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Molecular genetics and control of iron metabolism in hemochromatosis

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ABSTRACT

Background and Objectives. **Hereditary hemochromatosis (HC) is an inborn error of iron metabolism that leads to progressive iron overload. Considerable advances in the knowledge of molecular events in iron metabolism have been recently obtained. These molecular findings, the cloning of the gene responsible for HC (HFE gene) and the results of preliminary studies on the HFE protein prompted us to review this topic.**

Information Sources. **The material examined in this review article includes papers and abstracts published in the Journals covered by the Science Citation Index© and Medline©. The authors have been working in the field of HC for several years and have contributed eleven of the quoted papers.**

State of Art and Perspective. **HC is now recognized as the genetic disease characterized by the homozygosity for the Cys→Tyr substitution at position 282 (C282Y) of the HFE protein. The mutation abolishes the association of the HFE protein with β_2-microglobulin (β_2M), making the complex unable to gain the cell surface. Thus HC is an example of abnormal trafficking of the corresponding proteins. It is clear by the analysis of its structure that HFE protein is not an iron transporter itself, but has a regulatory role in iron metabolism. Its peculiar localization in the crypt cells of the small intestine suggests an important role in iron trafficking at this level. However, other proteins are involved in iron uptake, as the recently cloned Nramp2, the first iron transporter discovered in mammalians. Nramp2 has a recognized role both in the intestinal iron uptake and in the iron transport within the erythroblast. The relationships between HFE and Nramp2 are still unexplored. The recent association of HFE gene with transferrin receptor (TfR) in trophoblast cells opens new possibilities on its role in cellular iron uptake. The existence of other forms of genetic iron overload suggests that the scenario of iron proteins is not yet fully defined. Further studies in this field will contribute to our knowledge of iron metabolism regulation in humans.**

Key words: iron metabolism, hemochromatosis, HFE gene

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Hemochromatosis (HC) is an inherited recessive disorder causing high intestinal iron absorption and clinical symptoms related to excessive iron deposition in parenchymal cells of the liver, pancreas, heart and other organs. HC is the most common recessive disorder among Caucasians. For a complete review on all aspects of the disease the readers are referred to a recent paper in this journal.[1]

Considerable advances in the knowledge of the molecular events of iron delivery to cells by transferrin, the molecular control of cellular iron metabolism and of molecular genetics of disorders characterized by iron overload have been obtained over recent years. The major steps of these advances have been the following: a) the identification of the mechanisms of action of iron regulatory proteins (IRP1 and IRP2), which modulate in an iron-dependent way, the availability of iron proteins such as transferrin receptor and ferritin;[2] b) the cloning of HFE, the gene responsible for hemochromatosis, which surprisingly resulted to be an MHC-related gene;[3] c) the recognition of the first iron-transporter in mammalians, Nramp2, a member of the Nramp gene family, which revealed an unexpected link between iron transport and host defense.[4,5]

This review will focus on recent advances in molecular biology of iron metabolism and iron overload, also attempting to outline the molecular mechanism of intestinal iron absorption under physiological conditions and its derangement in HC. In addition, it will show that the genetic disorders causing iron overload extend beyond the HFE-related hemochromatosis.

The HFE gene and molecular genetics of HC

In August 1996 an American biotechnological company, Mercator Genetics, cloned the gene responsible for HC using a classical approach of positional cloning.[3] Formerly named HLA-H (H for Hemochromatosis), it was then redefined HFE by the *WHO Nomenclature Committee for Factors* of the HLA system.[6] HFE is an atypical HLA-class I-like gene, mapping approximately 4 Megabases telomeric to HLA-A.[3] A schematic structure of the gene is

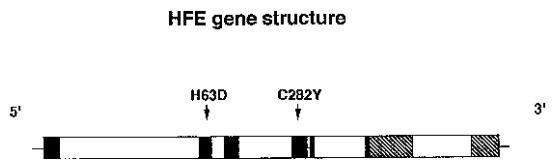

Figure 1. Structure of the HFE gene and localization of the two mutations associated with HC.

Table 1. Main features of the HFE gene.

Gene type	MCH class-I atypical gene
Genomic localization	6p22
Genomic structure	12 Kb DNA 7 exons (the 7th is non coding)* Housekeeping gene promoter ?°
Expression	Ubiquitous (low levels), except brain Liver and small intestine (higher levels)
Type of transcript	Unique transcript (about 4 Kb)
Homologies	HLA-A2, HLA G Fc-receptor

**It contains one poly (A) addition signal (3); °the 5' upstream region lacks classical TATA and CAAT boxes and shows a relatively GC rich region (55).*

shown in Figure 1 and its main features are reported in Table 1. HFE is expressed ubiquitously at low levels; its expression is slightly more evident in liver and small intestine, tissues believed to be affected in HC.[3] Since the first report it was considered as a strong candidate gene for HC, as most patients were carriers, at the homozygote state, of a *missense* mutation, changing cysteine at position 282 to tyrosine (C282Y), a change introduced by the substitution G→A at position 845 of the gene.[3] Although this change could have been a simple polymorphism in linkage with the causal mutation, the substitution introduces a non-conservative change in the HFE molecule, that disrupts an S-S bond between the α3 domain of the molecule and β_2-microglobulin (β_2M). The causality for the mutation was indirectly supported by the animal model of β_2-M knock out mice, who develop a type of iron overload indistinguishable from human hemochromatosis.[7-9]

Several studies have confirmed that C282Y is the most prevalent mutation in hemochromatosis.[10-14] However, the frequency of the mutation is variable. Studies on selected Australian families of Scottish and Irish origin have shown a 100% segregation of the mutation with the disease.[10] Among patients with severe iron overload homozygosity for C282Y attains approximately 90% in Northern Europe[11] and about 83% in two American series.[3,12] In Southern Europe it accounts for 64-76% of severely iron-loaded patients.[13,14] Studies on normal populations have shown that the incidence of C282Y mutation reflects the distribution of the disorder.[15]

A second mutation was identified in the original work in the coding sequence of the gene, a C→G substitution at position 187 within the gene, changing histidine at position 63 to aspartic acid (H63D). The localization of the two mutations is shown in Figure 1. H63D occurs in the α1 domain of the molecule and does not hamper the interaction with β_2M, as shown in a model of human embryonic kidney cells transfected with normal and mutant HFE cDNAs.[16] However, H63D is found with an increased frequency in the affected subjects heterozygous for C282Y in different populations.[3,11,12,13] Recently, it has been shown that also homozygotes for H63D are also enriched among HFE patients.[17] Thus, there is a modestly increased risk to develop iron overload associated with H63D.[18] Since this mutation is frequent in normal patients, it represents an example of a common variant with a modest effect that rarely produces an affected phenotype.[18] It is possible that it leads to iron overload only in cooperation with other genetic or environmental factors.[19] Since the H63D mutant protein does not hamper the association with β_2M,[16] the iron absorption deregulation caused by this mutation must be achieved through a mechanism different from the C282Y mutation.

Extensive sequencing of the HFE gene in patients with severe iron overload, negative for C282Y failed to reveal other mutations in the coding sequences of the gene or in intron-exon boundaries.[3,13,20] The only exception is a frameshift deletion (deletion of a cytosine) in the coding sequence, which leads to a premature stop codon downstream in the deletion; this change has been found at the heterozygous state in a single male patient aged 60 from England,[21] but the inheritance of the mutation could not be demonstrated in the family.

The identification of the HFE gene has stimulated research on the corresponding protein. The data in so far obtained allow us to revisit the issue of iron absorption deregulation in HC.

The molecular defect in HFE

In the absence of an active excretory mechanism, body iron homeostasis is primarily dependent on regulation of iron absorption from the small intestine. In genetic HC this regulation is defective, and dietary iron absorption is high and inappropriate to the levels of body iron stores.[22] The molecular pathways responsible for the tight control of iron absorption in humans and, particularly, for the derangement of intestinal iron uptake-transfer in HC, are still unknown. In this context, understanding the role of the HFE protein in intestinal iron absorption may be instrumental for clarifying the whole scenario of iron metabolism in humans.

Iron proteins and intestinal iron absorption

Traditionally, transferrin (Tf), transferrin receptor, (TfR), and ferritins are considered as the key proteins in iron metabolism involved in serum transport, cellular uptake and tissue storage of the metal, respectively.[23] Iron uptake involves Tf-iron binding to its specific cellular receptor (TfR), Tf-TfR complex entering in the cell by endocytosis and apotransferrin-TfR recycling after iron release.[24] Inside the cell, iron is stored as soluble ferritin, a multimeric protein composed of heavy (H) and light (L) chains. Intracellular iron homeostasis is maintained through the regulation of the synthesis of ferritin and transferrin receptor in a coordinate and opposite manner.[25] This task is achieved through the functioning of two cytoplasmic proteins called *iron regulatory proteins* 1 and 2 (IRP-1 and IRP-2) which bind in an iron-dependent way to *iron responsive elements* (IRE) in untranslated regions of ferritin and transferrin receptor mRNA. IRP-1 has two mutually exclusive functions, which are switched by changes in an 4Fe-4S cluster: under conditions of iron deficiency in the cellular labile iron pool (LIP) the cluster is disassembled, IRP-1 binds to IRE and decreases the synthesis of ferritin but enhances that of transferrin receptor, thus providing the cell with readily available iron. Conversely, when iron is abundant, the cluster is reconstituted, IRP-1 dissociates from IRE acquiring aconitase function and iron sequestration prevails over iron uptake.[2, 26-27] IRP-2 controls the expression of ferritin and transferrin receptor mRNAs with a specificity and an efficacy similar to those of IRP-1, but lacks an iron sulfur cluster, is regulated by iron through proteolysis and is differentially expressed in various tissues. In addition, IRP-2 is differentially modulated under some pathophysiological situations.[28-29]

The intestinal (e.g. duodenal) epithelial cell is the main physiologic site of iron absorption and has a central role in governing body iron homeostasis.[29] Entry of ionic iron in the duodenal mucosal cell is possibly mediated by a specific brush border receptor. In spite of several studies, clear evidences for a major iron-uptake carrier have not been presented. Recently, by using a positional cloning strategy to identify the causative mutation in mice with microcytic anemia and severe defects in intestinal iron absorption (*mk*), a strong candidate gene for *mk* has been identified.[4] The phenotype is a consequence of a *missense* mutation (G185R) in *Nramp2*, a previously identified gene of unknown function. More recently, the same G185R mutation found in *mk* mice has been identified in *Nramp2* gene of *Belgrade* rat, which has an autosomal recessive microcytic anemia similar to the *mk* mouse.[30] Previous studies on the biochemical defect in *Belgrade* rat had shown an inability of transferring iron out of the transferrin cycle endosome within the erythroid cell.[30] These data indicate that *Nramp2* is essential both for normal iron uptake and for transport of iron in the erytroblast.[4,30] *Nramp2* is homologous to *Nramp1*, a gene active in host defense.[31] The normal function of *Nramp1* in host defense is unknown. The gene is expressed only in macrophages, is localized to late endocytic compartment and could have a role in phagocytic clearance of infecting organisms.[4] *Nramp2* may be a main protein involved in intestinal iron transport although its role in HC is still undefined. It now appears clear that *Nramp* or other related proteins might play a central role in iron uptake at the intestinal lumen. On the contrary, the role played by TfR is definitely not as an iron-carrier at the luminal site. In fact, the absence of Tf mRNA in duodenal cells and TfR on the brush border membrane,[32,33] and the conflicting data on the ability of ^{59}Fe-transferrin to donate its iron to the mucosal cells,[34,35] argues against a direct role of TfR in the uptake of iron from the intestinal lumen (mucosal uptake). However, the preeminent localization of TfR in the basal-lateral membrane region of crypt and villous cells,[33,36] may be strategically important to facilitate the transport of iron out of the cell into the circulation (carcass transfer) or, in reverse, from the bloodstream into the intestinal cell. Carcass transfer of iron is typically influenced by the hypoxic stimulus[37] and, even more, by the increased erythroid demand.[38] However, in this case TfR, after complexing plasma-derived iron-poor Tf should deliver iron-rich Tf back to the plasma. Such a model, however, does not appear plausible in view of the present knowledge on TfR receptor cycle. On the other hand, as allowed by the traditional model of Tf-TfR cycle, TfR could facilitate the entry of iron into the cell from plasma by internalyzing plasma-derived iron-poor Tf. Thus, it is more plausible to postulate that Tf derived from plasma, although not involved in the uptake of iron at the luminal membrane, has a role in transferring iron into the mucosal cell from the bloodstream.

The HFE gene product and intestinal iron absorption

The human HFE protein predicted from the cDNA sequence is composed of 343 amino acids.[3] It is most homologous to major histocompatibility complex (MHC) class I molecules that are integral membrane proteins with three extracellular loops (α1, α2, and α3), a transmembrane region, and a short cytoplasmic tail. The C282Y mutation was predicted to disrupt a critical disulfide bond in the α3 loop of the HFE protein and to abrogate binding of the mutant HFE protein to β_2M and its transport to and presentation on the cell surface. These hypotheses correlate well with the β_2M knock-out mouse studies, where an iron overload resembling HC has been described.[7-9] Thus the mutation C282Y may be equivalent from a functional point of view to a disrupted β_2M. Feder *et al.*[16] confirmed these predictions by demonstrating the failure of the C282Y mutant HFE protein to associate with endogenous β_2M in human embryonic kid-

ney cells (293 cells), which were stably transfected with the mutant cDNA. Yet, a recent study by Waheed *et al*.[39] demonstrated that the wild-type HFE protein expressed in transfected COS-7 cells associates with coexpressed β_2M and is transported to the cell surface, but these capabilities are lost by the C282Y mutant HFE protein. Much of the C282Y mutant protein remains in high Mr aggregates, as usually misfolded glycoproteins and MHC class I protein molecules do, and fails to undergo late Golgi processing. The delayed delivery of the C282Y mutant protein from the ER to the middle Golgi is likely to be the cause of the accelerated degradation of a large fraction of the newly synthesized C282Y protein.[39] These authors suggested that the C282Y mutation, which prevents this association with β_2M, results in a reduction in the amount of mutant protein delivered from ER to Golgi, and also prevents delivery to the cell surface because of a block in the transit of the protein from the middle to trans Golgi compartment. Unlike the C282Y mutant protein, the H63D mutant protein associates with β_2M. In addition, its synthesis, intracellular transport, oligosaccharide processing, and cell surface expression in COS-7 cells are all similar to those of wild-type HFE protein. Despite its apparently normal behavior in all these functional criteria, genetic evidence[17-18] suggests that it can contribute to HC.

The antigen presenting function of the classical MHC class I molecules is well known, but other roles have been found for these molecules such as differentiation antigens or hormone receptors.[40] On the other hand, a clear definition and functions are still awaited for the non-classical MHC class I gene such as HLA-G, HLA-E, HLA-F. There are many hypotheses on their functions, including a signal-transducing activity, with their ligation leading to the appearance of a series of second messengers.

One of the best studied example is the neonatal Fc receptor (FcRn), which in the newborn binds IgGs from the ingested mother's milk and transcytoses them into the bloodstream, where the IgGs are freed via a pH dependent mechanism.[41] This family of molecules have been shown to be very versatile, with the ability of triggering various biological responses upon binding antibody-antigen complexes. In conclusion, the structure of HC-gene product may be very versatile ranging from signal transduction to peptide transporter. The fact that HFE protein does not show iron binding domains raises the question as to whether HFE interacts with other iron-proteins or iron-sensors. Interestingly, HFE protein has been recently localized by immunohistochemistry in the gastrointestinal tract, but, surprisingly, it was mainly expressed in cryptic duodenal cells and absent at luminal site of villous cells.[42] The unique localization of the HFE protein in the crypts of the small intestine suggests a special role for the HFE protein in these cells and, at the same time, a special importance of iron metabolism of cryptic cells in maintenance of iron homeostasis. In a recent study, human placenta was used with immunohistochemistry to define the site of the HFE protein expression and, by using immunoaffinity chromatography and immunoprecipitation, to identify some of the proteins with which it is associated.[43]

The study demonstrated that the HFE protein is expressed on the apical plasma membrane of the syncytiotrophoblasts, where iron is normally transported to the placenta via transferrin receptor-mediated endocytosis. It also showed that the HFE protein is physically associated with the TfR, that plays a central role in iron transport across the placenta. Thus, the novel HFE protein could be one link in regulating transfer of iron from maternal blood to the fetus. Seemingly, HFE could associate to TfR at the basolateral membrane and modulate iron availability in cryptic cells. Iron absorption is a tightly regulated process and depends on many factors, including the body's demand for iron.[22] Mature epithelial cells of the mid to upper villus are the site of dietary iron absorption, while the cells of the intestinal acquire iron from the circulation through TfR and, only after cells migrate and become mature enterocytes in the villus, they absorb iron from the diet. Therefore, it might be possible that HFE in cryptic cells might respond to body iron stores and demands and that a failure in this feedback regulatory loop might cause a dissociation between iron absorption and body iron-stores in HC. A *faulty primed* cryptic cell, once reaches the tip of the villi, should trigger high iron-absorption transfer.

What's the nature of the signal in mucosal cell for enhanced iron absorption? A possible signal might be the iron-content of enterocytes. Under normal conditions, intestinal iron absorption is inversely related to body-iron stores and, possibly, to mucosal cell-iron content. Indeed, the concept of a *paradoxical iron-deficiency* in HC enterocytes was proposed after biochemical and molecular biology studies showed a reduced accumulation of Ft, up-regulation of TfR and enhanced IRP activity in intestine of HC patients.[32,44] IRP activation, particularly, responsible for enhanced TfR expression and decreased ferritin synthesis, indicated that the duodenal labile iron pool in HC is reduced as in iron-deficiency.[44] However, after the original proposal by Hahn and Granick of the *mucosal block* where ferritin functioned as a block for unwanted iron,[45,46] other investigators challenged the role of mucosal ferritin in regulating iron absorption,[47,48] and a clear evidence for such a feedback mechanism exerted on iron absorption by the mucosal cell iron status has not been presented yet.

Future perspectives

While we have recently witnessed a dramatic enhancement of our knowledge on the mechanisms whereby the C282Y mutation might impair the func-

tion of the mutant HFE protein, many questions are still unaswered. How is HFE protein related to the maintenance of iron homeostasis? How does the C282Y mutation produce the full HC phenotype? Has the H63D mutation any biochemical implication? Has the biochemical link between TfR and HFE established in placenta a functional implication in the intestine? If so, through which mechanisms HFE influences TfR function and intestinal cell iron metabolism at the basolateral membrane? It is easy to predict that in the near future many of these questions will be answered.

It must also be considered that other genetic disorders causing primary iron overload do exist. A form of systemic siderosis, reported in Japan, characterized by diffuse iron accumulation, neurological abnormalities and elective storage in the liver and basal ganglia is caused by ceruloplasmin deficiency.[49,50] However, genes responsible for other genetic forms of iron overload are not yet identifed. The siderosis reported in Central Africa results from the interaction of an HLA-independent genetic component and environmental factors[51] and the rare juvenile form of hemochromatosis, is both unrelated to HFE and unlinked to the short arm of chromosome 6.[52] Also, familial cases of hemochromatosis unrelated to HFE and without juvenile expression have been observed (refs. #53, 54 and unpublished results). All these *non-HFE-related hemochromatosis* constitute a field of active research for new iron genes.

The existance of distinct genetic diseases causing iron overload strengthens the idea that a network of proteins play a role in iron absorption and metabolism. The elucidation of the genetic defects in all these conditions will certainly contribute to further understand the molecular mechanism of iron metabolism and to unravel its derangement in HC.

Note added in proofs

It has recently been shown that HFE protein assocaition with TfR occurs in cultured embryonic kidney cells transfect - ed with the wild type HFE protein. This association would decrease the affinity of TfR for Tf. This regulatory function is absent in C282Y abd reduced in H63D mutant proteins. These results establish a link between HFE and iron metab - olism and suggest a causative role for H63D (Feder et al. Proc Natl Acad Sci USA 1998; 95:1472-7).

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References

1. Camaschella C, Piperno A. Hereditary hemochromatosis: recent advances in molecular genetics and clinical management. Haematologica 1997; 82:77-8.
2. Klausner RD, Rouault TA, Harford JB. Regulating the fate of mRNA: the control of cellular iron metabolism. Cell 1993; 72:19-28.
3. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nature Genet 1997; 13:399-408.
4. Fleming MD, Trenor CC, Su MA, et al. Microcytic anaemia mice have mutation in Nramp2, a candidate iron transporter gene. Nature Genet 1997; 16:383-90.
5. Gunshin H, Mackenzie B, Berger UV, et al. Cloning and characterization of a mammalian proton coupled metal ion transporter. Nature 1997; 388:482-8.
6. Bodmer JG, Parham P, Albert ED, Marsh SGE. Putting an hold on 'HLA-H'. Nature Genet 1997; 15:234-5.
7. de Sousa M, Reimao R, Lacerda R, Hugo P, Kaufmann SH, Porto G. Iron overload in beta-2-microglobulin-d e ficient mice. Immunol Lett 1994; 39:105-11.
8. Rothenberg BE, Voland JR. β2 knockout mice develop parenchymal iron overload: a putative role for class I genes of the major histocompatibility complex in iron metablism. Proc Natl Acad Sci USA 1996; 93:1529-34.
9. Santos M, Schilham MW, Rademakers LHP, Marx JJM, de Sousa M, Clevers H. Defective iron homeostasis in β2 microglobulin deficient mice recapitulates hereditary hemochromatosis in man. J Exp Med 1996; 184:1975-85.
10. Jazwinska EC, Mac Cullen L, Busfield F, et al. Haemochromatosis and HLA-H. Nature Genet 1996; 14:249-50.
11. Jouanolle AM, Gandon G, Jezeque P, et al. Haemochromatosis and HLA-H. Nature Genet 1996; 14:251-2.
12. Beutler E, Gelbert T, West C, et al. Mutation analysis in hereditary hemochromatosis. Blood Cells Molec Dis 1996; 22:187-94.
13. Carella M, D'Ambrosio L, Totaro A, et al. Mutation analysis of HLA-H gene in Italian hemochromatosis patients. Am J Hum Genet 1997; 60:828-32.
14. Borot N, Roth MP, Malfroy L, et al. Mutations in the MHC class I-like candidate gene for hemochromatosis in French patients. Immunogenetics 1997; 45:320-4.
15. Merryweather-Clarke AT, Pointon JJ, Shearman JD, Robson KJH. Global prevalence of putative haemochromatosis mutations. J Med Genet 1997; 34:275-8.
16. Feder J, Tsuchihashi Z, Irrinki A, et al. The hemochromatosis founder mutation in HLA-H disrupts β2 microglobulin interaction and cell surface expression. J Biol Chem 1997; 272:14025-8.
17. Beutler E. The significance of the 187G (H63D) mutation in hemochromatosis. Am J Hum Genet 1997; 61:762-4.
18. Risch N. Haemochromatosis, HFE and genetic complexity. Nature Genet 1997; 17:375-6.
19. Piperno A, Sampietro M, Pietrangelo A, et al. Heterogeneity of hemochromatosis in Italy. Gastroenterology 1998; in press.
20. Beutler E, West C. New diallelic markers in the HLA region of chromosome 6. Blood Cells Mol Dis 1997; 23:219-29.
21. Pointon JJ, Shearman JD, Merryweather-Clarke AT, Robson KJH. A single nucleotide deletion in the putative haemochromatosis gene in a patient who is negative for both the C282Y and H63D mutations

[abstract]. International Symposium "Iron in Biology and Medicine". Saint Malo, France, June 16-20, 1997:268.
22. Bothwell TH, Charlton RW, Cook JD, Finch CA. Iron metabolism in man. Oxford, England: Blackwell Scientific, 1979.
23. Aisen P, Listowsky I. Iron transport and storage proteins. Annu Rev Biochem 1980; 49:357-93.
24. Bleil JD, Bretscher MS. Transferrin receptor and its recycling in HeLa cells. EMBO J 1982; 1:351-5.
25. Leibold EA, Guo B. Iron-dependent regulation of ferritin and transferrin receptor expression by the iron-responsive element binding proteins. Annu Rev Nutr 1992; 12:345-68.
26. Hentze MW, Kuhn LC. Molecular control of vertebrate iron metabolism: mRNA-based regulatory circuits operated by iron, nitric oxide, and oxidative stress. Proc Natl Acad Sci USA 1996; 93:8175-82.
27. Cairo G, Pietrangelo A. Transferrin receptor gene expression during rat liver regeneration. Evidence for post-transcriptional regulation by iron regulatory factor B, a second iron-responsive element-binding protein. J Biol Chem 1994; 269:1-5.
28. Cairo G, Pietrangelo A. Nitric-oxide-mediated activation of iron-regulatory protein controls hepatic iron metabolism during acute inflammation. Eur J Biochem 1995; 232:358-63.
29. McCance RA, Widdowson EM. Absorption and excretion of iron. Lancet 1987; 233:680-4.
30. Fleming MA, Romano LM, Garrick LM, Garrick MD, Andrews NC. Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for nramp2 in endosomal iron transport. Proc Natl Acad Sci USA 1998; 95:1148-53.
31. Vidal S, Malo D, Vogan K, Skamene E, Gros P. Natural resistance to infection with intracellular parasites: isolation of a candidate for Bcg. Cell 1993; 73:469-85.
32. Pietrangelo A, Rocchi E, Casalgrandi G, et al. Regulation of transferrin, transferrin receptor, and ferritin genes in human duodenum. Gastroenterology 1992; 102:802-9.
33. Levine JS, Seligman PA. The ultrastructural immunocytochemical localization of transferrin receptor (TFR) and transferrin (TF) in the gastrointestinal tract of man. Gastroenterology 1984; 86:1161.
34. Cox TM, Mazurier J, Spik G, Montreuil J, Peters TJ. Iron binding proteins and influx of iron across the duodenal brush border. Evidence for specific lactotransferrin receptors in the human small intestine. Biochim Biophys Acta 1979; 588:120-8.
35. Simpson RJ, Osterloh KRS, Raja KB, Snape S, Peters TJ. Studies on the role of transferrin and endocytosis in the uptake of Fe3+ from Fe-nitrilitriacetate by mouse duodenum. Biochim Biophys Acta 1986; 884: 166-71.
36. Banerjee D, Flanagan PR, Cluett J, Valberg LS. Transferrin receptors in the human gastrointestinal tract. Relationship to body iron stores. Gastroenterology 1986; 91:861-9.
37. Hatrorn MKS. The influence of hypoxia on iron absorption. Gastroenterology 1971; 60:76-81.
38. Mendel GA. Studies on iron absorption. The relationship between the rate of erythropoiesis, hypoxia, and iron absorption. Blood 1961; 18:727-36.
39. Waheed A, Parkkila S, Yan Zhou X, et al. Hereditary hemochromatosis: effects of C282Y and H63D mutations on association with β2-microglobulin, intracellular processing, and cell surface expression of the HFE protein in COS-7 cells. Proc Natl Acad Sci USA 1997; 94:12384-9.
40. Bjorkman PJ, Parham P. Structure, function, and diversity of class I major histocompatibility complex molecules. Annu Rev Biochem 1990; 59:253-88.
41. Burmeister WP, Huber AH, Bjorkman PJ. Crystal structure of the complex of rat neonatal Fc receptor with Fc. Nature 1994; 372:379-83.
42. Parkkila S, Waheed A, Britton RS, et al. Immunohistochemistry of HLA-H, the protein defective in patients with hereditary hemochromatosis, reveals unique pattern of expression in gastrointestinal tract. Proc Natl Acad Sci USA 1997; 94:2534-9.
43. Parkkila S, Waheed A, Britton RS, et al. Association of the transferrin receptor in human placenta with HFE, the protein defective in hereditary hemochromatosis. Proc Natl Acad Sci USA 1997; 94:13198-202.
44. Pietrangelo A, Casalgrandi G, Quaglino D, et al. Duodenal ferritin synthesis in genetic hemochromatosis. Gastroenterology 1995; 108:208-17.
45. Hahn PF, Bale WF, Ross JF, Balfour WM, Wipple GH. Radioactive iron absorption by gastro-intestinal tract: influence of anemia, anoxia, and antecedent feeding distribution in growing dogs. J Exp Med 1943; 78:169-88.
46. Granick S. Ferritin. IX. Increase of the protein apoferritin in the gastrointestinal mucosa as a direct response to iron feeding. The function of ferritin in the regulation of iron absorption. J Biol Chem 1946; 164:737-46.
47. Brown EB, Dubach R, Moore CV. Studies on iron transportation and metabolism. XI. Critical analysis of mucosal block by large doses of inorganic iron in human subjects. J Lab Clin Med 1958; 52:335-55.
48. Brittin GM, Raval D. Duodenal ferritin synthesis in iron-replete and iron deficient rats: response to small doses of iron. J Lab Clin Med 1971; 77:54-8.
49. Harris ZL, Takahashi Y, Miyahma H, Serizawa M, MacGillivray RTA, Gitlin JD. Aceruloplasminemia: molecular characterization of this disorder of iron metabolism. Proc Natl Acad Sci USA 1995; 92:2539-43.
50. Yoshida K, Furihata K, Takeda S, et al. A mutation in the ceruloplasmin gene is associated with systemic hemosiderosis in humans. Nature Genet 1995; 9:267-72.
51. Gordeuk V, Mukiibi J, Hasstedt SJ, et al. Iron overload in Africa. Interaction between a gene and dietary iron content. N Engl J Med 1992; 326:95-100.
52. Camaschella C, Roetto A, Cicilano M, et al. Juvenile and adult haemochromatosis are distinct genetic disorders. Eur J Hum Genet 1997; 5:371-5.
53. Adams PC, Campion ML, Gandon G, LeGall JY, David V, Jouanolle AM. Clinical and family studies in genetic hemochromatosis: microsatellite and HFE studies in five atypical families. Hepatology 1997: 26:986-90.
54. Piperno A, Sampietro M, Pietrangelo A, et al. Genetic heterogeneity and genotype/phenotype correlations in Italian patients with hemochromatosis [abstract]. International Symposium "Iron Biology and Medicine". Saint-Malo, June 16-20, 1997; 254.
55. Totaro A, Roetto A, Rommens J, et al. Generation of a transcription map of 1 Mbase region containing the HFE gene (6p22). Eur J Hum Genet 1998, in press.