HOMOZYGOSITY FOR THE C282Y SUBSTITUTION IN THE *HFE* GENE: THE INCOMPLETE PENETRANCE AND VARIABLE EXPRESSIVITY

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ABSTRACT

The syndrome of hepatic cirrhosis diabetes and skin pigmentation ('Bronze diabetes') has been well documented, including its propensity to lead to hepatocellular cancer. However, this picture of advanced disease is much less common nowadays with increased awareness and early diagnosis. However, in addition to this, it has been increasingly recognised that in contrast to other diseases inherited as autosomal recessive traits, subjects carrying the genetic predisposition infrequently develop overt disease. This is due only in part to physiological and pathological blood loss, and further relevant genetic mutations have been anticipated. Indeed, an international consortium has recently identified that the genetic variant (*GNPAT*) has been identified as predisposing to iron overload related disease. Further mutations can be anticipated and will assist in early diagnosis and treatment as well as identifying subjects predisposed to significant iron overload.

Keywords: Haemochromatosis, iron metabolism, *HFE* haemochromatosis, secondary iron overload.

INTRODUCTION

The clinical syndrome of haemochromatosis has been recognised in its advanced state for >100 years, and in the last two decades clinical and molecular research into this disease has accelerated.[1] Following identification of the mutation in the *HFE* gene, which is responsible for >90% of cases of classic haemochromatosis,[2] it was recognised that the genotypic predisposition is common among those of Northern European ancestry but that the incidence of biochemical abnormalities and clinical disease is less frequent. The recognition of the *HFE* mutation has allowed other forms of iron overload to be diagnosed as distinct entities, typically related to mutations in other iron-regulatory molecules. Largely as a result of the identification of the *HFE* gene, but also because of activities of patient support groups such as Haemochromatosis Australia, there is now a much better awareness of the disorder and subjects and their family members are increasingly recognised at an early stage, e.g. simply with raised serum ferritin (SF) levels and transferring saturation. It has also been increasingly recognised, that in contrast to other diseases inherited as autosomal recessive traits, subjects homozygous for C282Y do not always develop overt disease. In fact, it is now established that approximately only 75% of homozygous subjects develop increased SF levels, only 50% biochemical abnormalities, only 25% increased liver iron, and fewer than 5% develop iron overload related diseases (Figure 1[3-5]).

This was emphasised by Beutler et al.[6] in 2005, when he stated that "*HFE* mutations are necessary but not sufficient to cause haemochromatosis". This has led to a global search for further genes and mutations that could lead to iron overload. As discussed below, several mutations in iron transport molecules have been clearly identified and more

recently a mutation in *GNPAT* has been identified as predisposing to iron overload related disease. It should also be noted that certain transferrin genotypes or polymorphisms have been shown to affect disease expressivity,[7] in addition to which heterozygous mutations in non-*HFE* genes have also been shown to affect disease severity in C282Y homozgotes. Further mutations can be anticipated. Recent research in the field of iron metabolism has deepened our understanding of the molecular processes of iron transport and regulation and how this is disturbed in haemochromatosis. At the same time, population studies better describe the risk to an individual with a genetic mutation, and clinical investigations have improved the tools available for assessment and monitoring. One aspect of this condition that has changed the least is the treatment, with the ancient practice of bloodletting still the main therapy available. With new therapeutic agents under trial, this to may soon be changing. This review focuses on the recent developments in the field of genetic haemochromatosis, including the molecular basis of iron metabolism, relevant genetic mutations, and advances in investigations and therapy, and places these in a global perspective.

IRON ABSORPTION AND THE ROLE OF HEPCIDIN

Iron is absorbed from the gastrointestinal tract in one of two ways depending on whether it is in the haem or non-haem form. Transporters involved in haem iron absorption are not fully understood, however two carriers have been implicated – haem carrier protein 1 and haem responsive gene 1 protein.[8] In contrast, non-haem iron absorption is well characterised. Dietary ferric iron (Fe^{3+}) is reduced to ferrous iron (Fe^{2+}) by ferrireductases mainly, duodenal cytochrome B.[8] Activity of duodenal cytochrome B is facilitated by ascorbate, which acts as an electron donor. This allows ferrous iron uptake through the divalent metal transporter.[8,9] Once inside the cell, iron may be stored in the polymeric protein, ferritin. Once there, it is released into the circulation through ferroportin – a transport protein on the basolateral surface of enterocytes, macrophages, and other cells.[10] Ferroportin further interacts with feroxidases: hephaestin and ceruloplasmin to oxidise ferrous iron back to ferric, prior to release into circulation.[11] Ferroportin is the only known iron channel allowing export of iron.[12] Once released into the serum, free iron will bind transferrin (Tf). This complex can be carried to and taken up by cells expressing transferrin receptor 1 (TfR1).[8,13] Transferrin saturation is utilised in iron sensing pathways and acts as negative feedback to regulate ferroportin expression, through hepcidin signalling, in hepatocytes, macrophages, and other cells.

Hepcidin is the key regulator of iron metabolism. It is a small 25 amino acid protein synthesised in the liver and exerts its action by regulating ferroportin expression.[14-16] Hepcidin binds to an extracellular loop of ferroportin facilitating endocytosis and proteolysis of the channel, thus reducing the number of transporters available for iron export.[8,17] Normally, excess iron stimulates hepcidin expression in the hepatocytes, leading to a subsequent decrease in serum iron.[12] Regulation of hepcidin is not fully understood, however multiple pathways of regulation have been described. Experimental evidence suggests that hepcidin regulation occurs at a transcriptional level.[18] Mediators influencing hepcidin levels include the *HFE* gene product, transferrin receptor 2 (TfR2), haemojuvelin (HJV), bone morphogenic protein 6 (BMP-6), and matriptase-2.[19] The HFE protein is thought to be expressed primarily in Kupffer cells and bile duct epithelia, and exerts its effects on hepatocytes to induce hepcidin production.[20] It has been suggested that *HFE* interacts with TfR1 and as serum iron increases, it displaces *HFE* allowing it to interact with TfR2 and mediate hepcidin production.[19] HJV is thought to act as a ligand for BMP-2, 4, and 6 leading to increased hepcidin mRNA expression.[21] It has been shown that *HFE* interacts with HJV, suggesting that they form a complex together.[19,22] BMP-6 is thought to play a significant role in iron metabolism given that mouse models with BMP-6 ablation show very low levels of hepcidin mRNA expression.[22]

NOMENCLATURE OF IRON OVERLOAD STATES

The naming of haemochromatosis subtypes is based on the molecules affected. To date four main subdivisions named I, II, III, and IV, which affect the molecules *HFE*, HJV/hepcidin, TfR2, and ferroportin respectively, have been described in literature.[23,24] Further to this, Types 2 and 4 are subdivided into 2A, 2B, 4A, and 4B. As haemochromatosis involving *HFE* is by far the most prevalent, the alternative classification of Type 1 as *HFE* associated haemochromatosis and Types 2-4 as non-*HFE* haemochromatosis still persists.

Types 1-3 are autosomal recessive and affect hepcidin synthesis and regulation where Type 4 differs, being both autosomal dominant and not having a principal effect on hepcidin.[25] The phenotypic expression of typical disease likewise is similar between Types 1-3 and is described in the next section.

HFE (Type 1)

Type 1 haemochromatosis resulting from mutations in the *HFE* gene is the commonest and best established subtype. With an autosomal recessive inheritance pattern it shows the typical phenotype commonly associated with disease. Often presenting with nonspecific symptoms of fatigue and lethargy progressing to hepatic fibrosis, endocrine dysfunction, and cardiomyopathy.[26] Principally three mutations, C282Y, H63D, and S65C, at various loci in the *HFE* gene have been associated with disease. Of these, only the C282Y homozygotes, heterozygotes, and H63D/C282Y compound heterozygotes are of clinical interest. Recently, the role of S65C in disease has been shown to be limited to a mild risk factor. Further to which, compound heterozygosity is only considered clinically relevant in the presence of other risk factors such as heavy alcohol consumption. Even though the role of the membrane protein encoded by *HFE* remains unclear, it is known that aberrations in this gene cause iron overload by interfering with iron sensing in the liver.[8] This leads to decreased hepcidin regulation by iron and subsequent increased gastrointestinal iron uptake.

NON-HFE

Juvenile Haemochromatosis (Types 2A and 2B)

Characterised by earlier onset (typically by the second and third decades) Type 2 is a more severe form of haemochromatosis with higher mortality than Type 1.[27] Two distinct molecular origins have been identified; Types 2A and 2B affecting the *HJV* and hepcidin (*HAMP*) genes respectively.[28] *HJV* is a crucial modulator in the BMP/SMAD pathway of hepcidin regulation, and exerts a stronger influence over this pathway than the *HFE* gene product. Rather than influencing hepcidin release, Type 2B represents a mutation in *HAMP* itself.[29,30]

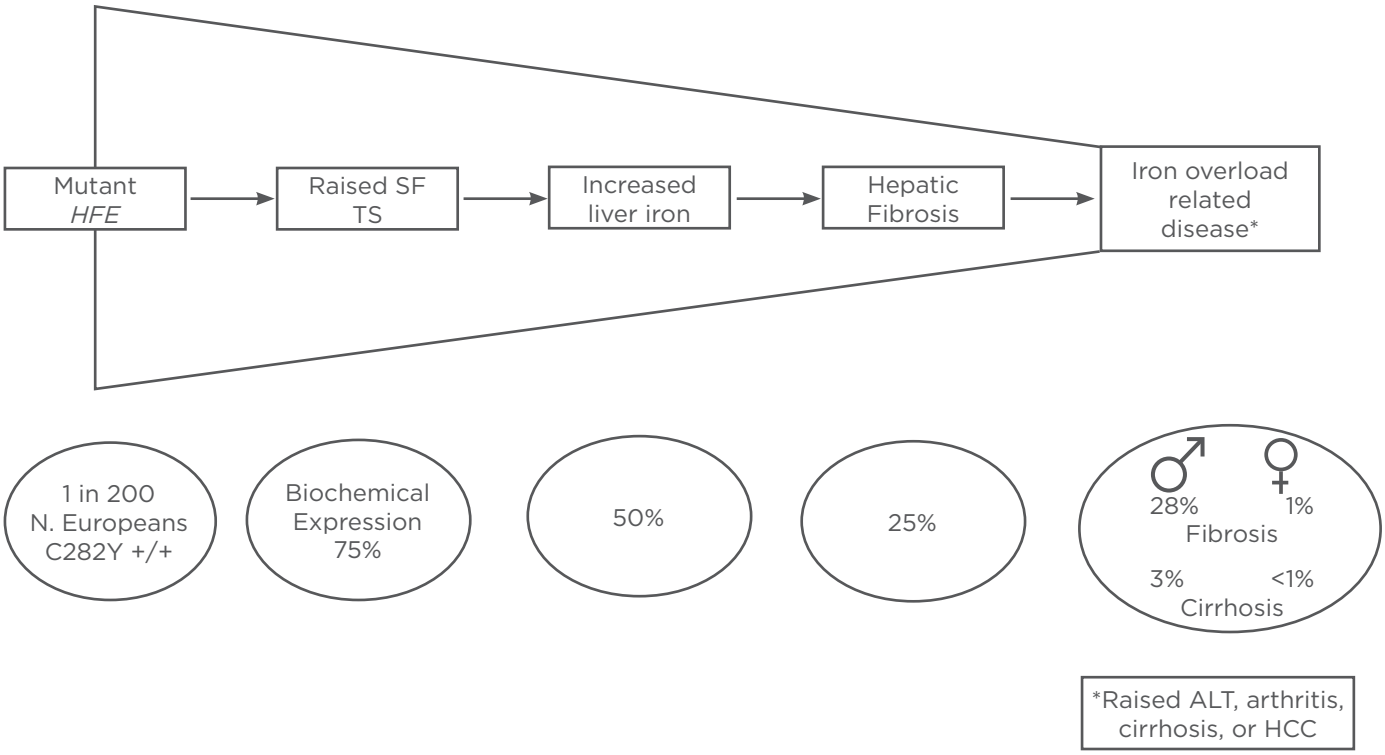

Figure 1: The natural history and disease burden of HFE associated haemochromatosis.
In addition to the above, note that in practice HFE genotyping is usually carried out in selected patients with elevated transferrin saturation (TS) or serum ferritin (SF) levels.
HCC: hepatocellular carcinoma; ALT: alanine transaminase.
Reproduced with permission[3-5]

Both subtypes are inherited in an autosomal recessive pattern. Clinically rapid iron deposition overload preferentially damages the heart and endocrine organs and causes diabetes, cardiomegaly, and hypogonadotropic hypogonadism.[25,26,31] Presentation is abdominal pain, hypogonadism, and arrhythmia with untreated patients commonly dying of congestive cardiac failure or arrhythmia.[26,32]

TfR2 Haemochromatosis (Type 3)

Described in 2000 TfR2, haemochromatosis was the first subtype attributed to mutations outside of *HFE*.[33] It is rare with symptomatic severity of falling between those of Types 1 and 2. Onset can be both adult and juvenile and clinical symptoms are similar to typical *HFE* haemochromatosis. TfR2 is homologous to TfR1 and is suspected to function as an iron sensing molecule in the liver. Defects in TfR2 are thought to affect the BMP/SMAD pathway and cause aberrant iron regulation by hepcidin.[34]

Ferroportin Haemochromatosis (Type 4A and B)

Ferroportin is the only known exporter of iron from cells in the body. It is also involved in the pathogenesis of the only autosomal dominant subtype of haemochromatosis. Ferroportin disease also differs in its clinical presentation. Both 4A and 4B are caused by mutations in the gene encoding *FPN*.[32] However, clinical presentations of 4A and 4B vary considerably. Type 4B is associated with mutations that affect the affinity of ferroportin for hepcidin and mediate hepcidin resistance. In 4A, an inactivation of ferroportin leads to loss of iron export function, causing increased hepatic and tissue iron deposition with low serum transferrin. Normal-low serum Tf, high SF, iron-loaded macrophages, and iron-deficiency anaemia[32,35] is the common biochemical picture. Complications are mild, with minimal sinusoidal fibrosis (extending to hepatocytic fibrosis with advanced age), but phlebotomy is poorly tolerated.[25,32,36] Conversely, 4B is a gain-of-function mutation, with ferroportin becoming insensitive to hepcidin.[31,35] Type 4B shows a more typical clinical picture with elevated transferrin saturation.[23]

THE TYPICAL NATURAL HISTORY AND DISEASE BURDEN OF HAEMOCHROMATOSIS

Gan et al.[37] outlines a four-stage process of iron overload disease: 1) Genetic predisposition to iron overload disease, and mildly raised serum TF; 2) Asymptomatic iron overload (0-5 g of parenchymal iron overload, >300 ug/l SF); 3) Nonspecific symptoms of iron overload (lethargy, arthralgia, malaise, etc.); 4) Iron-overload related disease (diabetes, cirrhosis, arthritis) Clinical complications of untreated iron overload are bronze pigmentation, insulin-dependent diabetes mellitus, hypopituitarism, and subsequent hypogonadism, arthropathies (classically the second and third metacarpophalangeal joints), cardiomyopathy, and hepatic cirrhosis.[25,38] Laboratory measures of liver function may show raised alanine transaminase (ALT) and aspartate aminotransferase (AST).[6] However, Adams et al.[39] note, that the stereotypical presentation of 'bronze diabetes' is quite rare (1%). Instead, it is more common to detect disease either on familial screens or when patients present with nonspecific symptoms.[25] Hepatic iron deposition histologically starts in the acinar zones 1 and 2 (peri-portal), and eventually spreads to central veins. With disease progression iron granules become denser and larger, and begin to involve kupffercells.[35] As mentioned above, identical pathophysiology is seen in Types 2 and 3. Hepatocellular carcinoma (HCC) is a late stage complication of cirrhosis in 6% of male and 1.5% of female patients.[37] A 30-year study by Fracanazani et al.[40] found that of 70 recorded deaths, 56% were due to HCC. Additionally, there is an increased risk of colorectal and breast cancer in C282Y homozygotes, and a tripled risk of colorectal cancer in H63D homozygotes also positive for mismatch repair mutations.[37,41]

HFE haemochromatosis appears to have a risk window. A Norwegian study screened 65,000 adults for iron overload, identifying new cases in 177 males and 92 females. 46% of the male cases were in the 40-49 year range, with an additional 34% being 50-59 years. Females were older, with 25% being 50-59 years and 23% being 60-69 years.[42] This matches work by other groups, which suggest an onset of symptoms around the fourth to fifth decades of life, with women being affected several years older.[25,26,42,43] At the time of diagnosis, men with stage 0-1 fibrosis averaged 40 years, and women 42 years. Men and women with stage 2-4 averaged 45 years and 50 years respectively.[7] There is no generalised association between serum iron and age,[41] but patients with raised SF show a correlation, with patients reaching 500-1,000 ug/l by 41.8 years and plateauing >3,000 ug/l by 54.7 years of age.[7] Most cases manifest by 55

years, and it is rare for SF to rise significantly after this age.[5] Not all C282Y homozygotes progress to end-organ disease. In many SF remains normal.[7,39-41] The Melbourne Collaborative Cohort Study showed that only 50% of patients with SF 300-1,000 ug/l progressed to clinical iron overload (>1,000 ug/l) in a 12 year study.[41] Unfortunately, the rarity of non-HFE strains precludes us from knowing their penetrance, but it is believed to vary between types.[31]

The incomplete penetrance of disease in persons with associated genetic predispositions means that from genotype to phenotype, iron overload and subsequent disease cannot be explained by any single mutation. This is demonstrated in the variable disease prevalence and progression, exemplified by 60-80% of C282Y homozygotes displaying raised SF, but only 24-43% of men, and 1-14% of women, showing clinical iron overload.[6,7,38,41] Known risk factors cause one of either: increased iron availability, reduction of hepcidin activity, or acceleration of hepatic fibrosis.[25,26,37,44] Protective factors include consumption of non-citrus fruit, female sex, Ca2+ channel-blockers, occult/menstrual bleeding, and chronic malabsorptive states.[25,26,35,44] However, Fracanzani et al.[40] found 27% of *HFE* and 39% of non-*HFE* hereditary haemochromatosis had no known environmental risk factors implicating other unknown genetic influences.

MANAGEMENT AND TREATMENT

Treatment for haemochromatosis with venesection and phlebotomy has remained unchanged over the years. Early intervention, prior to the onset of symptoms, improves patient prognosis.[45] Furthermore, venesection in symptomatic individuals improves certain symptoms, such as skin pigmentation, while not having an effect on others such as cirrhosis and arthropathy.[45] Current guidelines suggest that phlebotomy should be used when SF is above the reference range, but further study into the area is required. The aims of venesection are 2-fold, firstly, to directly reduce serum iron by depleting haemoglobin levels, and secondly, to mobilise stored iron from tissues to replace the depleted circulating levels. The current treatment guidelines suggest yearly follow-up in patients whose SF levels are within normal range.

Table 1: Types of hereditary haemochromatosis.

TYPE	GENE	FUNCTION	PREVALENCE	PENETRANCE	ASSOCIATED FEATURES
TYPE 1	*HFE*	Hepcidin upregulation	Most common form worldwide; varies by race	Autosomal recessive: 2-28% penetrance	Classical haemochromatosis
TYPE 2A	*HJV* (haemojuvelin)	Hepcidin upregulation	Rare. More common than Type 2B	Autosomal recessive	Severe, early onset. Associated with hypogonadal hypogonadism and cardiomyopathy.
TYPE 2B	*HAMP* (hepcidin)	Inhibition of enterocyte iron uptake	Rare	Autosomal recessive	
TYPE 3	*TFR2* (transferrin receptor 2)	Hepatic transferrin, possible hepcidin upregulation.	Rare. Commonest form in Japan, also seen in Italy and Brazil.	Autosomal recessive: high, but possibly confounded by observer bias.	Can be either juvenile or adult onset. Most cases are adult, with a slightly earlier and more severe course than Type 1.
TYPE 4A	*SLC40A1* (ferroportin)	Iron export	Rare	Autosomal dominant: high	Reduced end-organ damage and serum iron. Increased tissue sequestration. Reduced serum levels.
TYPE 4B	Ferroportin	Iron export	Rare	Autosomal dominant	Similar to classical haemochromatosis.

However, in those with elevated SF, venesection is to be used to bring SF down to maintenance levels.[46] There has been some debate in the literature with regards to the ideal level at which SF should be maintained. With reports that overzealous treatment may have unintended deleterious effects, the traditional suggestion of maintaining ferritin levels below 50 ug/l has been updated. Although the issue has not been settled conclusively, current guidelines suggest maintenance levels between 50-100 ug/l.[25,46] It is known that 1 unit of blood contains approximately 200-250 mg of iron. The amount of iron removed at each venesection, however, is highly variable.[45] This means that venesection intervals and treatment regimens need to be personalised to each patient informed on a case by case basis. The aforementioned treatment strategies work best with Types 1-3 and also with Type 4B. However, due to the aberrant iron export from cells in Type 4A patients may not tolerate venesection well.[47] Hence management of patients with Type 4A haemochromatosis is not so straightforward.

NEW RESEARCH AND INTERNATIONAL CONSORTIUM

Once the *HFE* locus was identified[48] it became apparent that it alone did not explain all cases of haemochromatosis. Parallels have been drawn between C282Y in haemochromatosis and Wilson's disease. However C282Y is a common polymorphism where *ATP7B* mutations in Wilson's disease are rare and usually deleterious for protein function. Studies resulted in the eventual identification of hepcidin, Transferrin receptor 2, HJV, and ferroportin.[29,30,33,48,49] Although mutations in these genes are much less common than in *HFE* they do explain many cases of haemochromatosis that are non-*HFE* or *HFE* negative (Table 1) and thus cases that were previously puzzling clinically. However, once again, clinicians looking after patients with iron overload were puzzled. In stark contrast to other familial liver diseases that are inherited as autosomal recessive traits, virtually all subjects with genetic mutations leading to the other diseases developed full blown disease, e.g. Wilson's disease. In 2010, an international consortium was formed to study the 'black swans' of this disease i.e. rare cases of advanced disease that stand out from the majority.[50] This group of eight centres fom the USA,Canada, and Australia is now funded by the National Institutes of Health and, so far, has discovered one significant gene (*GNPAT*) which appears to predispose to significant expression of this disease. The precise function and possible role *GNPAT* plays in iron metabolism is currently not known. Further modifying genes may be anticipated which assist clinicians in predicting those individuals predisposed to severe disease.

SUMMARY

The low penetrance of symptomatic haemochromatosis in those with HFE mutations and the worldwide prevalence of haemochromatosis in the absence of HFE mutations both have clarified the necessity to study other factors contributing to disease. The study of those who show iron overload without an underlying change in HFE has led to the discovery and classification of the non-HFE or HFE negative haemochromatosis; juvenile haemochromatosis, ferroportin disease, and transferrin receptor 2 associated haemochromatosis. Unfortunately, the reasons behind the incomplete penetrance of disease phenotype in those with HFE mutations has not yet been fully explained. Even though various environmental factors have been shown to influence the natural progression, it has become evident that further genetic factors must also play a part. To this end the discovery of GNPAT as a significant player in disease expression opens up the door to future investigation into this area. It also opens up future avenues for early detection and treatment of haemochromatosis.

REFERENCES

1. Von Recklinghausen F. Uber haemochromatose. Tageblatt Versammlung Dtsche Naturforscher Arzte Heidelberg. 1889;62:324-5.
2. McDonald CJ et al. Iron storage disease in Asia-Pacific populations: the importance of non-HFE mutations. J Gastroenterol Hepatol. 2013;28(7): 1087-94.
3. Wood MJ et al. The global burden of iron overload. Hepatol Int. 2009;3(3): 434-44.
4. Allen KJ et al. Iron-overload-related disease in HFE hereditary hemochromatosis. N Engl J Med. 2008;358(3):221-30.
5. Powell LW et al. Screening for hemochromatosis in asymptomatic subjects with or without a family history. Arch Intern Med. 2006;166(3):294-301.
6. Beutler E et al. Penetrance of

845G--> A (C282Y) HFE hereditary haemochromatosis mutation in the USA. Lancet. 2002;359(9302):211-8.

7. Guillemot J et al. Implication of the proprotein convertases in iron homeostasis: proprotein convertase 7 sheds human transferrin receptor 1 and furin activates hepcidin. Hepatology. 2013;57(6):2514-24.

8. Ganz T. Systemic iron homeostasis. Physiol Rev. 2013;93(4):1721-41.

9. Kanamori Y et al. Hepcidin expression in liver cells: evaluation of mRNA levels and transcriptional regulation. Gene. 2014;546(1):50-5.

10. Przybyszewska J, Zekanowska E. The role of hepcidin, ferroportin, HCP1, and DMT1 protein in iron absorption in the human digestive tract. Prz Gastroenterol. 2014;9(4):208-13.

11. Anderson GJ et al. The ceruloplasmin homolog hephaestin and the control of intestinal iron absorption. Blood Cells Mol Dis. 2002;29(3):367-75.

12. Donovan A et al. The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. Cell Metab. 2005;1(3): 191-200.

13. Silvestri L et al. The extrahepatic role of TFR2 in iron homeostasis. Front Pharmacol. 2014;5:93.

14. Krause A et al. LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. FEBS Lett. 2000;480(2-3):147-50.

15. Park CH et al. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem. 2001;276(11):7806-10.

16. Pigeon C et al. A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. J Biol Chem. 2001;276(11):7811-9.

17. Rochette L et al. The iron-regulatory hormone hepcidin: a possible therapeutic target? Pharmacol Ther. 2014;pii:S0163-7258(14)00166-1.

18. Flanagan JM et al. In vivo imaging of hepcidin promoter stimulation by iron and inflammation. Blood Cells Mol Dis. 2007;38(3):253-7.

19. D'Alessio F et al. The hemochromatosis proteins HFE, TfR2, and HJV form a membrane-associated protein complex for hepcidin regulation. J Hepatol. 2012;57(5):1052-60.

20. Bridle KR et al. Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homoeostasis. Lancet. 2003;361(9358):669-73.

21. Babitt JL et al. Modulation of bone morphogenetic protein signaling in vivo regulates systemic iron balance. J Clin Invest. 2007;117(7):1933-9.

22. Camaschella C. BMP6 orchestrates iron metabolism. Nat Genet. 2009;41(4): 386-8.

23. Bardou-Jacquet E et al. Non-HFE hemochromatosis: pathophysiological and diagnostic aspects. Clin Res Hepatol Gastroenterol. 2014;38(2):143-54.

24. Wallace DF, Subramaniam VN. Non-HFE haemochromatosis. World J Gastroenterol. 2007;13(35):4690-8.

25. Kanwar P, Kowdley KV. Diagnosis and treatment of hereditary hemochromatosis: an update. Expert Rev Gastroenterol Hepatol. 2013;7(6):517-30.

26. Siddique A, Kowdley KV. Review article: the iron overload syndromes. Aliment Pharmacol Ther. 2012;35(8): 876-93.

27. De Gobbi M et al. Natural history of juvenile haemochromatosis. Br J Haematol. 2002;117(4):973-9.

28. Pietrangelo A. Hereditary hemochromatosis—a new look at an old disease. N Eng J Med. 2004;350(23): 2383-97.

29. Roetto A et al. Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. Nat Genet. 2003;33(1):21-2.

30. Papanikolaou G et al. Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. Nat Genet. 2004;36(1):77-82.

31. Bardou-Jacquet E et al. Variable age of onset and clinical severity in transferrin receptor 2 related haemochromatosis: novel observations. Br J Haematol. 2013;162(2):278-81.

32. Camaschella C, Poggiali E. Rare types of genetic hemochromatosis. Acta Haematol. 2009;122(2-3):140-5.

33. Camaschella C et al. The gene TFR2 is mutated in a new type of haemochromatosis mapping to 7q22. Nat Genet. 2000;25(1):14-5.

34. Worthen CA, Enns CA. The role of hepatic transferrin receptor 2 in the regulation of iron homeostasis in the body. Front Pharmacol. 2014;5:34.

35. Bassett ML et al. The changing role of liver biopsy in diagnosis and management of haemochromatosis. Pathology. 2011;43(5):433-9.

36. Brissot P. Hereditary hemochromatosis. Hematology. 2013;18(6):370-1.

37. Gan EK et al. Natural history and management of HFE-hemochromatosis. Semin Liver Dis. 2011;31(3):293-301.

38. Kanwar P, Kowdley KV. Metal storage disorders: Wilson disease and hemochromatosis. Med Clin North Am. 2014;98(1):87-102.

39. Adams PC. The natural history of untreated HFE-related hemochromatosis. Acta Haematol. 2009;122(2-3):134-9.

40. Fracanzani AL et al. Hemochromatosis in Italy in the last 30 years: role of genetic and acquired factors. Hepatology. 2010;51(2):501-10.

41. Nadakkavukaran IM et al. Screening for hereditary haemochromatosis. Pathology. 2012;44(2):148-52.

42. Asberg A et al. Screening for hemochromatosis: high prevalence and low morbidity in an unselected population of 65,238 persons. Scand J Gastroenterol. 2001;36(10):1108-15.

43. Pietrangelo A. Hereditary hemochromatosis: pathogenesis, diagnosis, and treatment. Gastroenterology. 2010;139(2):393-408, 408.e1-2.

44. Adams PC et al. Hemochromatosis and iron-overload screening in a racially diverse population. N Eng J Med. 2005;352(17):1769-78.

45. Bacon BR. Diagnosis and management of hemochromatosis: 2011 practice guideline by the American Association for the Study of Liver Diseases. Hepatology. 2011;54(1):328-43.

46. European Association for the Study Of the Liver. EASL clinical practice guidelines for HFE hemochromatosis. J Hepatol. 2010;53(1):3-22.

47. Pietrangelo A. The ferroportin disease. Blood Cells Mol Dis. 2004;32(1):131-8.

48. Feder JN et al. A novel MHC class I–like gene is mutated in patients with hereditary haemochromatosis. Nat Genet. 1996;13(4):399-408.

49. Montosi G et al. Autosomal-dominant hemochromatosis is associated with a mutation in the ferroportin (SLC11A3) gene. J Clin Invest. 2001;108(4):619-23.

50. Emond MJ et al. Exome sequencing identifies genes and variant alleles associated with severity of iron overload in hemochromatosis HFE C282Y homozygotes. Blood. 2013;122(21):179.