DMT1

carrier protein, the final divalent pathway for inorganic iron uptake by intestinal mucosa. The mRNA species encoding murine DMT1 exist in two isoforms, one of which contains an iron-response element in its 3′ region, which would allow for the post-transcriptional regulation of protein expression controlled by intracellular iron status. A similar translational control of transferrin receptor expression has been described with the 3′ iron-response element in the mRNA encoding the human transferrin receptor. Since the isoform of DMT1 containing the iron-response element is preferentially expressed in the duodenum, it seems likely that changes in intracellular iron status regulate the expression of this carrier protein in iron deficiency and haemochromatosis. Studies in Hfe knockout mice indicate that the functional expression of the DMT1 protein is enhanced in the murine model of haemochroma- tosis, leading to increased iron uptake across the brush-border membrane of iron presented in the ferrous form. The action of rate- limiting ferrireductases at the brush-border membrane functionally coupled to DMT1 activity appears to explain the enhanced isotopic uptake of ferric iron in this model of haemochromatosis. Delivery of iron from enterocytes to the systemic circulation is mediated by the basolaterally expressed membrane protein ferroportin. Ferroxidases including hephaestin, encoded on the X chromosome and mutated in the sex-linked anaemic mouse sla, me- diate the transfer of iron across the intestinal mucosa in conjunction with ferroportin. It seems likely that, in hereditary haemochroma- tosis and physiological iron deficiency, post-transcriptional control of carrier proteins responsible for the uptake and transfer of iron occurs in the absorptive epithelium on the tips of the intestinal villi. Thus, homeostatic mechanisms in the proximal intestine operate to bring about the coordinated transfer of iron presented in the in- testinal lumen specifically to meet body requirements. Although functional interactions of HFE molecules with the identified com- ponents of the absorptive pathway have yet to be clarified, the HFE protein probably influences iron status in intestinal stem cells within the crypt. By these means, the expression of key absorptive proteins such as DMT1 may be imprinted, thus influencing their subsequent functional activity during ascent up the villus. How the antimicro- bial peptide hepcidin interacts with this machinery is uncertain. It is proposed that hepcidin is a negative stimulator of intestinal iron absorption and that hepatic synthesis increases in response to iron overload and inflammation but decreases in the iron-deficient state; hepcidin acts as a ligand for ferroportin whereby binding is thought to abrogate iron export into the circulation. Genetics and molecular biology The principal determinant of adult haemochromatosis has long been known to be tightly linked to the human MHC loci on the short arm of chromosome 6. In 1996, mutations in the HLA class I- linked haemochromatosis gene, HFE, were shown to predispose to the adult form of the disease. The most common mutation in

12.7.1 Hereditary haemochromatosis 2105 the nonclassical MHC class I HFE protein affects a key cysteine residue, which contributes to the formation of the conserved α- 3 helix that interacts cotranslationally with the β2-microglobulin protein. This association is required for the cell surface expression of all class I MHC molecules. Most patients with haemochroma- tosis are thus homozygous for a cysteine to tyrosine mutation at codon 282 (C282Y) of the nascent HFE protein. An increased fre- quency of this mutation, in association with the more common H63D missense mutation, also occurs in adult haemochromatosis (Fig. 12.7.1.5). A minor variant, affecting the same region in the α1 helix, S65C, is also occasionally associated with the C282Y al- lele in compound heterozygotes with adult iron storage disease and indeed a number of uncommon pathogenic variants in HFE have since been reported. Apart from reducing cell surface expression of the mutant C282Y polypeptide, and thus the abundance of this protein within a population of cytoplasmic vesicles, a functional explanation for the qualitative abnormality of iron metabolism

that characterizes haemochromatosis remains putative. Structural studies have pro- vided a molecular basis for an interaction between HFE and trans- ferrin receptor proteins. HFE may bind transferrin receptors, alter the affinity for the receptor to transferrin, and in turn affect the de- livery of transferrin-bound iron into cells. This interaction may be more specific in the case of TFR2, expression of which is restricted to locations such as the hepatocyte membrane; here, an iron- sensing role is postulated. The recently identified peptide hepcidin has become the focus of attention as the potential circulatory signal for body iron status with a key role in the disturbed iron homeostasis of hereditary haemo- chromatosis. Rather than the expected compensatory increase, hepcidin expression is paradoxically decreased and unresponsive in haemochromatosis caused by mutations in HFE, TFR2, and HJV. The proteins encoded by these genes are predominantly synthesized within the liver. Given that these types of haemochromatosis are qualitatively similar and differ mainly in severity, it has been ar- gued that these observations point to a common pathway in hep- atocytes which signals hepcidin production downstream. In support of this argument, the distribution of tissue iron in HFE-related haemochromatosis can be altered after inducing experimental overexpression of hepcidin. Many recent studies suggest that hepcidin production in hep- atocytes is linked to a haemojuvelin-dependent signalling pathway which is responsive to plasma iron saturations. Recent studies also suggest an effector function of hepcidin, whereby direct interaction with ferroportin at the cell surface results in its internalization and degradation with consequent reduced export of iron from macro- phages and enterocytes. SLC40A1 mutations appear either to abro- gate iron export function or interfere with the ability of membranous ferroportin to bind hepcidin; differential effects on cellular iron ex- port correlate with what appear to be the two discrete phenotypes observed in type 4 haemochromatosis. Serum hepcidin concentra- tions appear to be increased in ferroportin iron overload as opposed to other forms of haemochromatosis. In summary, a reduction in circulating hepcidin as a consequence of haemochromatosis gene defects, or directly as a result of HAMP gene mutations, appears to enhance plasma iron and subsequent tissue loading; it has been proposed that attenuated inhibition of macrophage and enterocyte ferroportin activity is responsible for this effect. An action of hepcidin as an effector molecule after hepatic signalling is central to the current favoured model of iron homeostasis (Fig. 12.7.1.6), but how the crypt programming hy- pothesis aligns with this model is not readily explained. Recent experiments are unravelling the detailed pathways that influence hepcidin synthesis within hepatocytes. A number of cell surface interactions result in downstream signalling of hepcidin synthesis via SMAD complexes (Fig. 12.7.1.7). Experimental work is focusing on possible novel molecular therapies—for example, interfering RNAs targeting TMPRSS6 have been shown to ameliorate iron over- load in mouse models of haemochromatosis by increasing hepcidin expression. Pathology of iron storage Heavy deposits of iron in the tissues are associated with fibrosis and cell death. Simple inspection reveals an overt rust-like discol- ouration of the liver, spleen, pancreas, heart, and lymph nodes. The liver is usually enlarged and haemosiderin is found in all cell types with the formation of fibrous septa and hyperplastic nodules. These nodules, which may be the forerunners of hepatocellular carcin- omas, contain little stainable iron, unlike the adjacent parenchyma. The dominant site of iron deposition during the early phases is within hepatocytes, but soon iron loading may be observed in all cell types, including the lining cells of biliary canaliculi, Kupffer cells, and stellate cells (Figs. 12.7.1.3 and 12.7.1.4). Similarly, in the pancreas there is fibrosis and iron deposition in the acini, ducts, and islets of Langerhans. Staining with Perls’ reagent reveals marked haemosiderin deposition in the exocrine and endo- crine glands, including many cell types in the testes. Haemosiderin is also markedly increased in the chief cells of the parathyroid, the an- terior pituitary, the zona

glomerulosa of the adrenal, and the thyroid. In the joints, there is loss of the intra-articular space with chondrocalcinosis and deposits of haemosiderin in the synovium. Electron microscopy shows selective deposits of ferritin and haemosiderin within type B synoviocytes. Radiological examination of the joints shows collapse of articular surfaces, subchondral cyst formation, and prominent formation of periarticular osteo- phytes. In the heart, pericardial constriction with fibrosis may
Fig. 12.7.1.3 Low-power needle-biopsy appearance of liver specimen stained with haematoxylin and eosin from a 67-year-old man with adult haemochromatosis due to homozygosity for the HFE C282Y mutation. Note the large hyperplastic nodules and fibrosis.

section 12 Metabolic disorders 2106 occasionally be observed, but the principal abnormality is seen in the myocardium with degeneration and vacuolation of cardiac myocytes and intermyocyte fibrosis that involves conducting tissue in the septa. Surviving myocytes show eosinophilic degeneration and evidence of hypertrophy. Microscopical examination shows that, in established cases of haemochromatosis, all tissues except the choroid plexus are affected by the iron storage process. In the past, it was considered that transfusional and other types of sec- ondary iron storage disease predominantly affected the cells of the mononuclear macrophage system, such as the Kupffer cells of the liver, rather than the parenchymal cells. Iron deposits in the Tf LIVER MACROPHAGES GUT Fe Hepcidin HFE Haemojuvelin TfR2 Fpn Fpn Fig. 12.7.1.6 Molecular regulation of iron homeostasis. This is maintained by hepcidin, a peptide released by hepatocytes into the circulation under stimulatory control of a common pathway involving HFE, haemojuvelin, and transferrin receptor 2. Hepcidin normally inhibits iron export from enterocytes and macrophages via its interaction with ferroportin. Mutations in haemochromatosis genes reduce hepcidin expression and allow excess iron to enter the plasma compartment and bind to transferrin with consequent tissue iron loading. Fig. 12.7.1.4 High-power micrograph of the liver biopsy specimen shown in Fig. 12.7.1.3 stained with Perls' reagent. Note the extensive deposits of ferric iron in all cell types including Kupffer cells, cells lining small biliary radicles, and in a punctate distribution within parenchymal hepatocytes. Liver cells are hyperplastic. C282Y Plasma membrane Cytosol HOOC S S S S S S H63D NH2 2 3 1 2-Microglobulin (2m) Fig. 12.7.1.5 Diagram of nonclassical MHC class I-like HFE molecule shown in juxtaposition with the β2-microglobulin. The location of the two frequent amino acid substitutions (C282Y and H63D) that predispose to the development of adult haemochromatosis is indicated by the arrows. BMP-R SMAD 1/5/8 Haemojuvelin BMP6 Neogenin TFR2 HFE HAMP (hepcidin) transcription IL-6 TMPRSS6 sHJV TWSG1 NUCLEUS HEPATOCYTE MEMBRANE GDF15 IRON TFR1 ? Fig. 12.7.1.7 Molecular signalling of hepcidin synthesis in hepatocytes. Iron stimulates the binding of bone morphogenetic protein 6 (BMP6) to the membrane-bound BMP receptor for which haemojuvelin is a coreceptor. This in turn activates the phosphorylation of SMAD proteins which translocate to the nucleus to promote transcription of the HAMP gene and synthesis of pro-hepcidin. The BMP pathway is also activated by neogenin but inhibited by the TMPRSS6 gene product matriptase-2, soluble haemojuvelin (sHJV), and twisted gastrulation 1 (TWSG1). Growth and differentiation factor 15 (GDF15) inhibits SMAD phosphorylation. HFE and transferrin receptor 2 (TFR2) act as an iron sensors and stimulate the BMP/SMAD pathway through an as yet unknown mechanism, although recent studies suggest an interaction with the BMP type 1 receptor ALK3. IL-6 stimulates hepcidin production during inflammation, independently of iron status. Figure adapted from Medicine, Vol 39/10, W. J. H. Griffiths, Haemochromatosis, pp. 597–601, Copyright (2011), with permission from Elsevier.

12.7.1 Hereditary haemochromatosis 2107 macrophage system may be less damaging than in other cell types, but it is difficult at present to relate evidence of iron-mediated in- jury to its cellular distribution. Progressive tissue injury follows the long-term cumulative toxicity of iron storage and its consequen- tial effects on organ structure and cellular function. A striking, but unexplained, feature of iron storage disease in the liver and other tissues is the absence of overt necrosis. In cancer cell biology, the novel term ‘ferroptosis’ describes iron-dependent death distinct from apoptosis, necrosis, and autophagy. Quantitative aspects of iron storage disease Chemical determination of tissue iron content yields useful infor- mation about the severity of iron loading in haemochromatosis. In normal individuals, the total concentrations of liver iron do not ex- ceed 0.15% by dry weight, but in established haemochromatosis, the value is usually 1% or more. In severely affected patients with un- treated hereditary haemochromatosis or secondary haemochroma- tosis, the amount of iron may exceed 5% of the dry weight of tissue. The overall burden of body iron in patients with haemochromatosis is usually in excess of 5 g in hereditary disease, a figure that rises with age. Estimates indicate that the total burden in patients with advanced haemochromatosis can be as much as 40 to 60 g, most of this accumulating in the liver. The pancreas and other organs such as the lymph, thyroid, pituitary, and salivary glands typically show an increase of more than 10 times the normal iron content. Other methods of iron quantification, other than the crude estima- tion offered by serum ferritin concentrations, include histochemical iron grading using Perls’ reagent, MRI techniques, and quantitative phlebotomy. Clinical features Adult haemochromatosis The clinical features of adult haemochromatosis include skin pig- mentation. The pigment may be manifest as a generalized slate-grey coloration, due principally to melanin, or localized bronzed pig- mentation particularly of the lower limbs, associated with iron de- posits in adnexal dermal structures, as well as melanin. Histological examination of the skin reveals increased melanocyte activity in conjunction with iron deposits, particularly in cutaneous sweat and apocrine glands. Increased skin pigmentation is a common, but not invariable, manifestation of haemochromatosis. It increases as the disease progresses and may be a late manifestation of the condition. Absence of pigmentation should consequently never be regarded as a contraindication to the diagnosis of iron storage disease. Iron storage disease invariably affects the liver, which is usually enlarged and may be cirrhotic, but portal hypertension and spleno- megaly are rare end-stage features of haemochromatosis. The enlarged liver, even in the absence of cirrhosis, may contain single or multifocal hepatocellular carcinomas. Hypogonadism is often present and is typ- ically preceded by a long history of fatigue, sexual asthenia, and impo- tence, as well as premature menopause and loss of libido in women. In men, there is gynaecomastia, circumoral vertical skin wrinkling, and loss of body hair; the genitalia show premature atrophy. Many patients with haemochromatosis suffer from arthritis at an early phase in the illness and this may indeed be the sole manifestation of the condition for many years. The arthritis typic- ally affects the second and third metacarpophalangeal joints of the hands and feet (Fig. 12.7.1.8). These joints show painful swelling without obvious inflammatory changes. Distal interphalangeal joint disease is also recorded and is usually considered to be typical of osteoarthritis. Many joints, including the wrist, elbow, shoulder, and knee, may be affected and the changes in these joints are typ- ically associated with chondrocalcinosis that is detected radio- logically. The affected joints show loss of joint space, subchondral cysts, and, especially in the digits, prominent osteophyte formation (Fig. 12.7.1.9). Recent studies show that premature and disabling arthritis in the hip and other large joints is a characteristic feature of haemochromatosis. The symptoms of haemochromatosis are notoriously nonspecific and slow in their progression. Fatigue is often reported and may be a manifestation of hypogonadism and the onset of diabetes

mellitus. Fig. 12.7.1.8 Arthropathy in a man with adult haemochromatosis forced to stop manual work because of painful arthritis, especially in the second and third metacarpophalangeal joints. Note the increased skin pigmentation. Fig. 12.7.1.9 Radiograph of hands in a 51-year-old woman with haemochromatotic arthropathy of the hands for many years. Note the loss of joint space, especially in metacarpophalangeal joints with subchondral cyst formation and osteophyte growth. Chondrocalcinosis is present in the ulnar fibrocartilage at the wrist.

section 12 Metabolic disorders 2108 Atrial fibrillation may be an early manifestation of cardiomyopathy. Later, paroxysmal arrhythmias and cardiac failure supervene, leading to shortness of breath and fatigue. Occasional patients with haemochromatosis present with isolated features, such as abnormal liver-related tests detected during a routine examination for health insurance, or with arthralgia and signs of arthropathy in association with diabetes, impaired libido, or sexual failure. Cardiomyopathy with heart failure or isolated arrhythmias is an unusual lone presen- tation of the disease. The differential diagnosis of haemochromatosis is very wide, but the presence of diabetes with abnormal liver function or hepatomegaly, or an association with endocrine failure or arthropathy, should prompt consideration of iron storage dis- ease. Likewise, the presence of seronegative polyarthropathy with pigmentation, hepatomegaly, or any of the associated endocrino- logical changes should initiate immediate testing for evidence of haemochromatosis. In young patients with hypogonadism or cardiomyopathy, iron storage disease should be considered. Juvenile haemochromatosis is often neglected by endocrinologists investigating young patients for infantilism or hypogonadotropic hypogonadism. The condi- tion may be responsible for cases of undiagnosed seronegative polyarthropathy. Haemochromatosis should be considered in any patient with signs and symptoms of chronic liver disease, including those with sustained mild elevation of serum transaminase activ- ities, particularly since the liver is affected early in the course of the iron overload. In fully established cases, skin pigmentation which may be either of a grey colour, as a result of increased melanin, or, especially on the shins, a yellow-brown bronze colour. Pigmentation in association with diabetes with or without arthropathy and hepatomegaly almost always signifies established iron storage disease. Diagnosis It is critically important to establish a diagnosis of haemochroma- tosis at the earliest opportunity. There is strong evidence that if treat- ment to remove iron before established structural injury occurs, then tissue function and symptoms improve. Several studies indicate that removal of iron from patients diagnosed in the precirrhotic phase of adult haemochromatosis is associated with a normal or near-normal life expectancy. Laboratory investigations In adult haemochromatosis, the diagnosis can be usually established by demonstrating abnormalities of iron metabolism (fasting serum transferrin saturation with iron >55% in males and 45% in females) together with a measurement of serum ferritin concentration that provides evidence of increased iron stores. Molecular analysis of the HFE gene for homozygosity for the common (C282Y) predisposing allele to the development of adult haemochromatosis may be very useful in patients of European ancestry. There is an increased frequency of compound heterozygotes for the C282Y/H63D or, more rarely, C282Y/S65C genotypes in patients with evidence of iron storage disease. For patients with elevated ferritin but normal or low transferrin saturation, ferroportin disease should be considered. Given the genetic variants that are now recognized as causes of haemochromatosis, it is clear that if any doubt exists as to the diagnosis, or molecular analysis of the HFE gene or of non-HFE iron overload genes fails to identify known pathogenic mutations, then tissue diagnosis is indicated. This is usually carried out by liver bi- opsy with histochemical determination, and preferably chemical quantification, of tissue iron content. Although a liver biopsy is asso- ciated with small but definable risks, it does offer a key

opportunity for the evaluation of liver structure and of the injury consequent upon iron deposition. The finding of cirrhotic change carries with it a worse prognosis. Cirrhotic change is also a major predictor of the occurrence of hepatocellular carcinoma, which occurs rarely in noncirrhotic subjects with iron storage disease (Fig. 12.7.1.10). For C282Y homozygotes, liver biopsy may be reserved for those at risk of significant liver fibrosis. When serum aminotransferase values are normal, hepatomegaly is absent and the serum ferritin is below 1000 µg/litre, the risk of significant fibrosis is negligible. This validated tool applies also to asymptomatic individuals identified through family screening or routine blood testing. More recently, transient elastography has been shown to reduce the requirement for biopsy in at-risk patients and can accurately classify severe fi- brosis in around 60% of homozygotes with ferritin above 1000 µg/ litre and raised transaminases. Serum iron-saturation determinations, and particularly serum ferritin concentrations, may signify conditions other than iron storage disease. Serum ferritin is elevated in inflammatory states, in certain malignancies such as Hodgkin's disease and in any condi- tion associated with significant necrosis of parenchymal liver cells. Under these circumstances liver biopsy is recommended, since it is most likely to provide a definitive diagnosis of iron storage dis- ease. Sometimes, however, liver biopsy is not possible, either be- cause the patient will not consent to it, or because of the presence of ascites and a bleeding disorder, especially thrombocytopenia. Under these circumstances, MRI of the liver can demonstrate iron storage if moderate or severe. A reduced signal on T2-weighted Fig. 12.7.1.10 Adult haemochromatosis. Section of liver lobe after surgical resection to remove a primary hepatocellular carcinoma arising in an iron-loaded but, unusually, noncirrhotic liver in this disorder. The patient, aged 62 years, had been partially treated by venesection but recently noticed increasing lethargy. A raised serum α-fetoprotein concentration led to the diagnosis. Moderate histochemical evidence of iron storage was found in the nonmalignant tissue excised at surgery.

 imaging correlates with significant iron deposition and a crude as- sessment of hepatic iron concentration is possible with dedicated data manipulation. If a liver biopsy is not possible and MRI of the liver does not reveal increased ferromagnetic signals indicative of iron storage, there are two further options: measurement of urinary iron excre- tion after parenteral administration of desferrioxamine, and, where the patient will tolerate it, quantitative phlebotomy. Injection of 500 mg of desferrioxamine intramuscularly in a patient with iron overload will usually induce the daily excretion of more than 2 mg of iron as the ferrioxamine complex in the urine. Ferrioxamine excretion may be increased in patients with haemolytic anaemia but, when elevated, is generally indicative of iron storage disease. Weekly phlebotomy of 500 ml will remove approximately 225 mg of iron, and thus provides a means of estimating the amount of iron removed from the storage compartment when undertaken to in- duce a mild hypochromic anaemia of approximately 10.5 to 11.0 g of haemoglobin/dl or a serum ferritin concentration of less than 30 µg/litre. Iron overload exists when the estimated iron removed by this method exceeds 1.5 g. Diagnosis in family members The diagnosis of haemochromatosis, whether it be of the adult or juvenile form, has immediate implications for that individual's first- degree relatives. All forms of haemochromatosis have a strong her- editary basis and even some forms of neonatal haemochromatosis may, in some families, be inherited as an autosomal recessive trait. A dominant transmission pattern has been established in the case of type 4 haemochromatosis. Although the penetrance and expressivity of homozygosity for the various alleles that predispose to haemochromatosis is not yet established, the risks of the disease in first-degree family members is sufficiently high to warrant systematic study. Clearly, the impli- cations for asymptomatic or

undiagnosed relatives of the index case are potentially very large. Hence, considerable care and sensi- tivity are needed in the means of informing them about the condi- tion through the identified index case. In large families there may be formidable difficulties, so that the help of genetic counselling services, as well as formal assistance from physicians practised in medical genetics, may be needed. There can be little doubt, however, that at-risk relatives should be offered the opportunity for further diagnostic and clinical evaluation in relation to iron storage disease. The condition is readily susceptible to iron depletion therapy in its early stages. Moreover, there may be additional considerations for patients who wish to make reproductive choices and who will need to be reassured that appropriate testing can be carried out on their future offspring. In HFE haemochromatosis, molecular analysis of the HFE gene may assist in assessing the risk of disease, particularly in asymptom- atic siblings. Phenotypic screening, however, is useful at the level of clinical evaluation for evidence of liver disease, hypogonadism, arthritis, pigmentation, and diabetes. Determining the biochemical phenotype first involves assay of the serum parameters of disordered iron metabolism. Since the serum parameters may be abnormal be- fore iron-mediated tissue injury has occurred, liver biopsy should be considered particularly if the serum ferritin is greater than 1000 µg/ ml or the serum transaminases are raised. In first-degree relatives, in whom molecular analysis of the HFE or non-HFE iron overload genes indicates a genetic predisposition to the disease, periodic re-evaluation is needed by clinical and bio- chemical testing at intervals of not more than 5 years. In members of families affected by haemochromatosis due to mutations in the HFE or non-HFE iron overload gene who were not found to carry the predisposing mutations and whose ferritin and iron parameters are normal, liver biopsy is not mandatory and the risk of the devel- opment of significant iron storage disease in less than 5 or 10 years is extremely low. In patients with no known pregenetic disposition and normal tissue biopsy findings, further follow-up screening is not indicated. From the foregoing it can be seen that there is an urgent need to characterize the genotype–phenotype relationship in both HFE and non-HFE haemochromatosis. Unfortunately, no genetic locus has yet been identified for neonatal haemochromatosis, although this is a subject of continuing research. In at-risk pregnancies, neo- natal haemochromatosis may be occasionally recognized by MRI during the third trimester, which may show increased iron signals in the fetal liver. After birth, biopsy of the oral mucosa on the gums or inner lip may reveal histological evidence of iron storage in minor salivary glands of affected infants. Environmental cofactors and disease expression Many patients with adult haemochromatosis give a history of exces- sive current or prior alcohol consumption. In the past, physicians have been tempted to attribute evidence of excess tissue iron in these individuals solely to the consumption of alcohol. In practice, how- ever, it appears that those individuals who have biopsy-proven evi- dence of hepatic iron storage usually prove to carry two predisposing alleles of the HFE gene and therefore have true haemochromatosis. Although no clear predictors for the expression of disease in first- degree relatives at risk are available, disease expression is reduced in women of reproductive age. Most practising clinicians consider that age and alcohol consumption are the main identifiable environ- mental factors that contribute to disease expression in predisposed homozygotes. Other comorbid factors, including heritable factors, that may influence the expression of HFE mutations in homozy- gous subjects, include the presence of adult coeliac disease. There are few data that define the relationship between haemochromatosis and coeliac disease, but subclinical coeliac disease may ameliorate the long-standing effects of iron loading in C282Y homozygotes. Cosegregation of haemochromatosis and coeliac disease has re- cently been reported in a large Swedish study. Discriminatory polymorphisms in genes which modulate oxidative stress and other fibrogenic cytokine responses have been postulated as influencing expression of haemochromatosis in C282Y

homozygotes. Recently, discrete genome-wide association studies in HFE haemochroma- tosis have identified the transferrin gene as a significant modifier of iron status and proprotein convertase subtilisin/kexin type 7 (PCSK7) as a host risk factor for liver cirrhosis. The A736V TMPRSS6 polymorphism may also influence the clinical expression of HFE haemochromatosis as may a variant in the glyceronephosphate O- acyltransferase (GNPAT) gene. The identification, since HFE, of several genes associated with haemochromatosis has provided some insight into the pheno- typic variation of primary iron overload. The phenotype of type 4

section 12 Metabolic disorders 2110 haemochromatosis certainly appears distinct. It is now apparent, how- ever, that individuals with juvenile mutations may present later and with milder disease than described historically for juvenile haemo- chromatosis patients. Those with TFR2 mutations have occasionally presented young with a severe iron overload phenotype more rem- iniscent of juvenile haemochromatosis. Iron overload with varying severity has been accounted for by compound heterozygous forms of HFE and juvenile mutations, termed digenic inheritance. Moreover, C282Y homozygotes with the most severe iron overload may carry an additional juvenile mutation to account for increased disease ex- pression. The classical haemochromatosis phenotypes overlap and combinations of genetic alteration contribute to a spectrum of disease.

Management Since it is the toxicity of iron that is responsible for the manifest- ations of all forms of haemochromatosis, treatment is directed to the removal of iron at the earliest possible stage.

Venesection In adult and juvenile haemochromatosis, the preferred method of treatment is iron depletion by means of phlebotomy. This is best instituted by the removal of approximately 500 ml of venous blood each week by needle puncture of peripheral veins in the antecubital fossa. In young patients, it may be possible to increase the frequency of venesection to twice per week after several once-weekly proced- ures. In elderly patients and those with hypoalbuminaemia as well as end-organ failure and heart disease, the frequency of venesection should be commuted to within the rate tolerated. Coincidental in- flammatory disease may impede the erythropoietin-mediated drive to haemopoiesis, and, particularly in the early phases of treatment, mild haemorrhagic anaemia may ensure. Thus, adjustments need to be made according to the early responses to venesection therapy, and regular monitoring of the haemoglobin concentration or haem- atocrit is advisable. Difficulties may arise in delivering this deceptively simple treat- ment as a result of poor organization of health service provision and of unavailability of suitable healthcare personnel to carry out the venesection procedure. Every practical effort should be made to ensure that the procedure is convenient for the patient, who is often a young or middle-aged person in full-time employment, and who may find regular access to the treatment centre problematic. In cold weather, or in patients with poor circulation or inconspicuous superficial venous access, the use of topical local anaesthetics such as lidocaine cream or even local diffusable preparations of glyceryl tri- nitrate, applied 30 to 60 min before the venesection procedure, may greatly improve venous access. Likewise, the simple technique of immersing the arm in warm water to improve peripheral blood flow may be critical for establishing confidence in treatment staff. Since patients with haemochromatosis usually harbour a large burden of iron, requiring repeated phlebotomy over a period of several years, every effort should be made to preserve the integrity of their per- ipheral veins. In the authors' view, the use of a local anaesthetic is usually unwarranted since it involves further tissue invasion in the region of the antecubital fossa with needles. Moreover, repeated in- jections of the irritant fluid often lead to sclerosis around the venous access site. Where blood transfusion services can assume some, if not all, responsibility for the phlebotomy of haemochromatosis pa- tients, the inconvenience of hospital-based services can be circum- vented

and blood supplies can be enhanced safely. Not all patients require immediate treatment but it is vital to inter- vene if ferritin concentrations are above or approaching 1000 µg/ litre. Some patients will be at lower risk of morbidity and mortality, typically those presenting with lower iron indices, in older age or those who are female. Such patients could be observed initially or indeed recommended to undergo blood donation with monitoring if otherwise eligible. Of note, since October 2012 the National Blood Service in England and Wales has accepted selected haemochroma- tosis patients for donation at up to 6-week intervals. An Australian study in homozygotes with ferritin concentrations of between 300 and 1000 µg/litre comparing iron reduction with sham treatment is currently underway. Duration of venesection therapy One 500 ml unit of peripheral blood contains approximately 225 mg of elemental iron. Thus most patients with established haemochromatosis will require weekly phlebotomy for a period of 1 to 2 years. The objective of this treatment is to restore serum ferritin concentrations to within the low normal range and, if pos- sible, to induce a mild iron deficiency anaemia of approximately 11.5 g haemoglobin/dl. Having thus achieved a satisfactory deple- tion of body iron stores, interval maintenance phlebotomy, carried out according to ferritin measurements, four to six times per year is usually sufficient to maintain normal iron stores with a serum ferritin concentration less than 100 µg/litre. Some authorities sug- gest that serum ferritin values below 30 µg/litre should ideally be achieved. In patients with juvenile haemochromatosis, who have a higher than normal intestinal iron absorption, more frequent phlebotomy may be needed to maintain a healthy iron balance. Proton pump inhibitors appear to significantly reduce the need for venesection, by reducing iron absorption through alkalization of the stomach, although currently are not recommended for this sole purpose. Iron chelation therapy Alternative methods of iron removal are needed for patients with severe clinical manifestations of haemochromatosis, such as life-threatening cardiac arrhythmias and those with severe liver dis- ease and hypoalbuminaemia, who are incapable of withstanding frequent phlebotomy. The preferred alternative involves chelation therapy with the parenteral agent desferrioxamine. As indicated in Chapter 22.6.4, the subcutaneous administration of desferrioxamine brings about the removal of a maximum of 20 to 25 mg of iron daily and is thus generally less efficient than vigorous weekly phlebotomy. However, desferrioxamine may gain access to cellular pools of iron that are important in the pathogenesis of tissue injury in established iron storage disease, and therefore may offer particular benefit in pa- tients critically ill with arrhythmias due to haemochromatotic car- diomyopathy. Although the nature of this so-called chelatable iron pool is unknown, there is strong circumstantial evidence that its depletion by means of intravenous desferrioxamine treatment may reverse the life-threatening consequences of terminal iron storage disease in patients with haemochromatosis. Moreover, the removal

 of 140 mg of chelatable iron per week represents about two-thirds of the amount that can be removed by weekly phlebotomy. A biological advantage may also be gained by therapeutic access to a reactive, low molecular weight, chelatable fraction responsible for the injurious effects of cellular iron overload. Parenteral desferrioxamine may be given intravenously for life- threatening cardiac disease, as described in Chapter 22.6.4, or, in the nonemergent situation, by subcutaneous infusion using portable infusion pumps for 12 to 14 h, five or six times per week. It must be stressed, however, that chelation therapy is not the preferred option for the treatment of established haemochromatosis and should be re- stricted to those patients unable to tolerate phlebotomy as a result of anaemia or hypoalbuminaemia, or in whom life-threatening cardio- myopathy or liver disease is present. Newer oral iron chelators with

promising safety profiles are becoming established for secondary iron overload. One such chelator, deferasirox, has been shown to be efficacious in an early phase study in genetic haemochromatosis, re- peated in a further phase II study published very recently.

General measures Attention should be given in patients with haemochromatosis to the diagnosis and treatment of end-organ failure. This particu- larly applies to the management of diabetes mellitus by diet and insulin where necessary, as well as hormone replacement therapy for hypogonadism (see Chapter 13.6.2). In men, intramuscular depot injections of testosterone enantate (250 mg every 2–3 weeks) are recommended to improve libido and inhibit the development of premature osteoporosis. Similarly, conventional sex hormone replacement therapy should be used in women with premature gonadal failure as a result of haemochromatosis. Cardiac failure in patients with haemochromatosis due to cardiomyopathy and hepatic failure consequential upon pigmentary cirrhosis should be treated by standard methods. Organ transplantation may be used successfully, but correction of systemic iron overload should be undertaken as soon as practicable to restore normal function in all organ systems. Rarely, end-organ hormone deficiencies re- sult from thyroid infiltration and parathyroid and adrenocortical disease. These deficiencies should be vigorously sought for in the clinical evaluation of the patient at presentation. The appearance of lethargy, faintness due to postural hypotension, or symptomatic hypocalcaemia demands immediate investigation and institution of appropriate replacement therapy. Patients with cirrhosis should undergo 6-monthly surveillance by ultrasonographic examination and α-fetoprotein estimation for early detection of hepatocellular carcinoma. Prognosis The main causes of death in untreated patients with haemo- chromatosis are hepatocellular failure, primary carcinoma of the liver (including hepatocellular carcinoma), and, rarely, cholangiocarcinomas. Cardiac failure due to haemochromatotic cardiomyopathy and untreated diabetes also contribute to death. Although not categorically proven, evidence from retrospective surveys suggest that life expectancy is improved by removing iron from patients with haemochromatosis of whatever cause and the subsequent maintenance of normal iron homeostasis. Most patients experience an improvement in well-being on iron depletion therapy and, during its early phases, there is evidence that hypogonadotropic hypogonadism may improve with this therapy. Similarly, the mani- festations of cardiomyopathy with intractable cardiac failure or tachyarrhythmias can improve after the removal of iron. The cirrhosis of haemochromatosis appears not to be reversible, although the earlier precirrhotic manifestations of hepatic disease improve greatly on the removal of iron with an apparent restoration of normal life expectancy. Indeed, there is mounting evidence that hepatic fibrosis, short of cirrhosis, can reverse following iron deple- tion. In all patients, there is at least a twofold increase in the survival rate at 5 years from the point of diagnosis with the introduction of phlebotomy. In patients studied during the 1950s and 1960s, the 5-year survival rate improved from 18% to more than 65% in all haemochromatosis subjects treated. In patients diagnosed with haemochromatosis but without cir- rhosis, iron depletion therapy is associated with a near normal or normal life expectancy compared with a sex- and age-matched con- trol cohort derived from the same population. It is notable, however, that the indolent nature of this storage disorder and the long-term survival of patients who are affected by it has, so far, rendered long- term controlled studies of the effects of phlebotomy on eventual out- come almost impossible to achieve. However, a wealth of evidence, based on the understanding of the pathogenesis and documented responses to iron depletion in individual patient cohorts, indicates that early removal of iron is highly desirable—indeed, it may be de- cisive in determining a good outcome from all forms of human iron storage disease, including all subtypes of hereditary haemochroma- tosis so far established. Hepatocellular carcinoma occurs mostly in patients with iron storage disease who have established

cirrhosis and the risk appears to persist despite removal of iron. Although hepatocellular carcinoma and cholangiocarcinoma have been reported in noncirrhotic pa- tients with haemochromatosis, these are rare phenomena. Systematic ultrasonographic surveillance is vital if liver cancer is to be detected at a stage where potentially curative treatment can be offered. Since all the evidence suggests that patients with haemochromatosis are more likely to have diabetes mellitus and other manifestations of the disease, every encouragement should be given to the prompt diag- nosis of the condition and early institution of iron depletion therapy. Increasingly, it has been recognized that the arthropathy of haemo- chromatosis can be disabling, whether or not it is associated simply with joint pain (arthralgia) or progressive and noninflammatory joint destruction. The disease is associated with a loss of cartilage and, in many large joints, chondrocalcinosis. Although the re- sponse of the arthropathy to iron depletion therapy is controver- sial, the weight of observation indicates that, once established, the arthropathy of haemochromatosis progresses independently of body iron status and of iron depletion treatment. It seems intrin- sically likely that effective removal of excess body iron stores before the development of joint symptoms will prevent their onset and pro- gression. However, at present only cross-sectional data are available to support this contention. In summary, observations in adult haemochromatosis suggest that once the disease is established in association with cirrhosis or diabetes mellitus, it diminishes life expectancy. In fact, a more recent

section 12 Metabolic disorders 2112 study demonstrated that among treated C282Y homozygotes those with serum ferritin levels greater than 1000 µg/litre at diagnosis had a fivefold relative risk of death. The prognosis for cardiomyopathy in juvenile haemochromatosis is very poor but it may be improved by early diagnosis and the early institution of vigorous iron deple- tion therapy. In several cases, the outcome has been improved by allogeneic cardiac transplantation. In adult patients with established pigment cirrhosis, hepatic transplantation has been undertaken and, provided the other systemic manifestations of haemochroma- tosis have been adequately treated, the procedure is associated with a good overall prognosis. Prevention and control The importance of early recognition and the institution of iron de- pletion therapy in all forms of haemochromatosis cannot be over- emphasized. Molecular analysis of the HFE gene, together with biochemical characterization using serum transferrin iron satur- ation estimations and serum ferritin concentrations, has the power to assist greatly in the detection of presymptomatic first-degree rela- tives of patients with haemochromatosis. In relation to whole populations in which mutations in the HFE gene are frequent, the health implications based on mass screening remain contentious. Superficially, adult hereditary haemochroma- tosis due to mutations in the HFE gene appears to be an ideal con- dition for DNA-based mass population screening. The condition is attributable to a single gene, and a single mutation of diagnostic significance is prevalent (gene frequency 5–10%). Disease-related mutations in HFE (especially C282Y) are easily tested for by means of techniques based on the polymerase chain reaction. At the same time, HFE-mediated haemochromatosis has a long incubation period without symptoms, and all the evidence suggests that the in- stitution of treatment for presymptomatic disease is cheap, simple, and effective. On the other hand, genetic identification of at-risk individuals is associated with problems of stigmatization, increased anxiety, and potential life insurance weighting, all of which are familiar aspects in well-rehearsed debates about genetic testing in the gen- eral population. These aspects must be considered, together with the age-related penetrance of the homozygous state for HFE C282Y variants and, as yet, unknown combined genetic and environmental influences on disease expression. Uncertainty as to the significance of these factors has held back the introduction of mass population screening by DNA-

based methods. In light of the present state of knowledge, it is clear that homozygosity for the C282Y allele of HFE cannot be considered to be tantamount to a diagnosis of hereditary haemochromatosis. More information is needed from outbred populations, rather than from homozygotes identified as a result of screening family members of index cases having full-blown clinical disease. Family studies provide a false measure of disease expressivity, presumably as a result of shared environments and of the cosegregation of po- tential disease-modifying genes within defined pedigrees. Finally, it must be emphasized that difficulties also occur for the evaluation of the burden of haemochromatosis in the population at large. Although there are definitions of iron storage disease that reflect the abnormal biochemical genotype, the manifestations of the clinical disease are variable and protean. Moreover, as pointed out earlier, no internationally agreed case definition of haemochromatosis exists, which creates additional difficulties for the introduction of public health measures and appropriate policy review of nationwide screening procedures. Future directions Although startling progress has been made in the discovery of many components that serve to regulate iron homeostasis in hu- mans, more information is needed before a full molecular under- standing of the mechanisms of iron homeostasis can be achieved. The genetic basis of some neonatal and further variant forms of adult haemochromatosis has yet to be fully explored. The inter- actions between iron regulatory molecules on the hepatocyte membrane are not fully resolved but this interplay and the asso- ciated downstream signalling pathway for hepcidin appear key to body iron homeostasis—thus a promising target for future therapeutic intervention. A challenging task will be the detailed understanding of how environmental cofactors determine the ex- pression of iron storage disease in genetically predisposed indi- viduals. Alcohol is a long-standing candidate, but the mechanism by which it leads to increased delivery of toxic iron to the tissues is, at present, poorly understood. Recognizing genetic modifiers of disease expression may, in future, inform natural history and treatment decisions in asymptomatic individuals at risk from iron storage disease. Greater understanding of these issues and of pene- trance in particular populations will determine local screening practices for disease prevention. Newly identified iron storage diseases By general agreement, the term haemochromatosis is used to de- scribe systemic syndromes of pathological iron storage that affect many tissues and disturb the function of diverse organ systems. Conversely, several distinct clinical syndromes of local iron tox- icity have been identified, especially in the eye and brain. Although these syndromes are individually rare, they are important because they are potentially accessible to measures that reduce cellular free iron (e.g. metal chelation, mentioned earlier), and because they demonstrate the central importance of metabolic iron in selected tissues. A fuller understanding of these disorders, and the cognate cell metabolic pathways they affect, may well shed light on ill- understood aspects of tissue iron physiology. Additional informa- tion is available by reference to the OMIM website at http://www.ncbi.nlm.nih.gov/omim. Hereditary hyperferritinaemia cataract syndrome (OMIM 600886) The sole clinical manifestation of this condition is of congenital bilateral ferrugineous nuclear cataracts due to the disposition of excess ferritin light chain polypeptide in the ocular lenses. The serum ferritin concentrations are moderately elevated but no evi- dence of systemic iron storage is found. The disorder is caused

 by mutations in the 5′ noncoding iron-response element of the ferritin light-chain (FTL) gene that leads to unregulated transla- tional overexpression of ferritin light chains. These polypeptides accumulate in the lenses and disturb their tissue organization and refractile properties. The hyperferritinaemia cataract syndrome is, as expected for

an overexpression disease, inherited as a dom- inant trait. Measurement of serum ferritin concentrations may identify at-risk family members. The gene encoding ferritin light chain polypeptide maps to chromosome 19q3.3-qter. Latterly, a syndrome without cataracts has been identified due to mutations in exon 1 of the FTL gene—this forms an important differential in individuals with unexplained hyperferritinaemia with normal transferrin saturation. Adult-onset basal ganglia disease (OMIM 606159) A single pedigree has been identified with a dominantly inherited disorder showing features of late-onset extrapyramidal dysfunction resembling parkinsonism or Huntington's disease. Imaging and aut- opsy studies revealed cavitation of the basal ganglia with deposition of iron and ferritin protein in adjacent tissue, especially in the putamen and the globus pallidus. The macroscopic appearances showed widespread reddish discolouration of affected tissues. This disorder was mapped to chromosome 19q13.3 and a single mutation, a point insertion of a single adenine at nucleotide 461, was identified in exon 4 of the FTL gene. The mutation is predicted to disrupt the C- terminal sequence of the ferritin light-chain molecule and disturb the iron-binding core of the hetero- or homomeric protein. Serum ferritin concentrations were found to be abnormally low in affected heterozygotes. Although this disorder has so far only been identified in a single large pedigree, it further illustrates the importance of ferritin in tissue iron metabolism and, especially, in selective regions of the brain. This disorder has been termed a 'neuroferritinopathy' and may be the first of several diseases affecting cellular iron pathways in iron-rich brain tissue. Acaeruloplasminaemia with iron deposition (haemosiderosis) in basal ganglia (OMIM 277900) This disorder is associated with mild systemic iron deposition and deficiency of the plasma copper-binding protein, caeruloplasmin. Caeruloplasmin has long been known to possess ferroxidase activity and the ability to enhance the mobilization and delivery of iron to and from macrophages and hepatocytes. It promotes iron loading of intact ferritin micelles. Acaeruloplasminaemia, due to mutations in the gene encoding caeruloplasmin on chromosome 3q21–24, is an autosomal recessive trait. The deficiency is associated with diabetes mellitus, dementia, and extrapyramidal features including parkin- sonism, with choreoathetosis as well as cerebellar ataxia. MRI shows altered signals in the basal ganglia, and retinal degeneration may be apparent by fundoscopy. Excess systemic iron is demonstrable by examination of liver tissue and the serum ferritin concentra- tion is moderately elevated; however, low serum iron transferrin saturations with hypochromic microcytic anaemia, reminiscent of copper deficiency, are usually present. Infusions of plasma or purified caeruloplasmin may correct the systemic abnormalities of iron metabolism, but probably do not influence the dementia or the other neurological deficits, at least once these are established. The role of caeruloplasmin replacement or indeed parenteral chelation therapy with desferrioxamine or trientine, especially in the early evolution of the neurological syn- drome, has not yet been established. The interplay between copper and iron metabolism is well illustrated by this severely disabling illness. Acaeruloplasminaemia illustrates the particular sensitivity of the basal ganglia to disturbances of iron metabolism. In this con- text, it is notable that caeruloplasmin expression is abundant in glia in the brain microvasculature juxtaposed to the pigment-containing dopaminergic neurones of the substantia nigra and inner layer of the retina. Hallervorden–Spatz disease: pantothenate kinase- associated neurodegeneration (OMIM 234200) This disease has been familiar to neurologists and neuropatholo- gists since its original description by two, now discredited, German neuroscientists of the Nazi period. The clinical features indicate basal ganglia disease and dementia with retinal degeneration leading to optic atrophy. The disorder often presents with club foot deformity in children and adolescents; extrapyramidal rigidity pre- ceded by choreoathetosis usually follows rapidly. Dementia, optic atrophy, and generalized seizures occur in the later stages, and death

usually ensues by the age of 30 years. Although late-onset forms of the disease are known, a striking feature is the presence of iron pigment in the basal ganglia and substantia nigra, now easily recognized by MRI. The heredofamilial nature of this syndrome has been known since its first description. Hallervorden–Spatz dis- ease is now known to be an autosomal recessive trait due to mu- tations in the pantothenate kinase 2 gene (PANK2) that maps to chromosome 20p13. Pantothenate kinase 2 is abundant in the retina and target regions of the brain and regulates the formation of coenzyme A. Deficiency of pantothenate kinase 2 would deplete sensitive neural tissues with a high metabolic rate of coenzyme A; the defect may also lead to a consequential accumulation of cysteine, which normally condenses with the enzyme product, phosphopantothenate. In the presence of high concentrations of free iron, excess cysteine may accelerate the formation of cytotoxic oxygen free radicals. For some years, cysteine accumulation has been independently observed in the iron-rich nigrostriatal regions of the brain affected by this disorder. Identification of PANK2 mutations offers the hope of improved diag- nosis of this neurodegenerative disorder, and, more importantly, the prospect of specific therapy using supplementation to enhance local coenzyme A activity and phosphopantothenate concentra- tions in affected neural tissue. Latterly, the term ‘neurodegeneration with brain iron accumulation’ (NBIA) has been coined to encom- pass several inherited neurological disorders with basal ganglia involvement—nine genes have thus far been implicated including PANK2 and FTL. The accelerated interest in this field is leading to trials of iron chelation in Parkinson’s disease. Further practical information Many patients’ associations and societies exist to serve the needs of patients in their respective countries. In the United Kingdom, useful information can be obtained from The Haemochromatosis Society, Haemochromatosis UK, PO Box 6356, Rugby, CV21 9PA.

section 12 Metabolic disorders 2114 Haemochromatosis UK is a working name of The Haemo chromatosis Society (a registered charity). Office: office@huk.org.uk; Helpline:helpline@huk.org.uk; Telephone: +44 (0)3030401102. The society’s website (http://www.haemochromatosis.org.uk) in- cludes links to similar societies in other parts of the world. FURTHER READING Adams P, et al. (2009). Screening for iron overload: lessons from the HEmochromatosis and IRon Overload Screening (HEIRS) Study. Can J Gastroenterol, 23, 769–72. Adams PC, Speechley M, Kertesz AE (1991). Long-term survival analysis in hereditary haemochromatosis. Gastroenterology, 101, 368–72. Allen KJ, et al. (2008). Iron-overload-related disease in HFE hereditary hemochromatosis. N Engl J Med, 358, 221–30. Andersen RV, et al. (2004). Hemochromatosis mutations in the general population: iron overload progression rate. Blood, 103, 2914–19. Barton JC, et al. (2012). Increased risk of death from iron overload among 422 treated probands with HFE hemochromatosis and serum levels of ferritin greater than 1000 µg/L at diagnosis. Clin Gastroenterol Hepatol, 10, 412–16. Bulaj ZJ, et al. (2000). Disease-related conditions in relatives of patients with hemochromatosis. N Engl J Med, 343, 1529–35. Camaschella C, et al. (2000). The gene TfR2 is mutated in a new type of haemochromatosis mapping to 7q22. Nat Genet, 25, 14–15. Cancado R, et al. (2015). Deferasirox in patients with iron overload secondary to hereditary hemochromatosis: results of a 1-yr phase 2 study. Eur J Haematol, 95, 545–50. Cullis JO, et al. (2018). Investigation and management of a raised serum ferritin. Br J Haematol, 181, 331–40. De Gobbi M, et al. (2002). Natural history of juvenile haemochroma- tosis. Br J Haematol, 117, 973–99. De Tayrac M, et al. (2015). Genome-wide association study identifies TF as a significant modifier gene of iron metabolism in HFE hemo- chromatosis. J Hepatol, 62, 664–72. Dusek P, Schneider SA (2012). Neurodegeneration with brain iron ac- cumulation. Curr Opin Neurol, 25, 499–506. European Association for the Study of the Liver (2010). EASL clinical practice guidelines for HFE hemochromatosis. J Hepatol, 53, 3–22. Falize L, et al. (2006). Reversibility of hepatic fibrosis in treated genetic hemochromatosis: a study of 36

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