Our Current Knowledge of Iron Metabolism and Related Disorders

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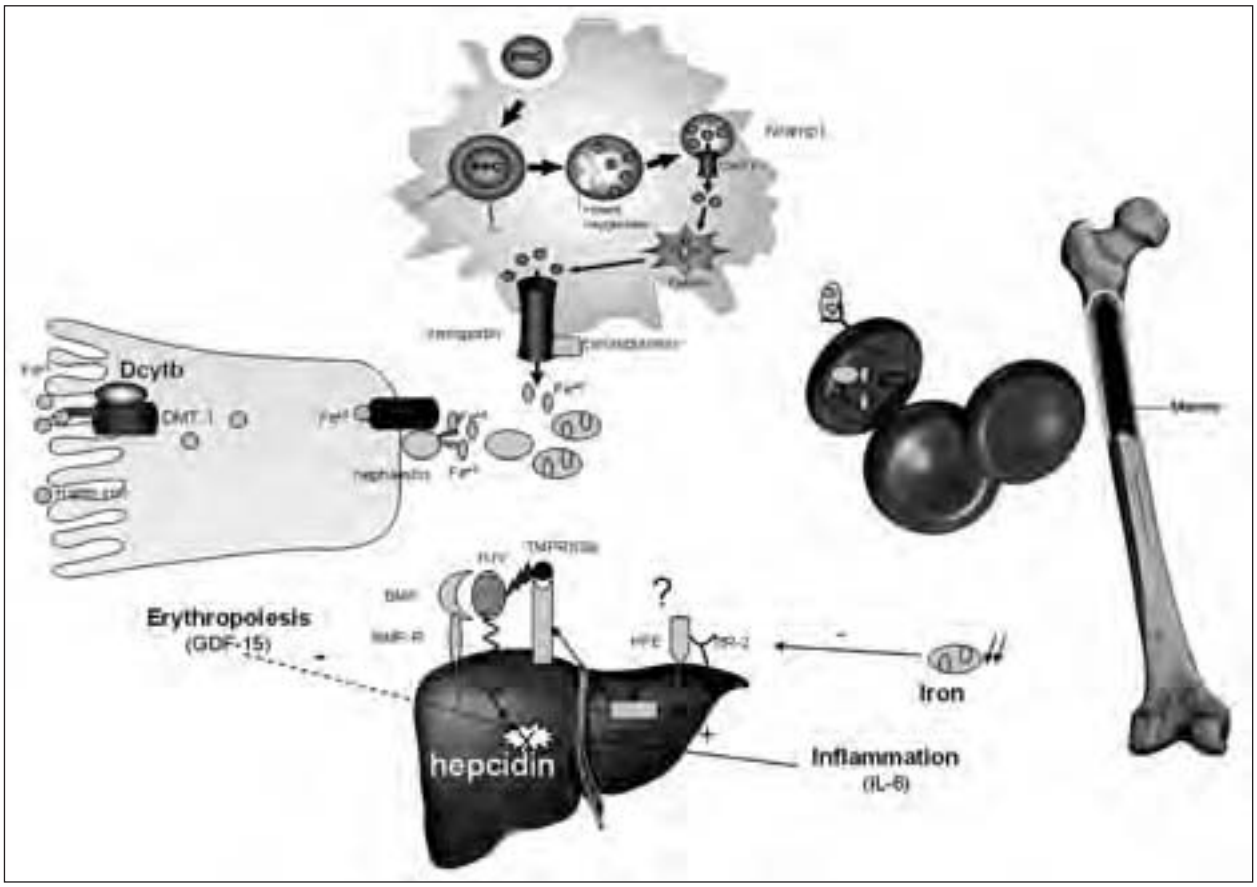
I. Iron absorption and Hepcidin regulation

In the recent years a major progress in the understanding of iron metabolism has been made. This was the result of the discovery of proteins interfering with iron absorption (DMT-1) (1) and release from the enterocyte and the macrophages (ferroportine) (2). However the major discovery was the identification of hepcidin considered today as the "iron hormone" (3). Hepcidin is produced in the liver and by binding to ferroportine regulates iron absorption from the duodenum and iron release from the macrophages, its action being a negative regulator of iron: high hepcidin leads to decreased iron absorption and release; low hepcidin leads to increased iron absorption from the gut and liberation from the macrophages.



Iron is transported in the plasma by binding to transferrin as Fe^{+3} and enters the cells by internalization of the TF-TfR-1 complex. In erythroblasts the great majority of iron goes to mitochondria where haeme is produced. Disturbances of iron metabolism in mitochondria lead to a group of diseases known as sideroblastic anaemias (see below). Hepcidin regulation is the object of intensive research. We now know that hepcidin is regulated both positively and negatively. Inflammation and serum iron positively regulate hepcidin gene expression while erythropoiesis negatively. Most recently a new protein, matriptase-2 has been discovered. It is a negative hepcidin regulator acting through degradation of hemojuvelin. Although we do not know exactly how HFE-TfR-2 works, we believe that it participates in the iron sensing pathway. In the preceding figure we try to give a link between serum iron and matriptase expression. We also schematically show the HJV-BMP complex positively regulating hepcifin expression via the SMAD signaling pathway. An excellent review of systemic iron metabolism is recently written by C. Beaumont and C. Delaby (4).

II. Disorders concerning iron absorption with microcytosis

Iron deficiency may be acquired (in the great majority of cases) and hereditary. Here, in the light of recent advances in iron metabolism, we describe the two new nosologic entities where iron absorption is disturbed leading to congenital microcytic anaemia with or without liver iron overload. These entities are secondary to mutations to the DMT-1 gene and to the very recently described TMPRSS6 gene.

DMT-1 (Nramp2) deficiency

DMT1 mutations were first described in animals where they create microcytic hypochromic anaemia without any response to oral or iv iron. In humans three patients have been described with microcytic-hypochromic anaemia from birth and liver iron overload secondary to DMT-1 mutations (see following table).

Mutations in the gene encoding DMT1

Hematological and biological data of the three described families with microcytic anemia secondary to DMT1 mutations.

origin	French[1]	Czech[2]	Italy[3]
Age (years)	6	20	5
Hb (g/L)	75	74	85
MCV (fL)	53	54	51
MCHC (g/L)	287	296	NA
Serum Fe (µM/L)	28	43	36.5
Transferrine sat (%)	68	NA	90
Ferritin µg/L	18	153	34

[1]Beaumont C, et al. Blood. 2006; 107:4168-4170; [2]Priwitzerova M, et al. Blood. 2004; 103:3991-3992; [3]Iolascon A, et al. Blood. 2006; 107:349-354.

DMT1 mutations lead to microcytic anaemia, normal or mildly elevated ferritinemia and liver iron overload. Although DMT-1 is not functional, iron absorption in the duodenum continues because the absorption of haeme iron is not disturbed. In fact, in meat-eating humans it is estimated that about 2/3 of absorbed iron comes from haeme. Thus in humans, a mutation in DMT1 protein may primarily affect iron utilization and not absorption, leading to severe microcytic iron deficiency anaemia with increased iron stores (5). Because of severe liver iron overload, chelation therapy was applied in one family; however deterioration of anaemia motivated authors to consider that this treatment is contra-indicated. Epo is effective because it increases Hb by reducing the degree of apoptosis of erythroid precursors (6).

IRIDA

The Mask Mouse is a chronically iron-deficient mouse with an unusual pattern of hair loss over the trunk but not the head (the mask phenotype) due to a homozygous recessive genetic mutation. Mask mice were shown to express inappropriately high levels of hepcidin mRNA in the liver, even when fed an iron-deficient diet. Using positional cloning techniques, Dr. Beutler's group was able to ascribe the mask phenotype to a splicing error in the Tmprss6 gene, which encodes a membrane-bound serine protease (7, 8).

	Adult reference values	Case 1	Children reference values	Case 2	CT1	CT2	CT3
Age, year(months) /sex		53 /M		3 /M	4.7 /M	5.7 /F	3.2 /M
Hb	110-160 g/l	114	115-135 g/l	77	130	129	115
MCV	78-92 fl	64	75-87 fl	56	75	78	80
MCHC	320-360 g/l	318	310-370 g/l	293	347	343	331
leukocytes	3.0-12.5 10^9/l	8.6	5-14.5 10^9/l	7.9	6.6	5	6
Platelets	150-450 10^9/l	262	105-392 10^9/l	427	339	214	380
Serum iron	6-30 µmol/l	3	9-21.5 µmol/l	1.8	10.6	19	10.3
Transferrin	44-87 µmol/l	2.61 g/l **	47.3-86 µmol/l	62	48	62	48
Transferrin saturation	15-50 %	5	15-50 %	3	22.1	31	21.5
Ferritin	26-420 mg/l	68	6-60 mg/l	46	11	11	17
sTfR	0.9-2.3 mg/l	2.33	1.5-3.3 mg/l	5.37	1.46	1.65	1.36
CRP	0-10 mg/l	<5	0-10 mg/l	<1	<1	<1	<1
hepcidin	F: 4.0 ±2.8 nM	9.8	NA*	12.2	<0.5	<0.5	0.8
	M: 5.8 ±4.0 nM						

NA*: There are no available normal values
**: normal values for transferrin: 2.5-4.3 g/l

Matriptase-2 (Tmprss6) plays an essential role in iron homeostasis as a hepcidin inhibitor. Iron Refractory Iron Deficiency Anaemia (IRIDA) is an autosomal recessive disease characterised by: 1) congenital hypochromic, microcytic anaemia; 2) very low MCV; 3) low serum iron and low transferrin saturation; 4) normal ferritin or in the lower limits of the normal; 5) no response to oral iron treatment and incomplete response to iv iron administration; 6) recessive pattern of inheritance and 7) inappropriately high levels of hepcidin (9). IRIDA has recently been shown to be caused by mutations in the gene TMPRSS6. Up to date, sixteen cases with IRIDA, without any common geographic or ethnic distribution, have been reported in the literature (9). All of them present different missense, nonsense, frameshift, splice junction and deletional mutations affecting almost all the domains of the *TMPRSS6* in homozygous or in double heterozygous state (except two cases where one mutation was found in the heterozygous state), suggesting that we are dealing with private mutations in familial sporadic cases. The previous table gives haematological findings and iron data in two cases with IRIDA described by our group recently (9).

III. Disorders concerning iron absorption leading to iron overload

Here we distinguish two groups of diseases: those leading to systemic iron overload with normal erythropoiesis [Hereditary Hemochromatosis, (HH)] and the "iron loading anaemias" which include hereditary and acquired forms. Only the hereditary forms of the "iron loading anaemias" will be mentioned briefly in part IV of the present review.

HH have increased transferrin saturation, serum ferritin and present parenchymal iron overload, especially in the liver. Progress in iron metabolism allowed the molecular characterization of this group of diseases: Type I, (classic HH) with mutations at *HFE* and an adult onset of the clinical phenotype. Type IIA, (juvenile HH) with mutations at *HJV* and severe early onset phenotype. Type IIB, (juvenile HH) with mutations at *HAMP* and severe early onset phenotype. The difference between IIA and IIB HH lies in the fact that in IIA hepcidin is low because of reduced HAMP activation by the mutated *HJV*, while in IIB there is low/absent hepcidin because of mutations in the HAMP gene. HH type III is characterized by mutations in *TFR2* leading to early onset phenotype with liver iron overload. Finally type IV is due to *SLC40A1* mutations (=ferroportin gene) with two possible phenotypes: a) **ferroportin disease** with reduced iron export and mainly accumulation of iron in the macrophages, and **hemochromatosis-like disease** with as a mechanism hepcidin resistance and liver iron overload (10).

HFE-related hemochromatosis is by far the most frequent form and most patients are homozygous for C282Y mutation or compound heterozygotes for C282Y/H63D mutations. Juvenile or type II hemochromatosis is a severe form presenting early in life. The phenotype of HJV and HAMP (form IIA and form IIB) mutations is identical in patients without a chronic inflammation.

IV. Hereditary forms of "iron loading anaemias"

Low hepcidin levels represent the most powerful stimulus for increased iron absorption in the duodenum. Papanikolaou G. and Kattamis A. were the first to describe that ineffective erythropoiesis is associated with low hepcidin levels. The hypothesis of an "erythroid regulator" of hepcidin's gene expression, which is active even in patients with iron overload, was thus reinforced. Tanno et al in 2007 identified GDF15, produced by the hyperplastic erythroid tissue in these forms of anaemias, as the down-regulator of hepcidin expression. Up to date low hepcidin levels together with high serum GDF15 were found in patients with pyruvate kinase deficiency, thalassaemia intermedia, hereditary sideroblastic anaemia and congenital dyserythropoietic anaemia type I. The exact mechanism of hepcidin inhibition by GDF15 is for the moment unknown. As GDF15 is a member of the bone morphogenetic family of proteins its function may be mediated by inhibition of this signalling pathway (11, 12, 13, and 14).

In conclusion

Important advances in our understanding of iron metabolism have been made in the past 10 years. These allowed us to discover new diseases like the hereditary forms of iron deficiency anaemias, some new rare forms of familial sideroblastic anaemias and to understand the different phenotypes of hereditary iron overload. Although not covered in this review, iron deregulation characterizes the anaemia of chronic disorders or of chronic inflammation too. Although we now know a lot about regulation of hepcidin's gene expres-

sion, we still ignore the exact function and regulation at a molecular level of fundamental proteins in iron metabolism, like HFE, HJV and TfR2. This knowledge is mandatory if we want to develop molecules acting as agonists and antagonists of hepcidin or as positive or negative regulators of *HAMP* expression. The clinical interest of such molecules for the treatment of iron overload, iron deficiency or anaemia of inflammation is more than evident.

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