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Mitochondrial involvement in genetically determined transition metal toxicity
I. Iron toxicity

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Abstract

Iron that is not specifically chaperoned through its essential functional pathways is damaging to biological systems, in major part by catalyzing the production of reactive oxygen species. Iron serves in several essential roles in the mitochondrion, as an essential cofactor for certain enzymes of electron transport, and through its involvement in the assembly of iron–sulfur clusters and iron–porphyrin (heme) complexes, both processes occurring in the mitochondrion. Therefore, there are mechanisms that deliver iron specifically to mitochondria, although these are not well understood. Under normal circumstances the mitochondrion has levels of stored iron that are higher than other organelles, though lower than in cytosol, while in some disorders of iron metabolism, mitochondrial iron levels exceed those in the cytosol. Under these circumstances of excess iron, protective mechanisms are overwhelmed and mitochondrial damage ensues. This may take the form of acute oxidative stress with structural damage and functional impairment, but also may result in long-term damage to the mitochondrial genome. This review discusses the evidence that mitochondria do indeed accumulate iron in several genetic disorders, and are a direct target for iron toxicity when it is present in excess. We then consider two classes of genetic disorders involving iron and the mitochondrion. The first include defects in genes directly regulating mitochondrial iron metabolism that lead to Friedreich's ataxia and the various sideroblastic anemias, with excessive mitochondrial iron accumulation. Under the second class, we discuss various primary hemochromatoses that lead to direct mitochondrial damage, with reference to mutations in genes encoding HFE, hepcidin, hemojuvelin, transferrin receptor-2, ferroportin, transferrin, and ceruloplasmin.
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1. Introduction

Iron is an essential element whose redox properties and coordination chemistry suit it for a number of catalytic and transport functions in living cells [1]. However, these same properties render iron toxic, to a large extent due to its ability to generate reactive oxygen species (ROS) [2,3]. Thus, mechanisms have evolved to allow the organism to handle essential iron safely [4,5]. At the same time, the possibility of genetic defects in these mechanisms gives rise to iron toxicity manifest in many different ways. It has long been known that in disorders of iron overload, the mitochondrion is a target for iron toxicity, with oxidative mitochondrial membrane damage and poisoning of enzymes of the tricarboxylic acid cycle and energy metabolism recognized as potential targets [6,7]. However, whether these effects are

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primary effects of iron on mitochondria, or secondary to more general aspects of tissue damage has not always been clear. In the subsequent paragraphs, we review the evidence for mitochondrial iron accumulation and direct toxicity, and then describe some genetic disorders that lead to mitochondrial iron overload or impaired mitochondrial iron homeostasis.

2. Mitochondrial iron homeostasis and accumulation

In recent years, a number of proteins involved in mitochondrial transport and storage have focused attention on the rich iron metabolism that occurs at this site. These include frataxin [8–10], which regulates mitochondrial iron levels; ABC-B7 [11,12], involved in export of iron–sulfur [Fe–S] clusters; the recently identified iron carrier mitoferrin [13]; and a specific mitochondrial ferritin [14,15] for iron storage. That mitochondria have special requirements for iron is clear from their biosynthetic functions. They are the only site of heme synthesis, and the ultimate site of insertion of iron into protoporphyrin by the inner mitochondrial membrane enzyme, ferrochelatase [12]. Not surprisingly, mitochondria take up the major proportion of iron acquired from transferrin in erythroid cells [12], but also acquire iron in non-erythroid cells where storage is mainly cytosolic under normal circumstances. Most, if not all, [Fe–S] clusters are assembled in the mitochondrion and either transported to the cytosol for incorporation into their target proteins, or utilized within the mitochondrion [16,17]. The interplay of required enzymes, scaffolding proteins, and transport proteins are better understood in yeast, but homologs of these proteins occur in human mitochondria [18]. Iron is also essential for a number of cytochrome intermediates in mitochondrial respiration and electron transport.

The special requirements of the mitochondrion for iron support the notion of a specific mechanism of mitochondrial iron delivery, and such a mechanism would then be a candidate for up-regulation of mitochondrial iron acquisition in iron-overload states. Quite recently, the major iron carrier necessary for delivery of iron to mitochondria for heme synthesis in hematopoietic tissue of mice has been identified as mitoferrin [13]. Defects in the homologous genes lead to impaired [Fe–S] cluster biogenesis in yeast and the anemic frascati mutant in the zebra fish. Storage forms of cytosolic iron are in equilibrium with a labile iron pool (LIP), a designation given to the small amount of non-sequestered iron presumed to be in an exchangeable, low molecular weight form [19–21]. However, the LIP has not been demonstrated to be a kinetic intermediate in mitochondrial iron acquisition [17,22]. In keeping with a privileged pathway for mitochondrial iron delivery, it has been reported that extracellular non-transferrin-bound iron enters mitochondria without detection by cytosolic fluorophores [23]. Ponka and coworkers have hypothesized a “kiss and run” mechanism of iron delivery. In this mechanism, iron newly acquired from receptor-mediated endocytosis of diferric transferrin and released in the endosome is delivered directly to the mitochondrion during transient physical contact of the endosome with the mitochondrion [11,12,17].

Thus, mechanisms exist in both erythroid and non-erythroid cells to target iron to the mitochondrion. But is there direct evidence for excess mitochondrial iron accumulation? Yes, and compelling evidence comes from studies of iron overload anemias (sideroblasic anemia, also known as sideroblastosis). In sideroblastic anemia patients, iron enters the developing red blood cells but fails to incorporate into protoporphyrin and form heme. As a result, iron accumulates in the mitochondria of erythroblasts (known as ring-erythroblasts) in the bone marrow in the form of characteristic ring-shaped structures. Wickramasinghe et al. [24] demonstrated electron dense deposits in mitochondria of sideroblasts from patients suffering from X-liked sideroblastic anemia. Acquired sideroblastic anemia often results from prolonged exposure to toxic substances (such as alcohol, lead, or drugs), nutritional imbalances (such as deficiency in folic acid or copper or excess in zinc) or inflammatory disease, cancer, kidney, endocrine, or metabolic disorders. Iron accumulation was reported in the cristae of the mitochondria from the bone marrow of refractory and alcoholic sideroblastic anaemia patients [25].

Erythropoietic protoporphyria is another genetic disorder arising from impaired incorporation of iron during heme synthesis. As in sideroblastic anemia, iron traffics to the mitochondrion but accumulates due to failed utilization. Ultrastructural examination of the bone marrow of nine patients with erythropoietic protoporphyria showed finely dispersed electron-dense deposits localized in mitochondria of erythroblasts in each one [26]. Not all patients were anemic, indicating that mitochondrial iron deposition is independent of iron status in these patients. Similar findings were reported for three sideroblastic anemia patients with secondary hemochromatosis in the same study, reinforcing the independence of mitochondrial iron deposition on iron status. Deposition is the same at the molecular level. Furthermore, electron energy-loss spectroscopy was also used in this study to demonstrate that the mitochondrial iron existed as identical chemical species in both disorders. In fact, Cazzola

et al. [27] have now used immunocytochemical methods to demonstrate that iron deposited in perinuclear mitochondria of ring-sideroblasts of X-linked sideroblastic anemia patients and other patients with refractory anemia accompanies the presence of mitochondrial ferritin. In these patients, there was a strong correlation between the presence of detectable mitochondrial ferritin and the number of circulating ring-sideroblasts. This study provided the molecular evidence showing that iron is deposited in the mitochondria of sideroblastic anemia patients. On the other hand, Napier et al. [17] argue that soluble ferritin is generally not detected by Perl's stain, and tissue iron deposits visualized by this histochemical method are more consistent with the occurrence of hemosiderin.

A number of other human diseases have also been shown to lead to mitochondrial iron accumulation. The myelodysplastic syndromes are a group of myeloid stem cell disorders that gradually affect the ability of a person's bone marrow to produce normal blood cells. Iron accumulation was associated with electron microscopic evidence of mitochondrial abnormalities in erythroblasts of patients [28]. Iron mitochondrial accumulation, described as paracrystalline inclusions on electron microscopy, was also reported in the muscle tissues and erythroblasts of patients with myopathy and sideroblastic anemia syndrome [29]. A histopathological and electron microscopy study of the livers of 30 cases of transfusional iron overload in thalassemic individuals showed the presence of an electron dense matrix in swollen and ruptured mitochondia [30]. Mitochondrial iron deposition has also been demonstrated histochemically in hearts, livers, and spleens of patients with Friedreich's ataxia [31,32] (see below).

All these studies with human samples provide compelling evidence that iron deposits in the mitochondrion. In addition, mitochondrial iron accumulation has been demonstrated in primary cell cultures or tissue sections obtained from the brains of chemically induced neurological disorders in animals. These animals were intended to generate models to mimic human neurological degenerative diseases including Alzheimer disease, Parkinson disease, and multiple sclerosis [33–37]. Furthermore, studies with yeast showed that defects in any of the enzymes of the [Fe–S] cluster assembly pathway cause mitochondrial iron accumulation [38,39].

3. Mitochondrial involvement in iron toxicity

While mitochondrial involvement in cell death is secondary to a variety of causes that result in loss of the mitochondrial membrane potential, impaired energy production, mitochondrial swelling, and loss of calcium homeostasis, substantial evidence points to direct toxic effects of mitochondrial iron. Mitochondria have been shown to be the site of anthracycline-mediated toxicity in cardiac myocytes; inner-membrane respiratory chain activity was impaired [40]. Iron potentiated the effect, and is generally believed to enhance anthracycline toxicity by facilitating redox cycling and ROS formation. Iron impairs hepatic mitochondrial respiration by decreasing cytochrome *C* oxidase activity, and disrupts calcium homeostasis by interfering with mitochondrial and microsomal calcium sequestration [41]. In cardiac myocytes, iron targets mitochondrial respiratory enzymes. In gerbils overdosed with iron dextran, significant decreases in hepatic mitochondrial NADH-cytochrome *C* reductase, cytochrome *C* oxidase, and succinate dehydrogenase were observed [42]. Furthermore, upon addition of the oxidizable substrate succinate, mitochondria from control gerbils developed a much higher transmembrane potential than those from iron-loaded gerbils (197 mV versus 167 mV, respectively), and oxygen consumption upon addition of ADP was decreased in the iron-loaded mitochondria. Significantly, administration of silybin (an antioxidant and potential iron chelator) decreased deferoxamine-chelatable iron in the mitochondrial fraction, and decreased mitochondrial lipid peroxidation and oxidative damage in the iron-loaded animals, without affecting total hepatic tissue iron [42]. These observations directly implicate mitochondrial iron in mitochondrial membrane damage accompanied by decreased respiratory enzyme activity and ATP production in hepatic iron overload.

Studies with cardiac myocytes reinforce the idea that mitochondrial iron is directly damaging to the mitochondrion [43,44]. Mitochondrial inner membrane enzyme activity was markedly decreased in cultured cardiac myocytes treated with ferric ammonium citrate, with a corresponding decrease in ATP levels. Although these studies do not directly demonstrate the role of mitochondrial iron, the targeting of the mitochondrial enzymes and the similarity to the results of Masini et al. [42], noted above, strongly support such a role. Furthermore, the authors [43,44] provide a compelling argument for the direct involvement of iron based on unique aspects of the mitochondrion: its high oxygen consumption makes it a major source of hydroxyl radicals through the iron-driven Fenton reaction; the high cardiolipin content of the mitochondrial membrane renders mitochondria susceptible to iron-catalyzed peroxidative damage, and low iron chelator concentrations that have no measurable affect on total cellular iron uptake or release nevertheless afford significant mitochondrial protection. Eaton and

Qian [45] have discussed the further possibility that the mitochondrial genome is a particularly sensitive target to local effects of iron. Mitochondrial DNA is much more susceptible to oxidative damage than nuclear DNA, and is preferentially damaged by hydrogen peroxide treatment [46,47]. A lower histone content and less effective repair mechanisms may in part account for this [45]. The presence of iron in mitochondrial ferritin, respiratory enzymes, nascent [Fe–S] clusters, and mobilized for heme synthesis would seem to be an obvious contributing factor to the generation of ROS during respiration.

4. Genetic disorders of mitochondrial and cellular iron transport

4.1. Friedreich's ataxia and frataxin

Friedreich's ataxia is an autosomal recessive neurodegenerative disorder involving both the central and peripheral nervous system. It is characterized by a progressive ataxia, sensory loss, and cardiomyopathy [48,49]. Frataxin, also called X25, is the gene responsible for Friedreich's ataxia. It is located in chromosome 9q13 [50] and encodes a 210-amino acid mitochondrial protein [51] involved in mitochondrial iron metabolism. The pathomechanism of the disease appears to involve mitochondrial iron overload [48,52,53], and the potential for isonicotinyl hydrazone-based iron chelators to target mitochondrial iron has been considered as a therapeutic strategy [52,54].

The major cause of Friedreich's ataxia is the expansion of a GAA trinucleotide repeat in the first intron of the frataxin gene. Mutations have also been reported. Most patients are homozygous for the expansion of a GAA triplet repeat within the frataxin gene, but a few show compound heterozygosity for a point mutation in addition to the GAA-repeat expansion [49,51,53,55]. The number of GAA repeats correlates with the age of onset of progressive disease. Durr et al. [56] studied 140 Friedreich's ataxia patients, with ages of onset ranging from 2 to 51 years and homozygous for a GAA expansion from 120 to 1700 repeats, and demonstrated that clinical variability in Friedreich's ataxia is related to the size of the expanded repeat. Milder forms of the disease (late-onset Friedreich's ataxia and Friedreich's ataxia with retained reflexes) were associated with shorter expansions. The expanded GAA repeats cause the formation of abnormal but highly thermostable DNA that impairs frataxin transcription and results in low level frataxin expression [57,58]. Gacy et al. [58] suggested that the GAA instability in Friedreich's ataxia is a DNA-directed mutation caused by improper DNA structures at the repeat region which leads to the cellular frataxin deficiency.

Mutations in the frataxin gene cause impaired protein translation. This was demonstrated in yeast. Cavadini et al. [10] studied mutants of YFH1, the yeast frataxin homolog, and showed that wildtype human frataxin cDNA can complement the YFH1 protein-deficient yeast with prevention of the mitochondrial iron accumulation and oxidative damage associated with loss of YFH1. Two clinically relevant mutants, G130V and W173G are unable to prevent these consequences. Both mutants affected protein stability, resulted in low levels of mature frataxin expression, and were unable to prevent mitochondrial iron accumulation. In addition to demonstrating that the mutation affects protein stability, this study provided experimental evidence for the role of human frataxin in mitochondrial iron metabolism.

Frataxin deficiency results in diminished activity of various mitochondrial iron–sulfur proteins. Since iron is not being used to form the proteins, it accumulates in mitochondria. Frataxin has an iron binding site and has been suggested to be a mitochondrial iron chaperone protein. Bulteau et al. [59] found that Frataxin interacted with aconitase in a citrate-dependent fashion and converted the inactive $[3Fe–4S]^{1+}$ enzyme to the active $[4Fe–4S]^{2+}$ form of the protein. They suggested that frataxin is an iron chaperone protein that protects the aconitase $[4Fe–4S]^{2+}$ cluster from disassembly and promotes enzyme reactivation. It also plays a role in Fe–S cluster assembly and transport and possibly in regulation of energy metabolism and oxidative phosphorylation [52,60].

4.2. X-linked sideroblastic anemia

X-linked sideroblastic anemia is caused by mutations in the δ-aminolevulinic acid synthase 2 (ALAS2) gene. There are two genes encoding ALAS. ALAS1 is expressed ubiquitously in all cells including erythroid cells, while ALAS2 is only expressed in the erythroid cells. The ALAS2 gene is located on the X chromosome in the region Xp21-q21, with the most likely location being on band Xp11.2 [61].

ALAS is the first enzyme in the heme synthetic pathway. Heme synthesis involves eight steps. Four of them are in the mitochondrion while the other four are in the cytosol (for review, see [12]). The first step, occurring in the mitochondrion, is the condensation of glycine and succinyl-CoA to form aminolevulinic acid (ALA). This step is catalyzed by ALAS. After several additional enzymatic transformations, coproporphyrinogen III is formed in the cytosol. It re-enters the mito-

chondrion, where iron is inserted by ferrochelatase and heme is formed. A defect in the ALAS2 gene inhibits heme synthesis. Consequently, iron is accumulated in the mitochondrion.

Aoki et al. [62] demonstrated that the isozyme encoded by the ALAS2 gene is decreased in activity in bone marrow of patients with X-linked hereditary sideroblastic anemia. More than 20 different mutations encompassing exons of the catalytic region of the ALAS2 gene have been described. A common feature of these mutations is that the enzyme activity of ALAS2 is significantly lower in patient bone marrow compared to the normal control. For example, a missense mutation 1754 A-to-G, located in exon 11, causes ALAS2 activity in bone marrow cells of the patient to drop to only 53.3% of that in normal controls [63].

4.3. X-linked sideroblastic anemia with ataxia

X-linked sideroblastic anemia with ataxia is characterized by an infantile to early childhood onset of nonprogressive cerebellar ataxia and mild hypochromic, microcytic anemia. It is a recessive disorder caused by gene mutations in the ABCB7 gene located at chromosome Xq13.1-q13.3 [64,65]. ABCB7 is a member of the large family of ATP-binding (ABC) transporters. It shares highest sequence similarity with the yeast ATM1 gene, which encodes an ABC half-transporter located in the mitochondrial inner membrane. ABCB7 was suggested to be a transporter for heme from the mitochondrion to the cytosol [66]. Mutations in ABCB7 cause the phenotype of X-linked sideroblastic anemia with ataxia. The first missense mutation (I400M) in ABCB7 was identified in X-linked sideroblastic anemia with ataxia patients by Allikmets et al. [64]. Bekri et al. [65] reported another missense mutation at exon 10 of the ABCB7 gene: a G-to-A transition at nucleotide 1305 of the full-length cDNA, resulting in a charge inversion caused by the substitution of lysine for glutamate at residue 433 C-terminal to the putative sixth transmembrane domain of ABCB7.

5. Other genetic disorders of iron overload

Primary hemochromatoses arising from genes other than those affecting mitochondrial iron metabolism give rise to iron overload disorders that ultimately lead to increased tissue iron deposition and consequent mitochondrial damage. The hereditary hemochromatoses are now classified into several subtypes, while rare defects in proteins of iron transport, including transferrin and ceruloplasmin, lead to similar pathology.

5.1. Hereditary hemochromatosis

Hereditary hemochromatosis (HH) is one of the most common autosomal inherited iron overload diseases. It is characterized by excessive dietary iron absorption and results in tissue iron deposition and toxicity. The clinical characteristics of HH include cirrhosis of the liver, diabetes, hypermelanotic pigmentation of the skin, and heart failure. Based on the genetic characteristics, HH is further classified into HH types I, II (A and B), III, and IV.

HH type-I is the classical hemochromatosis, caused by mutations in the gene called HFE. It is located on chromosome 6p21.3 [67]. HFE protein is similar to major histocompatibility complex (MHC) class I molecules which are associated with β2-microglobulin. The most common mutation in HFE associated with HH is the homozygous substitution C282Y of tyrosine for cysteine at amino acid 282. A second missense mutation, H63D, was reported to be enriched in HH patients with heterozygosity of the C282Y mutation [67]. The C282Y mutation abolishes its binding to β2-microglobulin and its expression on the cell surface, while the H63D mutation neither interrupts its binding to β2-microglobulin nor its expression on the cell surface [68]. Disruption of HFE surface expression results in abnormal dietary iron absorption.

HH type II is a juvenile onset hemocromatosis. The gene responsible for HH type IIA is called hemojuvelin (HJV) located on chromosome 1q21 [69]. A number of mutations have been reported in HH type IIA patients (see review [70]). HH type IIB is caused by mutation in the gene *hamp* encoding hepcidin, which is located on chromosome 19q13 [71]. Hepcidin is a small peptide with antimicrobial activity possibly as a result of macrophage iron deficiency. It binds to ferroportin, causing ferroportin to be internalized and degraded, thus limiting iron abosrption from the intestine [72]. Animal models indicate that hepcidin is a key regulator of iron absorption in mammals. The mutations cause disruption of this regulation and lead to iron overload [73,74].

HH type III (HFE3) is caused by mutation in the gene encoding transferrin receptor-2 (TFR2), which is located on chromosome 7q22 [75]. The TFR2 transcript is highly expressed in hepatocytes, and is not regulated by tissue iron status [76]. A number of mutations have been reported from HH patients that result in iron overload. Mattman et al. [77] studied a group of non-C282Y HH patients and identified several sequence variants, including a homozygous missense mutation in exon 17 of the TFR2 gene, which resulted in a Q690P glutamine-to-proline amino acid switch.

HH type IV is caused by mutation in the SLC40A1 gene which encodes ferroportin, located at chromosome 2q32 [78]. Ferroportin is an iron exporter located in the basolateral membrane of enterocytes [79]. Ferroportin expression is regulated by intracellular iron status. The 5′ untranslated region of the ferroportin mRNA contains a functional iron-responsive element. By studying an Australian family with autosomal dominant hemochromatosis, Wallace et al. [80] found heterozygosity for a 3 bp (TTG) deletion in exon 5 of the FPN1 gene, resulting in the deletion of valine at position 162. The deletion is a loss-of-function mutation that results in impaired iron homeostasis and leads to iron overload.

5.2. Hypotransferrinemia

Hypotransferrinemia [81], or even more rarely atransferrinemia, are disorders that impair the normal delivery of transferrin-bound iron to tissues for hematopoeisis and iron storage. The anemia that results from failed delivery of transferrin-bound iron for hematopoeisis is paradoxically accompanied by iron overload, as increased circulating non-transferrin-bound iron finds its way into soft tissues. Parenchymal iron overload is a consequence of decreased circulating apotransferrin. The lesson is that when iron is not delivered to tissues in a regulated manner, it is unavailable for appropriate hematopoeisis; nor is it appropriately excreted. Mice with congenital hypotransferrinemia dosed with soluble iron salts accumulate radiolabelled iron in the liver and pancreas, in contrast to deposition in the bone marrow and spleen in control animals [82]. The pattern of deposition is similar to that in hemochromatosis [82] at both the tissue and cellular levels. In the absence of sufficient transferrin, just as when transferrin is saturated in states of iron overload, it seems that there is a protective mechanism that clears non-transferrin-bound iron from circulation into the soft tissues [83].

5.3. Aceruloplasminemia

Ceruloplasmin was long thought to be a Cu transporter. Long known to have ferroxidase activity (for a historical perspective see [84]), this was recognized only recently to be its primary function [85]. Ceruloplasmin, in fact, functions as a systemic hephaestin [86,87], as a ferroxidase to generate ferric ion as needed. The consequences of decreased ceruloplasmin in Wilson disease are minimal, but rare occurrences of a complete absence of ceruloplasmin have been identified, which lead to increased cellular iron deposition. Aceruloplasminemia was initially identified in a few families in Japan and North America [88–91]. Patients present in mid life with neurological symptoms including ataxia, Parkinson-like movement disorder, dystonia and progressive dementia. Neuronal dropout is accompanied by iron deposition in both neurons and microglia [88], so this is really a disorder characterized as a hemochromatosis, and indeed hepatic iron overload is a feature [92]. The basis of the iron overload is not completely clear. Decreased ferroxidase activity may increase deposition because of increased plasma ferrous iron, and impaired tissue iron efflux is also presumably involved [92].

6. Conclusions

There is now compelling evidence that iron accumulates in mitochondria at levels significantly greater than in the cytosol in sideroblastic anemias, Friedreich's ataxia, and some animal models of iron overload. Accumulation of iron in mitochondria in certain genetic diseases is probably a consequence of the special requirements of the organelle to utilize iron for the synthesis of heme and iron–sulfur clusters, including several enzymes of the electron transport pathway. The transport mechanisms involved in iron acquisition are probably operating maximally in iron overload, but they are not well understood. What is known with more certainty is that the mitochondrial accumulation of iron has significant toxic consequences, both for the cell as a whole and for the mitochondrion itself. The phenomenon of mitochondrial damage in iron overload seems to be general in mammalian cells, whether excessive mitochondrial deposition is demonstrated or not. Because many iron complexes have redox potentials accessible to endogenous biological reductants, they can produce oxidative stress through Fenton/Haber Weiss chemistry that disrupts mitochondrial energy metabolism, compromises the integrity of the mitochondrial membrane system, inhibits mitochondrial enzyme systems, and damages the mitochondrial genome. Targeting mitochondrial iron with directed chelation therapy is an attractive strategy for managing a number of clinical disorders of iron imbalance.

References

[1] W.R. Harris, Iron chemistry, in: D.M. Templeton (Ed.), Molecular and Cellular Iron Transport, Marcel Dekker, New York, 2002, pp. 1–40.

[2] B. Halliwell, J.M.C. Gutteridge, Role of free radicals and catalytic metal ions in human disease: an overview, Methods Enzymol. 186 (1990) 1–88.

[3] B. Halliwell, J.M.C. Gutteridge, Biologically relevant metal ion-dependent hydroxyl radical generation. An update, FEBS Lett. 307 (1992) 108–112.

[4] M.W. Hentze, M.U. Muckenthaler, N.C. Andrews, Balancing acts: molecular control of mammalian iron metabolism, Cell 117 (2004) 285–297.
[5] J.J.M. Marx, R.C. Hider, Iron: an essential but potentially harmful nutrient, Eur. J. Clin. Invest. 32 (Suppl. 1) (2002) 1–2.
[6] C. Hershko, G. Link, I. Cabantchik, Pathophysiology of iron overload, Ann. N. Y. Acad. Sci. 850 (1998) 191–201.
[7] C. Hershko, D.J. Weatherall, Iron-chelating therapy, CRC Crit. Rev. Clin. Lab. Sci. 26 (1988) 303–346.
[8] G. Kispal, P. Csere, B. Guiard, R. Lill, The ABC transporter Atm1p is required for mitochondrial iron homeostasis, FEBS Lett. 418 (1997) 346–350.
[9] M. Babcock, D. De Silva, R. Oaks, S. Davis-Kaplan, S. Jiralerspong, L. Montermini, M. Pandolfo, J. Kaplan, Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin, Science 276 (1997) 1709–1712.
[10] P. Cavadini, C. Gellera, P.I. Patel, G. Isaya, Human frataxin maintains mitochondrial iron homeostasis in *Saccharomyces cerevisiae*, Hum. Mol. Genet. 9 (2000) 2523–2530.
[11] P. Ponka, Iron utilization in erythrocyte formation and hemoglobin synthesis, in: D.M. Templeton (Ed.), Molecular and Cellular Iron Transport, Marcel Dekker, New York, 2002, pp. 643–677.
[12] P. Ponka, Tissue-specific regulation of iron metabolism and heme synthesis: distinct control mechanisms in erythroid cells, Blood 89 (1997) 1–25.
[13] G.C. Shaw, J.J. Cope, L. Li, K. Corson, C. Hersey, G.E. Ackermann, B. Gwynn, A.J. Lambert, R.A. Wingert, D. Traver, N.S. Trede, B.A. Barut, Y. Zhou, E. Minet, A. Donovan, A. Brownlie, R. Balzan, M.J. Weiss, L.L. Peters, J. Kaplan, L.I. Zon, B.H. Paw, Mitoferrin is essential for erythroid iron assimilation, Nature 440 (2006) 96–100.
[14] S. Levi, B. Corsi, M. Bosisio, R. Invernizzi, A. Volz, D. Sanford, P. Arosio, J. Drysdale, A human mitochondrial ferritin encoded by an intronless gene, J. Biol. Chem. 276 (2001) 24437–24440.
[15] J. Drysdale, P. Arosio, R. Invernizzi, M. Cazzola, A. Volz, B. Corsi, G. Biasiotto, S. Levi, Mitochondrial ferritin: a new player in iron metabolism, Blood Cells Mol. Dis. 29 (2002) 376–383.
[16] J.N. Agar, C. Krebs, J. Frazzon, B.H. Huynh, D.R. Dean, M.K. Johnson, IscU is a scaffold for iron–sulfur cluster biosynthesis: sequential assembly of [2Fe–2S] and [4Fe–4S] clusters in IscU, Biochemistry 39 (2000) 7856–7862.
[17] I. Napier, P. Ponka, D.R. Richardson, Iron trafficking in the mitochondrion: novel pathways revealed by disease, Blood 105 (2005) 1867–1874.
[18] W.-H. Tong, T. Rouault, Distinct iron–sulfur cluster assembly complexes exist in the cytosol and mitochondria of human cells, EMBO J. 19 (2000) 5692–5700.
[19] F. Petrat, H. de Groot, R. Sustmann, U. Rauen, The chelatable iron pool in living cells: a methodologically defined quantity, Biol. Chem. 383 (2002) 489–502.
[20] O. Kakhlon, Z.I. Cabantchik, The labile iron pool: characterization, measurement, and participation in cellular processes, Free Radic. Biol. Med. 33 (2002) 1037–1046.
[21] M. Kruszewski, Labile iron pool: the main determinant of cellular response to oxidative stress, Mutat. Res. 531 (2003) 81–92.
[22] D.R. Richardson, P. Ponka, D. Vyoral, Distribution of iron in reticulocytes after inhibition of heme synthesis with succinylacetone: examination of the intermediates involved in iron metabolism, Blood 87 (1996) 3477–3488.
[23] M. Shvartsman, R.B. El, S. Epstejn, A. Shanzer, K. Raghavendra, A.M. Konijn, U. Rauen, R. Sustmann, I. Cabantchik, Iron can be delivered from plasma membrane to mitochondria via diffusion of chelator shielded complexes as viewed by live fluorescence imaging, First Congress of the International BioIron Society, Prague, May 22–26, 2005, Abstract 49.
[24] S.N. Wickramasinghe, M.J. Fulker, M.S. Losowsky, R. Hall, Microspectrophotometric and electron microscopic studies of bone marrow in hereditary sideroblastic anaemia, Acta Haematol. 45 (1971) 236–244.
[25] J.A. Grasso, J.D. Hines, A comparative electron microscopic study of refractory and alcoholic sideroblastic anaemia, Br. J. Haematol. 17 (1969) 35–44.
[26] L.H. Rademakers, J.C. Koningsberger, C.W. Sorber, H. Baart de la Faille, J. Van Hattum, J.J. Marx, Accumulation of iron in erythroblasts of patients with erythropoietic protoporphyria, Eur. J. Clin. Invest. 23 (1993) 130–138.
[27] M. Cazzola, R. Invernizzi, G. Bergamaschi, S. Levi, B. Corsi, E. Travaglino, V. Rolandi, G. Biasiotto, J. Drysdale, P. Arosio, Mitochondrial ferritin expression in erythroid cells from patients with sideroblastic anemia, Blood 101 (2003) 1996–2000.
[28] A.A. van de Loosdrecht, S.J. Brada, N.R. Blom, D.W. Hendriks, J.W. Smit, E. van den Berg, J.T. de Wolf, E. Vellenga, Mitochondrial disruption and limited apoptosis of erythroblasts are associated with high risk myelodysplasia. An ultrastructural analysis, Leuk. Res. 25 (2001) 385–393.
[29] A. Inbal, N. Avissar, M. Shaklai, A. Kuritzky, A. Schejter, E. Ben-David, S. Shanske, B.Z. Garty, Myopathy, lactic acidosis, and sideroblastic anemia: a new syndrome, Am. J. Med. Genet. 55 (1995) 372–378.
[30] K. Thakerngpol, S. Fucharoen, P. Boonyaphipat, K. Srisook, S. Sahaphong, V. Vathanophas, T. Stitnimankarn, Liver injury due to iron overload in thalassemia: histopathologic and ultrastructural studies, Biometals 9 (1996) 177–183.
[31] J.L. Bradley, J.C. Blake, S. Chamberlain, P.K. Thomas, J.M. Cooper, A.H. Schapira, Clinical, biochemical and molecular genetic correlations in Friedreich's ataxia, Hum. Mol. Genet. 9 (2000) 275–282.
[32] S.J. Tabrizi, A.H. Schapira, Secondary abnormalities of mitochondrial DNA associated with neurodegeneration, Biochem. Soc. Symp. 66 (1999) 99–110.
[33] H.M. Schipper, L. Bernier, K. Mehindate, D. Frankel, Mitochondrial iron sequestration in dopamine-challenged astroglia: role of heme oxygenase-1 and the permeability transition pore, J. Neurochem. 72 (1999) 1802–1811.
[34] H.M. Schipper, R. Vininsky, R. Brull, L. Small, J.R. Brawer, Astrocyte mitochondria: a substrate for iron deposition in the aging rat substantia nigra, Exp. Neurol. 152 (1998) 188–196.
[35] X. Wang, F. Manganaro, H.M. Schipper, A cellular stress model for the sequestration of redox-active glial iron in the aging and degenerating nervous system, J. Neurochem. 64 (1995) 1868–1877.
[36] D. Ham, H.M. Schipper, Heme oxygenase-1 induction and mitochondrial iron sequestration in astroglia exposed to amyloid peptides, Cell. Mol. Biol. (Noisy-le-grand) 46 (2000) 587–596.
[37] K. Mehindate, D.J. Sahlas, D. Frankel, Y. Mawal, A. Liberman, J. Corcos, S. Dion, H.M. Schipper, Proinflammatory cytokines promote glial heme oxygenase-1 expression and mitochondrial iron deposition: implications for multiple sclerosis, J. Neurochem. 77 (2001) 1386–1395.
[38] R. Lill, K. Diekert, A. Kaut, H. Lange, W. Pelzer, C. Prohl, G. Kispal, The essential role of mitochondria in the biogenesis of cellular iron–sulfur proteins, Biol. Chem. 380 (1999) 1157–1166.

[39] T.A. Rouault, W.H. Tong, Iron–sulphur cluster biogenesis and mitochondrial iron homeostasis, Nat. Rev. Mol. Cell. Biol. 6 (2005) 345–351.

[40] G. Link, R. Tirosh, A. Pinson, C. Hershko, Role of iron in the potentiation of anthracycline cardiotoxicity: identification of heart cell mitochondria as a major site of iron-anthracycline interaction, J. Lab. Clin. Med. 127 (1996) 272–278.

[41] R.S. Britton, K.S. Leicester, B.R. Bacon, Iron toxicity and chelation therapy, Int. J. Hematol. 76 (2002) 219–228.

[42] A. Masini, D. Ceccarelli, F. Giovannini, G. Montosi, C. Garuti, A. Pietrangelo, Iron-induced oxidant stress leads to irreversible mitochondrial dysfunctions and firosis in the liver of chronic iron-dosed gerbils: the effect of Silybin, J. Bioenerget. Biomembr. 32 (2000).

[43] G. Link, A. Saada, A. Pinson, A.M. Konijn, C. Hershko, Mitochondrial respiratory enzymes are a major target of iron toxicity in rat heart cells, J. Lab. Clin. Med. 131 (1998) 466–474.

[44] G. Link, A.M. Konijn, C. Hershko, Cardioprotective effect of alpha-tocopherol, ascorbate, deferoxamine, and deferiprone: mitochondrial function in cultured, iron-loaded heart cells, J. Lab. Clin. Med. 133 (1999) 179–188.

[45] J.W. Eaton, M.W. Qian, Molecular bases of cellular iron toxicity, Free Radic. Biol. Med. 32 (2002) 833–840.

[46] J.J. Salazar, B. Van Houten, Preferential mitochondrial DNA injury caused by glucose oxidase as a steady generator of hydrogen eroxide in human fibroblasts, Mutat. Res. 385 (1997) 139–149.

[47] F.M. Yakes, B. Van Houten, Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 514–519.

[48] F. Palau, Friedreich's ataxia and frataxin: molecular genetics, evolution and pathogenesis, Int. J. Mol. Med. 7 (2001) 581–589.

[49] M.B. Delatycki, J. Camakaris, H. Brooks, T. Evans-Whipp, D.R. Thorburn, R. Williamson, S.M. Forrest, Direct evidence that mitochondrial iron accumulation occurs in Friedreich ataxia, Ann. Neurol. 45 (1999) 673–675.

[50] L. Montermini, F. Rodius, L. Pianese, M.D. Molto, M. Cossee, V. Campuzano, F. Cavalcanti, A. Monticelli, F. Palau, G. Gyapay, et al., The Friedreich ataxia critical region spans a 150-kb interval on chromosome 9q13, Am. J. Hum. Genet. 57 (1995) 1061–1067.

[51] V. Campuzano, L. Montermini, M.D. Molto, L. Pianese, M. Cossee, F. Cavalcanti, E. Monros, F. Rodius, F. Duclos, A. Monticelli, F. Zara, J. Canizares, H. Koutnikova, S.I. Bidichandani, C. Gellera, A. Brice, P. Trouillas, G. De Michele, A. Filla, R. De Frutos, F. Palau, P.I. Patel, S. Di Donato, J.L. Mandel, S. Cocozza, M. Koenig, M. Pandolfo, Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion, Science 271 (1996) 1423–1427.

[52] V. Calabrese, R. Lodi, C. Tonon, V. D'Agata, M. Sapienza, G. Scapagnini, A. Mangiameli, G. Pennisi, A.M. Stella, D.A. Butterfield, Oxidative stress, mitochondrial dysfunction and cellular stress response in Friedreich's ataxia, J. Neurol. Sci. 233 (2005) 145–162.

[53] M.B. Delatycki, R. Williamson, S.M. Forrest, Friedreich ataxia: an overview, J. Med. Genet. 37 (2000) 1–8.

[54] D.R. Richardson, Friedreich's ataxia: iron chelators that target the mitochondrion as a therapeutic strategy? Expert Opin. Investig. Drugs 12 (2003) 235–245.

[55] S.M. Forrest, M. Knight, M.B. Delatycki, D. Paris, R. Williamson, J. King, L. Yeung, N. Nassif, G.A. Nicholson, The correlation of clinical phenotype in Friedreich ataxia with the site of point mutations in the FRDA gene, Neurogenetics 1 (1998) 253–257.

[56] A. Durr, M. Cossee, Y. Agid, V. Campuzano, C. Mignard, C. Penet, J.L. Mandel, A. Brice, M. Koenig, Clinical and genetic abnormalities in patients with Friedreich's ataxia, N. Engl. J. Med. 335 (1996) 1169–1175.

[57] S.I. Bidichandani, T. Ashizawa, P.I. Patel, The GAA triplet-repeat expansion in Friedreich ataxia interferes with transcription and may be associated with an unusual DNA structure, Am. J. Hum. Genet. 62 (1998) 111–121.

[58] A.M. Gacy, G.M. Goellner, C. Spiro, X. Chen, G. Gupta, E.M. Bradbury, R.B. Dyer, M.J. Mikesell, J.Z. Yao, A.J. Johnson, A. Richter, S.B. Melancon, C.T. McMurray, GAA instability in Friedreich's Ataxia shares a common, DNA-directed and intraallelic mechanism with other trinucleotide diseases, Mol. Cell 1 (1998) 583–593.

[59] A.L. Bulteau, H.A. O'Neill, M.C. Kennedy, M. Ikeda-Saito, G. Isaya, L.I. Szweda, Frataxin acts as an iron chaperone protein to modulate mitochondrial aconitase activity, Science 305 (2004) 242–245.

[60] H. Puccio, M. Koenig, Recent advances in the molecular pathogenesis of Friedreich ataxia, Hum. Mol. Genet. 9 (2000) 887–892.

[61] T.C. Cox, M.J. Bawden, N.G. Abraham, S.S. Bottomley, B.K. May, E. Baker, L.Z. Chen, G.R. Sutherland, Erythroid 5-aminolevulinate synthase is located on the X chromosome, Am. J. Hum. Genet. 46 (1990) 107–111.

[62] Y. Aoki, S. Muranaka, K. Nakabayashi, Y. Ueda, delta-Aminolevulinic acid synthetase in erythroblasts of patients with pyridoxine-responsive anemia. Hypercatabolism caused by the increased susceptibility to the controlling protease, J. Clin. Invest. 64 (1979) 1196–1203.

[63] H. Harigae, K. Furuyama, A. Kimura, K. Neriishi, N. Tahara, M. Kondo, N. Hayashi, M. Yamamoto, S. Sassa, T. Sasaki, A novel mutation of the erythroid-specific delta-aminolaevulinate synthase gene in a patient with X-linked sideroblastic anaemia, Br. J. Haematol. 106 (1999) 175–177.

[64] R. Allikmets, W.H. Raskind, A. Hutchinson, N.D. Schueck, M. Dean, D.M. Koeller, Mutation of a putative mitochondrial iron transporter gene (ABC7) in X-linked sideroblastic anemia and ataxia (XLSA/A), Hum. Mol. Genet. 8 (1999) 743–749.

[65] S. Bekri, G. Kispal, H. Lange, E. Fitzsimons, J. Tolmie, R. Lill, D.F. Bishop, Human ABC7 transporter: gene structure and mutation causing X-linked sideroblastic anemia with ataxia with disruption of cytosolic iron–sulfur protein maturation, Blood 96 (2000) 3256–3264.

[66] Y. Shimada, S. Okuno, A. Kawai, H. Shinomiya, A. Saito, M. Suzuki, Y. Omori, N. Nishino, N. Kanemoto, T. Fujiwara, M. Horie, E. Takahashi, Cloning and chromosomal mapping of a novel ABC transporter gene (hABC7), a candidate for X-linked sideroblastic anemia with spinocerebellar ataxia, J. Hum. Genet. 43 (1998) 115–122.

[67] J.N. Feder, A. Gnirke, W. Thomas, Z. Tsuchihashi, D.A. Ruddy, A. Basava, F. Dormishian, R. Domingo Jr., M.C. Ellis, A. Fullan, L.M. Hinton, N.L. Jones, B.E. Kimmel, G.S. Kronmal, P. Lauer, V.K. Lee, D.B. Loeb, F.A. Mapa, E. McClelland, N.C. Meyer, G.A. Mintier, N. Moeller, T. Moore, E. Morikang, C.E. Prass, L. Quintana, S.M. Starnes, R.C. Schatzman, K.J. Brunke, D.T. Drayna, N.J. Risch, B.R. Bacon, R.K. Wolff, A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis, Nat. Genet. 13 (1996) 399–408.

[68] J.N. Feder, Z. Tsuchihashi, A. Irrinki, V.K. Lee, F.A. Mapa, E. Morikang, C.E. Prass, S.M. Starnes, R.K. Wolff, S. Parkkila, W.S.

Sly, R.C. Schatzman, The hemochromatosis founder mutation in HLA-H disrupts beta2-microglobulin interaction and cell surface expression, J. Biol. Chem. 272 (1997) 14025–14028.

[69] M. Pissia, K. Polonifi, M. Politou, K. Lilakos, N. Sakellaropoulos, G. Papanikolaou, Prevalence of the G320V mutation of the HJV gene, associated with juvenile hemochromatosis, in Greece, Haematologica 89 (2004) 742–743.

[70] C. Lanzara, A. Roetto, F. Daraio, S. Rivard, R. Ficarella, H. Simard, T.M. Cox, M. Cazzola, A. Piperno, A.P. Gimenez-Roqueplo, P. Grammatico, S. Volinia, P. Gasparini, C. Camaschella, Spectrum of hemojuvelin gene mutations in 1q-linked juvenile hemochromatosis, Blood 103 (2004) 4317–4321.

[71] A. Krause, S. Neitz, H.J. Magert, A. Schulz, W.G. Forssmann, P. Schulz-Knappe, K. Adermann, LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity, FEBS Lett. 480 (2000) 147–150.

[72] T. Ganz, E. Nemeth, Iron imports. IV. Hepcidin and regulation of body iron metabolism, Am. J. Physiol. 290 (2006) G199–G203.

[73] A. Roetto, G. Papanikolaou, M. Politou, F. Alberti, D. Girelli, J. Christakis, D. Loukopoulos, C. Camaschella, Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis, Nat. Genet. 33 (2003) 21–22.

[74] A. Roetto, F. Daraio, P. Porporato, R. Caruso, T.M. Cox, M. Cazzola, P. Gasparini, A. Piperno, C. Camaschella, Screening hepcidin for mutations in juvenile hemochromatosis: identification of a new mutation (C70R), Blood 103 (2004) 2407–2409.

[75] H. Kawabata, R. Yang, T. Hirama, P.T. Vuong, S. Kawano, A.F. Gombart, H.P. Koeffler, Molecular cloning of transferrin receptor 2. A new member of the transferrin receptor-like family, J. Biol. Chem. 274 (1999) 20826–20832.

[76] R.E. Fleming, M.C. Migas, C.C. Holden, A. Waheed, R.S. Britton, S. Tomatsu, B.R. Bacon, W.S. Sly, Transferrin receptor 2: continued expression in mouse liver in the face of iron overload and in hereditary hemochromatosis, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 2214–2219.

[77] A. Mattman, D. Huntsman, G. Lockitch, S. Langlois, N. Buskard, D. Ralston, Y. Butterfield, P. Rodrigues, S. Jones, G. Porto, M. Marra, M. De Sousa, G. Vatcher, Transferrin receptor 2 (TfR2) and HFE mutational analysis in non-C282Y iron overload: identification of a novel TfR2 mutation, Blood 100 (2002) 1075–1077.

[78] S. Abboud, D.J. Haile, A novel mammalian iron-regulated protein involved in intracellular iron metabolism, J. Biol. Chem. 275 (2000) 19906–19912.

[79] A.T. McKie, P. Marciani, A. Rolfs, K. Brennan, K. Wehr, D. Barrow, S. Miret, A. Bomford, T.J. Peters, F. Farzaneh, M.A. Hediger, M.W. Hentze, R.J. Simpson, A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation, Mol. Cell. 5 (2000) 299–309.

[80] D.F. Wallace, P. Pedersen, J.L. Dixon, P. Stephenson, J.W. Searle, L.W. Powell, V.N. Subramaniam, Novel mutation in ferroportin1 is associated with autosomal dominant hemochromatosis, Blood 100 (2002) 692–694.

[81] A. Hayashi, Y. Wada, T. Suzuki, A. Shimizu, Studies on familial hypotransferrinemia: unique clinical course and molecular pathology, Am. J. Hum. Genet. 53 (1993) 201–213.

[82] C.M. Craven, J. Alexander, M. Eldridge, J.P. Kushener, S. Berstein, J. KKaplan, Tissue distribution and clearance kinetics of non-transferrin-bound iron in the hypotransferrinemic mouse: a rodent model for hemochromatosis, Proc. Natl. Acad. Sci. U.S.A. 84 (1987) 3457–3461.

[83] J.G. Parkes, D.M. Templeton, Transport of non-transferrin-bound iron by hepatocytes, in: D.M. Templeton (Ed.), Molecular and Cellular Iron Transport, Marcel Dekker, New York, 2002, pp. 451–466.

[84] P.L. Fox, The copper-iron chronicles: the story of an intimate relationship, Biometals 16 (2003) 9–40.

[85] E.D. Harris, Ceruloplasmin and iron: vindication after 30 years, Nutrition 15 (1999) 72–74.

[86] C.D. Vulpe, Y.M. Kuo, T.L. Murphy, L. Cowley, C. Askwith, N. Libina, J. Gitschier, G.J. Anderson, Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse, Nat. Genet. 21 (1999) 195–199.

[87] G.J. Anderson, D.M. Frazer, A.T. McKie, C.D. Vulpe, The ceruloplasmin homolog hephaestin and the control of intestinal iron absorption, Blood Cells Mol. Dis. 29 (2002) 367–375.

[88] H. Morita, S. Ikeda, K. Yamamoto, S. Morita, K. Yoshida, S. Nomoto, M. Kato, N. Yanagisawa, Hereditary ceruloplasmin deficiency with hemosiderosis: a clinicopaythological study of a Japanese family, Ann. Neurol. 37 (1995) 646–656.

[89] Z.L. Harris, L.J. Klomp, J.D. Gitlin, Aceruloplasminemia: an inherited neurodegenerative disease with impairment of iron homeostasis, Am. J. Clin. Nutr 67 (Suppl.) (1998) 972S–977S.

[90] M. Yonekawa, T. Okabe, Y. Asamoto, M. Ohta, A case of hereditary ceruloplasmin deficiency with iron deposition in the brain associated with chorea, dementia, diabetes mellitus and retinal pigmentation: administration of fresh-frozen human plasma, Eur. Neurol. 42 (1999) 157–162.

[91] T. Nittis, J.D. Gitlin, The copper-iron connection: hereditary aceruloplasminemia, Semin. Hematol. 39 (2002) 282–289.

[92] N.E. Hellman, M. Schaefer, S. Gehrke, P. Stegen, W.J. Hoffman, J.D. Gitlin, W. Stremmel, Hepatic iron overload in aceruloplasminaemia, Gut 47 (2000) 858–860.