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CERULOPLASMIN METABOLISM AND FUNCTION

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■ **Abstract** Ceruloplasmin is a serum ferroxidase that contains greater than 95% of the copper found in plasma. This protein is a member of the multicopper oxidase family, an evolutionarily conserved group of proteins that utilize copper to couple substrate oxidation with the four-electron reduction of oxygen to water. Despite the need for copper in ceruloplasmin function, this protein plays no essential role in the transport or metabolism of this metal. Aceruloplasminemia is a neurodegenerative disease resulting from inherited loss-of-function mutations in the ceruloplasmin gene. Characterization of this disorder revealed a critical physiological role for ceruloplasmin in determining the rate of iron efflux from cells with mobilizable iron stores and has provided new insights into human iron metabolism and nutrition.

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INTRODUCTION

Ceruloplasmin was first isolated from plasma and characterized as a copper-containing protein by Holmberg & Laurell in 1948 (39). Soon thereafter Scheinberg & Gitlin demonstrated a marked decrease in the concentration of this protein in serum samples from patients with Wilson disease, providing a biochemical test for this disorder that is still in clinical use today (90). Frieden (78) demonstrated

that ceruloplasmin is a ferroxidase, and this observation, taken together with Cartwright's (51) detailed nutritional studies in copper-deficient pigs, suggested a role for ceruloplasmin in iron homeostasis. In 1984 Putnam determined the complete amino acid sequence of human ceruloplasmin, revealing the single-chain structure of this molecule (94). Solution of the crystal structure of ascorbate oxidase by Messerschmidt permitted sequence alignment and delineation of the copper-binding amino acids in ceruloplasmin (60). Isolation and characterization of ceruloplasmin cDNA clones confirmed the amino acid sequence obtained by protein chemistry and demonstrated abundant expression of the ceruloplasmin gene in the liver (48, 101). An essential role for ceruloplasmin in iron metabolism was established in 1995 with the identification of patients with aceruloplasminemia (36, 105).

MULTICOPPER OXIDASES

Ceruloplasmin is a member of the multicopper oxidase family of enzymes. This evolutionarily conserved group of proteins is characterized by the presence of three types of spectroscopically distinct copper sites (59). Ceruloplasmin contains three type I copper sites, and charge transfer between the cysteine ligand sulfur and the copper at these sites results in strong absorption at 600 nm, conferring an intense blue color to this protein (Figure 1). A single type II copper is coordinated by four imidazole nitrogens and is in close proximity to two antiferromagnetically coupled type III copper ions that absorb at 330 nm. The type II and type III coppers form a trinuclear copper cluster that is the site of oxygen binding during the catalytic cycle (7). Resolution of the structure of human ceruloplasmin by X-ray crystallography has confirmed the presence of this trinuclear cluster as well as the identity of each of the amino acid copper ligands (107).

Multicopper oxidases utilize the facile electron chemistry of bound copper ions to couple substrate oxidation with the four-electron reduction of dioxygen. Electrons pass from the substrate to the type I copper and then to the trinuclear copper cluster and subsequently to the oxygen molecule bound at this site (60). Whereas the signature sequences encompassing the amino acid ligands for copper are highly conserved amongst all multicopper oxidases, the substrates, the number of type I coppers and precise mechanisms of intramolecular electron transfer vary from protein to protein (55). Unique members of this family of enzymes, which include the well-characterized proteins laccase and ascorbate oxidase, are present in bacteria, fungi, yeast, plants, worms, parasites, and mammals. Known substrates of the multicopper oxidases include manganese, iron, nitrate, bilirubin, phenols, and ascorbate. In addition to ceruloplasmin, several multicopper oxidases have been identified as playing a critical role in iron homeostasis. Fet3 is a ferroxidase essential for iron uptake in yeast, and hephaestin is a ceruloplasmin homologue that is required for efficient iron efflux from the placenta and enterocytes in mammals (6, 99).

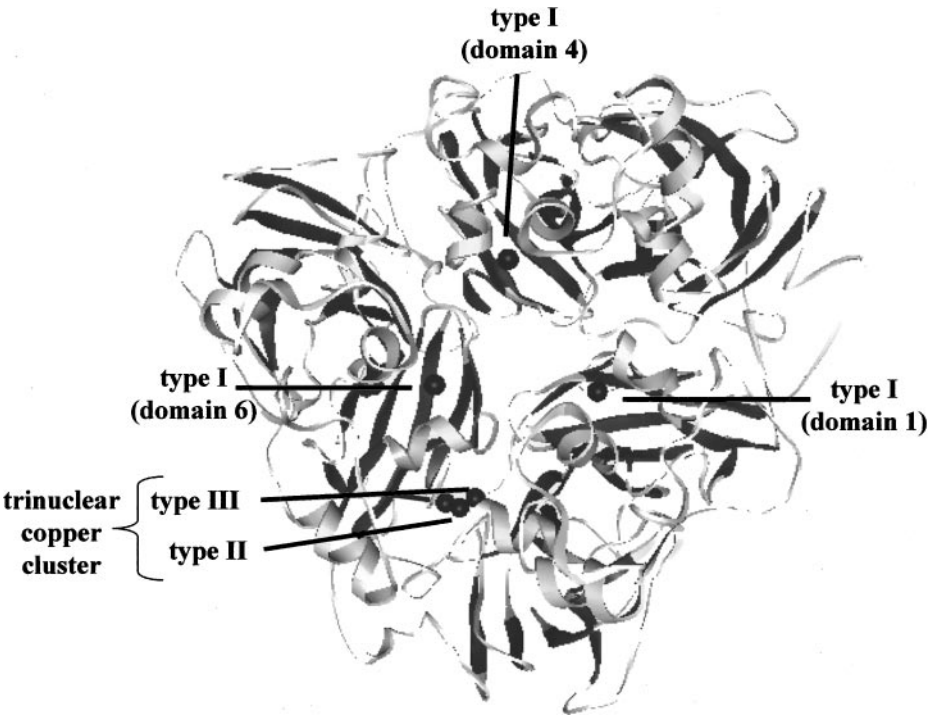


Figure 1 Structural model of human ceruloplasmin based on X-ray crystallographic data (107). The type I, II, and III coppers are indicated, as well as the site of the trinuclear copper cluster.

GENE STRUCTURE AND EXPRESSION

Human ceruloplasmin is encoded in 20 exons encompassing approximately 65 kb of DNA localized to chromosome 3q23-q24 (16, 37). A processed pseudogene for human ceruloplasmin encoding the carboxyl-terminal 563 amino acids has been identified and mapped by somatic cell hybridization to chromosome 8 (47). Although this pseudogene is not expressed, the presence of this sequence in the human genome must be considered in the design of any molecular diagnostic testing for aceruloplasminemia. In hepatocytes, the human ceruloplasmin gene is expressed as two transcripts of 3.7 and 4.2 kb, which arise from use of alternative polyadenylation sites within the 3′ untranslated region (48, 101). Abundant expression of these transcripts in the liver results in the 1046–amino acid protein detected in serum. Nucleotide and amino acid sequence comparisons suggest that serum ceruloplasmin and the serum clotting factors V and VIII constitute a family of structurally related proteins (9). Ceruloplasmin is an acute phase reactant,

and the serum concentration increases during inflammation, infection, and trauma largely as the result of increased gene transcription in hepatocytes mediated by the inflammatory cytokines (25). Cloning and characterization of ceruloplasmin from rat and mouse reveals 90% amino acid identity with the human sequence and similar patterns of gene expression in all three species (22, 44).

Although the liver is the predominant source of serum ceruloplasmin, extrahepatic ceruloplasmin gene expression has been demonstrated in many tissues including spleen, lung, testis, and brain (2, 22, 44, 100). Within the human central nervous system ceruloplasmin is expressed in astrocytic glia lining the brain microvasculature, surrounding dopaminergic neurons in the substantia nigra, and within the inner nuclear layer of the retina (45). Interestingly, recent studies demonstrate that ceruloplasmin is synthesized as a glycophosphatidylinositol (GPI)–anchored protein generated by alternative splicing of exons 19 and 20 in astrocytes and Sertoli cells (23, 37, 80, 81, 86). Although the precise function of this isoform is unknown, biosynthesis in these cell types suggests a role for this membrane-anchored form of ceruloplasmin in the oxidation and mobilization of iron at the blood-brain and blood-testis barriers. In addition to this GPI-linked isoform, a ceruloplasmin mRNA arising from alternative splicing of exon 18 and predicted to result in a protein with four additional amino acids has been detected in multiple extrahepatic cell types, although a protein product arising from this mRNA has not yet been characterized (102).

METABOLISM

Both the secreted and GPI-linked isoforms of ceruloplasmin are synthesized with six atoms of copper incorporated during biosynthesis late in the secretory pathway (37, 80, 87). Serum ceruloplasmin has a half-life of 5.5 days, and studies with radioactive copper demonstrate little or no copper exchange of ceruloplasmin-bound copper following synthesis (28, 93). Although copper has no effect on the rate of synthesis or secretion of ceruloplasmin, failure to incorporate this metal during synthesis results in the secretion of an unstable apoceruloplasmin moiety that is devoid of ferroxidase activity (30, 41, 42). In the normal adult about 10% of the total circulating ceruloplasmin is found as the apoprotein, which is rapidly catabolized with a half-life of about 5 hours (57). Consistent with these data, an increase in the hepatic copper pool results in a sustained increase in the serum ceruloplasmin concentration, whereas a decrease as occurs in nutritional copper deficiency, results in a marked decrease in serum ceruloplasmin (40, 77). Under normal circumstances the hepatic copper pool is not rate-limiting for holoceruloplasmin synthesis, as the serum ceruloplasmin concentration increases rapidly during infection, trauma, and pregnancy while the ratio of apo to holoceruloplasmin is maintained (57). Despite the fact that 90% of circulating serum ceruloplasmin is in the form of the holoprotein, the observed differences in the half-lives of apo and holoceruloplasmin indicate that under steady state conditions the rate of hepatic synthesis and secretion of both apo and holoproteins is equivalent.

Metabolic studies indicate that a single dose of dietary copper appearing in the portal circulation following gastrointestinal absorption is rapidly cleared by the liver with first pass kinetics. Within 24 hours about 10% of this dose reappears in the plasma in newly synthesized ceruloplasmin, a finding that simply reflects the relative abundance of this protein, as kinetic and metabolic studies reveal no direct role for ceruloplasmin in copper transport or delivery to tissues (28, 61). The only physiologically relevant mechanism for copper excretion is via the biliary tract, and the amount of copper appearing in bile is directly proportional to the size of the hepatic copper pool (29). Given the enormous capacity of the liver to increase biliary copper excretion, hepatic copper overload is a rare occurrence in the normal individual. As anticipated from earlier kinetic experiments, clinical and metabolic studies in aceruloplasminemia reveal no essential role for this protein in biliary copper excretion (26, 34).

Ceruloplasmin is detectable in the yolk sac and fetal liver of the human conceptus by the fifth week of gestation (27). The serum concentration of this protein increases throughout gestation, and kinetic studies indicate that this is entirely due to endogenous synthesis without any significant transfer of maternal ceruloplasmin across the placenta (27). Clinical and experimental studies demonstrate a significant diminution in biliary excretory capacity in the developing human fetus and newborn infant (5). Consistent with this, the fetal and newborn liver accumulates copper, and very little copper enters the secretory pathway of the hepatocyte (89). As a consequence, the newborn liver synthesizes and secretes mostly apoceruloplasmin, resulting in a decreased serum ceruloplasmin concentration in the newborn period. This decrease in serum ceruloplasmin concentration mitigates against the use of this protein as an indicator of nutritional or genetic abnormalities of copper homeostasis in the newborn period.

Electrophoretic variants of ceruloplasmin have also been identified that result in a two- to fourfold difference in serum ceruloplasmin concentration (70). The specific polymorphisms resulting in such variants have not been identified, and the mechanism for the differences in serum concentration are unknown. Ceruloplasmin is a serum glycoprotein and undergoes N-linked glycosylation within the hepatocyte prior to secretion. Heterogeneity of the carbohydrate chains of ceruloplasmin has been noted between individuals, as have variations in the carbohydrate structure in the same individual following the acute phase response (21, 56). Although N-linked glycosylation is not required for copper incorporation during biosynthesis, these changes in carbohydrate structure may have functional implications with regards to protein turnover or ferroxidase activity (87). Estrogen increases the synthesis of many hepatic proteins including ceruloplasmin, and it is well established that the serum ceruloplasmin concentration increases three- to fourfold during pregnancy, although the ratio of apo and holoceruloplasmin remains constant (50, 57). Reported associations of increased serum ceruloplasmin concentration and coronary vascular disease likely reflect the acute inflammatory nature of this disorder rather than any specific role for ceruloplasmin in vessel

pathology (52). Many experimental tumors and cancer lines demonstrate significant ceruloplasmin gene expression, and the serum ceruloplasmin concentration is increased in certain malignancies; however, the significance of these observations is unknown and the precise mechanisms for such increases are not well defined.

CELL BIOLOGY

The liver is the central organ of copper homeostasis, and within the liver hepatocytes are the primary site of copper metabolism. Hepatocytes are highly polarized epithelial cells that regulate copper excretion into the bile dependent upon the intracellular copper concentration. Wilson disease is an autosomal recessive disorder resulting in hepatic copper accumulation. Identification of the molecular defect in this disorder has permitted a detailed understanding of the cell biological mechanisms of copper homeostasis (54). Wilson disease results from the absence or dysfunction of a copper transporting ATPase localized to the *trans*-Golgi network of hepatocytes (88). This ATPase transports copper into the secretory pathway for subsequent incorporation into ceruloplasmin and excretion into bile. When the copper concentration in the hepatocyte increases, the Wilson ATPase traffics from the *trans*-Golgi network to a cytoplasmic vesicular compartment near the canalicular membrane. Copper is then sequestered in this vesicular compartment, and as the cytoplasmic copper concentration decreases, the Wilson ATPase returns to the *trans*-Golgi network while copper is excreted at the biliary canaliculus (43). This copper-dependent movement of the Wilson ATPase provides for a unique posttranslational mechanism that permits rapid and sensitive regulation of copper homeostasis by hepatocytes. This mechanism also accounts for the observation that loss-of-function mutations in the Wilson gene result in impaired biliary copper excretion and decreased serum ceruloplasmin (88).

Genetic and biochemical studies of *Saccharomyces cerevisiae* indicate that under physiological conditions intracellular copper availability is extraordinarily restricted (83). For this reason the delivery of copper to specific targets within the cell is mediated by a family of proteins termed copper chaperones that provide copper directly to specific proteins while protecting it from intracellular scavenging (85). The cytoplasmic copper chaperone atox1 is required for the delivery of copper to the secretory pathway via direct interaction with the Wilson ATPase in the *trans*-Golgi network (32, 49). Atox1 is thus directly in the pathway of copper delivery to ceruloplasmin; consistent with this, mice deficient in atox1 demonstrate increased mortality with evidence of impaired copper incorporation into cuproproteins in the secretory pathway of most cells (31).

The mechanism of copper incorporation into ceruloplasmin is not well defined. Genetic studies of *S. cerevisiae* demonstrate a requirement for both the H^+ transporting V-type ATPase and the CLC chloride channel Gef1 for copper incorporation into the homologous multicopper oxidase fet3 (20, 24). Although these experiments cannot distinguish between a requirement for these proteins in

copper transporting ATPase function and direct copper incorporation into fet3, biochemical studies reveal that an acidic environment equivalent to that found in mammalian endosomes is required for holofet3 synthesis (17). This observation is consistent with previous studies of the effect of decreased pH on copper exchange in ceruloplasmin in vitro (87). Interestingly these same studies also demonstrate that Cl^- is an allosteric modulator of copper incorporation into fet3, suggesting that similar factors may be required for this process in mammalian cells (17).

The nature of the secretory compartment in which copper is incorporated into ceruloplasmin is unknown. Fractionation studies and pulse-chase experiments indicate that copper is incorporated into ceruloplasmin late in the secretory pathway either at or beyond the *trans*-Golgi network (37, 74, 87, 96). Recent studies of a missense mutation that results in retention of ceruloplasmin in the endoplasmic reticulum support this concept and indicate that copper is not incorporated into nascent apoceruloplasmin in this compartment (37). Because quality control mechanisms ensure proper folding of newly synthesized ceruloplasmin prior to exiting the endoplasmic reticulum, these data on the site of copper incorporation suggest that additional events must occur to allow for metal acquisition by apoceruloplasmin late in the secretory pathway. Further work is needed to elucidate this process and to determine if common mechanisms exist for all cuproproteins in the secretory pathway.

FUNCTION

In vitro ceruloplasmin is capable of catalyzing the oxidation of a number of different substrates, a finding that has created some confusion as to the physiologic role of this protein. Frieden demonstrated that ceruloplasmin from human serum had considerable ferroxidase activity and that this protein was able to mobilize iron from perfused dog livers with the subsequent oxidation of ferrous iron and incorporation of the ferric product into apotransferrin (78, 79). In a series of elegant nutritional studies in pigs, Cartwright and his colleagues demonstrated that copper deficiency results in a marked decrease in circulating ceruloplasmin with concomitant iron accumulation in the liver and other tissues. The administration of oxidase-active ceruloplasmin to these animals resulted in the prompt release of iron into the circulation, which was detectable in circulating transferrin (51, 84). Although these biochemical and nutritional studies suggested a role for ceruloplasmin in iron homeostasis, the results were complicated by the presence of copper deficiency, which results in pleiotropic features.

Further evidence supporting a role for ceruloplasmin in iron homeostasis came from genetic studies designed to determine the mechanisms of iron uptake in yeast. High-affinity iron uptake in this organism is dependent upon the presence of the plasma membrane multicopper oxidase fet3 (6). This protein functions as a membrane ferroxidase, oxidizing iron in the extracellular milieu for subsequent uptake by the membrane iron permease ftr1 (92). Subsequent investigations have

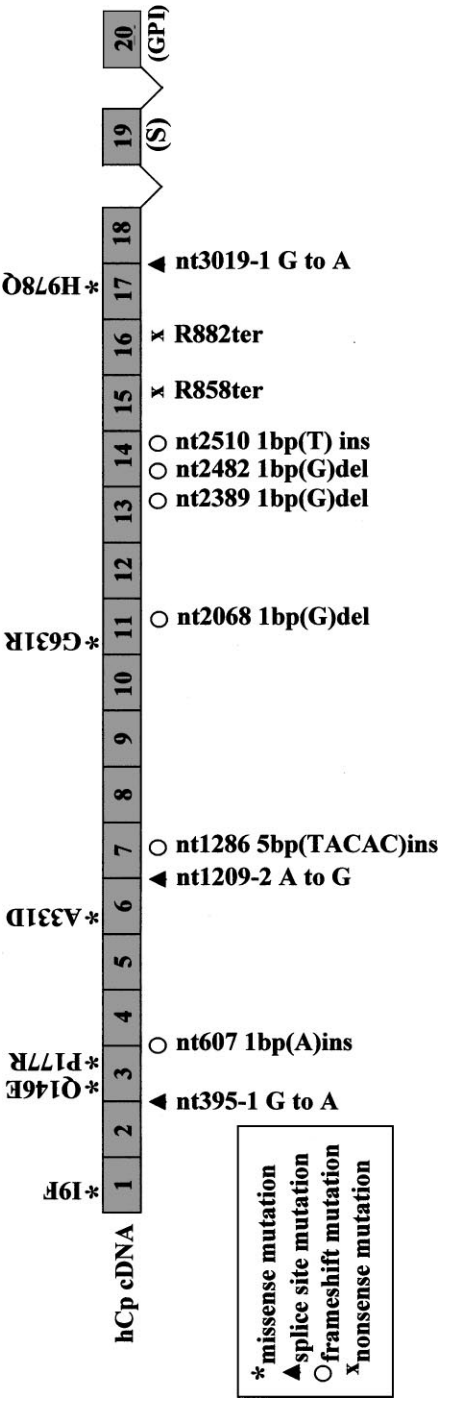
identified a second multicopper oxidase-permease complex that is required for iron transport in the intracellular vacuole of yeast (91, 98). *Sla* mice are affected with anemia secondary to impaired iron export from the intestine and placenta. Recognition that the defective gene in these animals encodes a multicopper oxidase with high homology to ceruloplasmin supported a role for these proteins in iron homeostasis in mammals (99). Definitive evidence in support of a physiologic role for ceruloplasmin in iron homeostasis came with the discovery of patients with aceruloplasminemia (36, 105).

ACERULOPLASMINEMIA

Early clinical studies of ceruloplasmin identified individuals with a heritable decrease in serum ceruloplasmin concentration to 50% of normal (8, 10, 19). This finding was not associated with any clinical abnormalities and was designated as hereditary hypoceruloplasminemia (19). However, in 1987 Miyajima and colleagues reported on a 52-year-old Japanese woman with diabetes, retinal degeneration, and basal ganglia symptoms in association with a complete absence of serum ceruloplasmin (67). Analysis of the serum ceruloplasmin concentration in family members of the proband revealed an autosomal recessive inheritance. Soon thereafter a report appeared on two brothers from Belfast with diabetes and dementia, both of whom lacked detectable serum ceruloplasmin, and a second Japanese family with similarly affected individuals (53, 73). Molecular genetic analysis of these original patients demonstrated loss-of-function mutations in the ceruloplasmin gene leading to the characterization of this disorder as aceruloplasminemia (36, 105). Subsequent studies have now identified a number of unique ceruloplasmin gene mutations in affected patients and family members (13, 15, 35, 37, 38, 46, 66, 76, 95, 103, 104) (Figure 2).

Patients with aceruloplasminemia usually present in the fourth or fifth decade of life with neurologic signs and symptoms that include various features of dementia, dysarthria, and dystonia (53, 67, 73). These neurologic features are progressive in most patients and are associated with significant iron accumulation in the basal ganglia that is detected as a low-intensity signal on T1- and T2-weighted magnetic resonance imaging. Affected individuals usually have a history of insulin-dependent diabetes mellitus at the time of presentation, resulting from iron accumulation in the endocrine pancreas with progressive loss of the β-cells in the islets of Langerhans (73). Although visual symptoms are less common, ophthalmologic examination

Figure 2 Mutations identified in aceruloplasminemia. The 20 exons of the ceruloplasmin gene are indicated in the boxes with the site of specific mutations as indicated. The nineteenth exon gives rise to the secreted isoform, and the twentieth exon, to the glycophosphatidylinositol (GPI)–linked isoform. All identified mutations are referenced in the text.



often shows evidence of photoreceptor degeneration in the peripheral retina as a consequence of iron deposition in this region. Despite limitation of the clinical signs and symptoms to this triad of diabetes, neurologic features, and retinal degeneration, all patients have evidence of abnormal iron homeostasis with marked parenchymal iron accumulation in most tissues. Consistent with this systemic abnormality in iron metabolism, most patients have a mild normochromic, normocytic anemia with a serum iron concentration decreased to about half of normal. Liver biopsy reveals significant iron accumulation in both hepatocytes and macrophages, often in the range observed in primary hemochromatosis (>1500 μg/g dry weight). Although this degree of iron accumulation results in significant hepatic injury and cirrhosis in hemochromatosis, patients with aceruloplasminemia have no evidence of liver injury and demonstrate normal hepatic architecture on biopsy.

Consistent with the findings of hepatic iron accumulation in all patients with aceruloplasminemia, the serum ferritin concentration is elevated at the time of diagnosis, often to the range of 1000–2000 ng/ml. By the third decade of life oral glucose tolerance tests are usually abnormal, even in asymptomatic patients, and the hemoglobin A_1C levels are elevated; however, there are no detectable antiinsulin antibodies. Results of liver function tests such as serum transaminases are also normal, and no other biochemical abnormalities are detected in the hematological, renal, or endocrine system. Cerebrospinal fluid examination shows no evidence of pleocytosis or hypoglycorrhachia but does reveal an elevation in iron content and total protein (64). Pathologic examination of the brain reveals cavitary degeneration of the basal ganglia, with obvious discoloration in this region (73). Histological findings from these affected regions include abundant spongiform degeneration and neuronal cell loss without evidence of inflammation or gliosos. A 10-fold increase in the iron content of the basal ganglia occurs, and Prussian blue staining of this tissue reveals iron accumulation within both neurons and glia (73).

ACERULOPLASMINEMIA: DIFFERENTIAL DIAGNOSIS

The association of basal ganglia symptoms with absent serum ceruloplasmin may lead to diagnostic confusion with Wilson disease. In such cases the finding of half-normal serum ceruloplasmin concentrations in parents or siblings, as well as low serum iron, elevated ferritin, diabetes, and evidence of brain iron accumulation on magnetic resonance imaging will help to confirm the diagnosis of aceruloplasminemia. Patients with aceruloplasminemia may also be misdiagnosed with hemochromatosis, especially if the abnormal iron parameters are noted prior to the onset of neurologic symptoms. It is essential to distinguish between these two diagnoses, as phlebotomy, the treatment of choice to reduce systemic iron in hemochromatosis, has no effect on iron accumulation in aceruloplasminemia and results in anemia, as patients are unable to mobilize reticuloendothelial cell iron (38). When hemochromatosis is a consideration, measurement of the serum transferrin saturation is useful, as this is elevated in hemochromatosis but decreased

in aceruloplasminemia (38). Autosomal dominant inheritance of mutations in the ferritin light chain gene also results in brain iron accumulation and a neurologic picture of extrapyramidal symptoms that may be confused with aceruloplasminemia (12). However, patients with this neuroferritinopathy have decreased serum ferritin concentration with no evidence of systemic iron accumulation or diabetes. Heterozygosity for aceruloplasminemia results in a half-normal serum ceruloplasmin concentration but is not usually associated with signs or symptoms of abnormal iron homeostasis. Although recent reports have noted neurologic abnormalities in individuals with a mutation in one ceruloplasmin allele, the relationship of this finding to the clinical features remains unclear (14, 65).

ACERULOPLASMINEMIA: MECHANISMS OF DISEASE

The impairment of iron homeostasis in patients with aceruloplasminemia is best understood by examining the role of ceruloplasmin in the systemic iron cycle (3) (Figure 3). Most of the iron used each day for hematopoiesis and other essential needs is recycled from heme as erythrocytes are turned over within the reticuloendothelial system. Iron transported in the plasma bound to transferrin must be oxidized prior to binding to this transport protein. Ceruloplasmin plays a critical role in the iron cycle by establishing a rate of iron oxidation sufficient for iron release from the reticuloendothelial system. The absence of serum ceruloplasmin in patients with aceruloplasminemia leads to a slow accumulation of iron in compartments where this metal is normally mobilized for recycling. This concept can be readily appreciated in a murine model of aceruloplasminemia in which an experimental situation is created in which either excess iron is delivered to the reticuloendothelial system or the hematopoietic requirement for iron is increased by phlebotomy (33). Consistent with these observations, the administration of ceruloplasmin as fresh frozen plasma to patients with aceruloplasminemia results in a rapid increase in serum iron (53, 104). Analysis of these experimental findings also illustrates why phlebotomy is an inappropriate and harmful approach to the iron overload in aceruloplasminemia. Patients with aceruloplasminemia have only mild anemia and are able to maintain the iron cycle to a degree sufficient for hematopoiesis, presumably owing to alternative oxidase sources in the plasma.

In addition to the slow accumulation of iron within the reticuloendothelial system, the absence of serum ceruloplasmin also results in increased ferrous iron in the plasma, which is rapidly removed from the circulation by the liver, pancreas, and other tissues. This process is analogous to what occurs in patients with atransferrinemia, in which serum transferrin is absent, or primary hemochromatosis, in which transferrin binding capacity is exceeded (11). This non–transferrin-dependent iron uptake is presumably mediated by DMT1, the divalent cation transporter expressed in most tissues, which has been shown to be required for normal intestinal iron uptake (4). This mechanism of iron uptake accounts for the accumulation of iron in hepatocytes and pancreatic β-cells observed in patients with aceruloplasminemia. In most situations 5% of the circulating serum ceruloplasmin

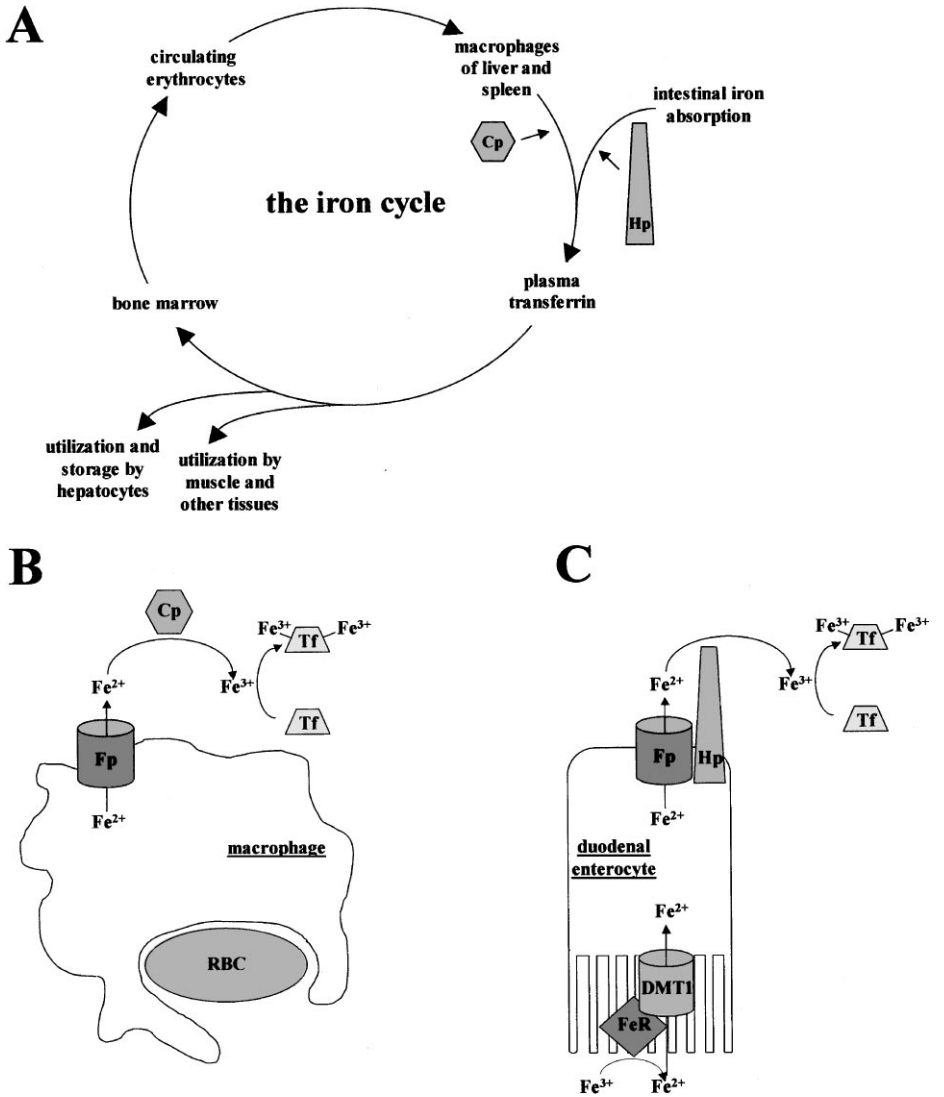


Figure 3 Role of the multicopper oxidases in the iron cycle. (*A*) The iron cycle is depicted with movement of iron between sites of storage and utilization. (*B*) In the reticuloendothelial system (macrophage) iron derived from heme is returned to the plasma via the membrane transporter ferroportin (Fp). Ceruloplasmin (Cp) plays an essential role in determining the rate of iron efflux via oxidation, which is required for binding to transferrin (Tf). (*C*) Iron absorption in the enterocyte requires the apical membrane reductase (FeR) and the divalent metal transporter-1 (DMT1), as well as the basolateral transporter Fp and the ceruloplasmin homologue hephaestin (Hp). The role of hephaestin in enterocyte iron efflux is analogous to that of ceruloplasmin in the reticuloendothelial system.

concentration is sufficient to sustain a normal plasma iron turnover rate, which is why abnormalities of iron homeostasis are rarely observed in patients with Wilson disease (84). Aceruloplasminemia does not result in impaired intestinal iron uptake and transport, as the oxidation of iron necessary for this process is accomplished by the homologous multicopper oxidase hephaestin (99).

Although ceruloplasmin is required for the efficient release of cellular iron, the actual mechanism of transport of iron from cells is mediated by the polytopic membrane protein ferroportin (IREG1, MTP1) (1, 18, 58) (Figure 3). Ferroportin plays a key role in the absorption of iron by enterocytes and the release of iron from the reticuloendothelial system; mutations in this gene result in an autosomal dominant form of hemochromatosis (72, 75). As anticipated from the model discussed above, affected patients with ferroportin mutations differ from those with primary hemochromatosis in that reticuloendothelial iron stores are increased, the transferrin saturation is decreased, and phlebotomy is poorly tolerated.

Iron accumulation in the central nervous system of patients with aceruloplasminemia does not arise from the increase in ferrous iron in the plasma, as accumulation of brain iron is not observed in other disorders with elevated plasma ferrous iron such as atransferrinemia or hemochromatosis. Ceruloplasmin does not cross the blood-brain barrier, which implies a direct role for locally synthesized ceruloplasmin in central nervous system iron metabolism. Early developmental studies have revealed biosynthesis of many plasma proteins in brain tissue, and consistent with this observation astrocyte-specific ceruloplasmin gene expression has been demonstrated throughout the cerebral microvasculature, surrounding dopaminergic neurons in the substantia nigra and in Müller glia cells in the retina (44, 45, 71). Biosynthetic studies indicate that ceruloplasmin is synthesized and secreted by astrocytes, confirming these gene expression studies and suggesting that local production of this protein is required for iron homeostasis in the central nervous system (44). Ceruloplasmin translated from brain mRNA in a Xenopus oocyte system is not secreted; consistent with this, the majority of ceruloplasmin expressed in the central nervous system is synthesized as a glycophosphatidylinositol (GPI)–linked isoform (71, 81). The role of this isoform in iron homeostasis has not been established, but the demonstration of copper incorporation into GPI-linked ceruloplasmin and the presence of ferroxidase activity by this protein suggests that this membrane association may be critical in determining the rate of iron release from storage cells within the central nervous system (37, 81).

The mechanisms of neurodegeneration in aceruloplasminemia are not well defined. Presumably ceruloplasmin functions both to oxidize ferrous iron for incorporation into transferrin following transport of iron through the microvasculature and to promote the release of iron from storage sites within the brain. This implies a cycle of iron in the brain similar to but largely separate from that present in the systemic circulation. Such a cycle would be useful in minimizing the effects of systemic iron deficiency on brain function. The absence of ceruloplasmin might result in glial cell injury from excessive iron accumulation with subsequent loss of critical glial-derived neurotrophic factors. Alternatively, the accumulation of

ferrous iron within the central nervous system might be expected to result in direct oxidant-mediated injury to neurons, and consistent with this, affected patients demonstrate evidence of increased lipid peroxidation and impaired fatty acid oxidation (62, 63, 69, 106). In either case, a role for iron accumulation is supported by studies that demonstrate an amelioration of neurologic symptoms in severely affected patients in association with a reduction of body iron stores following chelation therapy (68). Neuronal injury in this disease may also result in part from iron deficiency in specific cell types as the result of impaired mobilization of iron from storage pools. In certain circumstances specific missense mutations that result in impaired trafficking and ceruloplasmin retention within the endoplasmic reticulum may also contribute directly to the neuronal pathology through conformation-induced cellular injury (37).

CONCLUSION

Although ceruloplasmin has been recognized as the major copper-containing protein in plasma for more than half a century, the past few years have witnessed considerable progress in our understanding of the biological function of this protein. Given the critical role of iron in early cognitive development as well as recent studies that implicate copper in a number of neurodegenerative diseases, the recognition that ceruloplasmin plays an essential role in brain iron metabolism and neuronal survival provides much needed molecular insight into the mechanisms of iron homeostasis within the central nervous system (82, 97). Future studies must now address the molecular details of ceruloplasmin ferroxidase function including the mechanisms of ceruloplasmin-mediated iron release from cells and the specific pathways of iron trafficking and recycling within the central nervous system.

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LITERATURE CITED

1. Abboud S, Haile DJ. 2000. A novel mammalian iron–regulated protein involved in intracellular iron metabolism. *J. Biol. Chem.* 275:19906–12
2. Aldred AR, Grimes A, Schreiber G, Mercer JF. 1987. Rat ceruloplasmin. Molecular cloning and gene expression in liver, choroid plexus, yolk sac, placenta, and testis. *J. Biol. Chem.* 262:2875–78
3. Andrews NC. 1999. Disorders of iron metabolism. *N. Engl. J. Med.* 341:1986–95

4. Andrews NC. 2000. Iron homeostasis: insights from genetics and animal models. *Nat. Rev. Genet.* 1:208–17
5. Arrese M, Ananthananarayanan M, Suchy FJ. 1998. Hepatobiliary transport: molecular mechanisms of development and cholestasis. *Pediatr. Res.* 44:141–47
6. Askwith C, Eide D, VanHo A, Bernard PS, Li L, et al. 1994. The FET3 gene of *S. cerevisiae* encodes a multicopper oxidase required for ferrous iron uptake. *Cell* 76: 403–10
7. Calabrese L, Carbonaro M, Musci G. 1989. Presence of coupled trinuclear copper cluster in mammalian ceruloplasmin is essential for efficient electron transfer to oxygen. *J. Biol. Chem.* 264:6183–87
8. Cartwright GE, Markowitz H, Shields S, Wintrobe MM. 1960. Studies on copper metabolism. XXIX. *Am. J. Med.* 28:555–63
9. Church WR, Jernigan RL, Toole J, Hewick RM, Knopf J, et al. 1984. Coagulation factors V and VIII and ceruloplasmin constitute a family of structurally related proteins. *Proc. Nat. Acad. Sci. USA* 81:6934–37
10. Cox DW. 1966. Factors influencing serum ceruloplasmin levels in normal individuals. *J. Lab. Clin. Med.* 68:893–904
11. Craven CM, Alexander J, Eldridge M, Kushner JP, Bernstein S, et al. 1987. Tissue distribution and clearance kinetics of non-transferrin-bound iron in the hypotransferrinemic mouse: a rodent model for hemochromatosis. *Proc. Natl. Acad. Sci. USA* 84:3457–61
12. Curtis AR, Fey C, Morris CM, Bindoff LA, Ince PG, et al. 2001. Mutation in the gene encoding ferritin light polypeptide causes dominant adult-onset basal ganglia disease. *Nat. Genet.* 28:350–54
13. Daimon M, Kato T, Kawanami T, Tominaga M, Igarashi M, et al. 1995. A nonsense mutation of the ceruloplasmin gene in hereditary ceruloplasmin deficiency with diabetes mellitus. *Biochem. Biophys. Res. Commun.* 217:89–95
14. Daimon M, Moriai S, Susa S, Yamatani K, Hosoya T, et al. 1999. Hypocaeruloplasminaemia with heteroallelic caeruloplasmin gene mutation: MRI of the brain. *Neuroradiology* 41:185–57
15. Daimon M, Susa S, Ohizumi T, Moriai S, Kawanami T, et al. 2000. A novel mutation of the ceruloplasmin gene in a patient with heteroallelic ceruloplasmin gene mutation (HypoCPGM). *Tohoku J. Exp. Med.* 191:119–25
16. Daimon M, Yamatani K, Igarashi M, Fukase N, Kawanami T, et al. 1995. Fine structure of the human ceruloplasmin gene. *Biochem. Biophys. Res. Commun.* 208:1028–35
17. Davis-Kaplan SR, Askwith CC, Bengtzen AC, Radisky D, Kaplan J. 1998. Chloride is an allosteric effector of copper assembly for the yeast multicopper oxidase fet3p: an unexpected role for intracellular chloride channels. *Proc. Natl. Acad. Sci. USA* 95:13641–45
18. Donovan A, Brownlie A, Zhou Y, Shepard J, Pratt SJ, et al. 2000. Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. *Nature* 403:776–81
19. Edwards CQ, Williams DM, Cartwright GE. 1979. Hereditary hypoceruloplasminemia. *Clin. Genet.* 15:311–16
20. Eide D, Bridgham JT, Zhao Z, Matoon JR. 1993. The vacuolar H+ ATPase of *Saccharomyces cerevisiae* is required for efficient copper detoxification, mitochondrial function and iron metabolism. *Mol. Gen. Genet.* 241:447–56
21. Endo M, Suzuki K, Schmid K, Fournet B, Karamanos Y, et al. 1982. The structures and microheterogeneity of the carbohydrate chains of human plasma ceruloplasmin. A study employing 500-MHz 1H-NMR spectroscopy. *J. Biol. Chem.* 257: 8755–60
22. Fleming R, Gitlin JD. 1990. Primary structure of rat ceruloplasmin and analysis of tissue-specific gene expression during development. *J. Biol. Chem.* 265:7701–7

23. Fortna RR, Watson HA, Nyquist SE. 1999. Glycosyl phosphatidylinositol-anchored ceruloplasmin is expressed by rat Sertoli cells and is concentrated in detergent-insoluble membrane fractions. *Biol. Reprod.* 61:1042–49
24. Gaxiola RA, Yuan DS, Klausner RD, Fink GR. 1998. The yeast CLC chloride channel functions in cation homeostasis. *Proc. Natl. Acad. Sci. USA* 95: 4046–50
25. Gitlin JD. 1988. Transcriptional regulation of ceruloplasmin gene expression during inflammation. *J. Biol. Chem.* 263: 6281–87
26. Gitlin JD. 1998. Aceruloplasminemia. *Pediatr. Res.* 44:271–76
27. Gitlin D, Biasucci A. 1969. Development of gamma G, gamma A, gamma M, beta IC-beta IA, C 1 esterase inhibitor, ceruloplasmin, transferrin, hemopexin, haptoglobin, fibrinogen, plasminogen, alpha 1-antitrypsin, orosomucoid, beta-lipoprotein, alpha 2-macroglobulin, and prealbumin in the human conceptus. *J. Clin. Invest.* 48:1433–46
28. Gitlin D, Janeway CA. 1960. Turnover of the copper and protein moieties of ceruloplasmin. *Nature* 185:693
29. Gitlin D, Janeway CA. 1960. Absorption and excretion of copper in mice. *Nature* 188:150–51
30. Gitlin JD, Schroeder JJ, Lee-Ambrose LM, Cousins RJ. 1992. Mechanisms of caeruloplasmin biosynthesis in normal and copper-deficient rats. *Biochem. J.* 282:835–39
31. Hamza I, Faisst A, Prohaska J, Chen J, Gruss P, et al. 2001. The metallochaperone atox1 plays a critical role in perinatal copper homeostasis. *Proc. Natl. Acad. Sci. USA* 98:6848–52
32. Hamza I, Schaefer M, Klomp LWJ, Gitlin JD. 1999. Interaction of the copper chaperone HAH1 with the Wilson disease protein is essential for copper homeostasis. *Proc. Natl. Acad. Sci. USA* 96:13363–68
33. Harris ZL, Durley AP, Man TK, Gitlin JD. 1999. Targeted gene disruption reveals an essential role for ceruloplasmin in cellular iron efflux. *Proc. Natl. Acad. Sci. USA* 96:10812–17
34. Harris ZL, Klomp LW, Gitlin JD. 1998. Aceruloplasminemia: an inherited neurodegenerative disease with impaired iron homeostasis. *Am. J. Clin. Nutr.* 67:972S–77S
35. Harris ZL, Migas MD, Hughes AE, Logan JL, Gitlin JD. 1996. Familial dementia due to a frameshift mutation in the caeruloplasmin gene. *Q. J. Med.* 89:355–59
36. Harris ZL, Takahashi Y, Miyajima H, Serizawa M, MacGillivray RT. 1995. Aceruloplasminemia: molecular characterization of this disorder of iron metabolism. *Proc. Natl. Acad. Sci. USA* 92:2539–43
37. Hellman NE, Kono S, Miyajima H, Gitlin JD. 2002. Biochemical analysis of a missense mutation in aceruloplasminemia. *J. Biol. Chem.* 277:1375–80
38. Hellman NE, Schaefer M, Gehrke S, Stegen P, Hoffman WJ, et al. 2000. Hepatic iron overload in aceruloplasminaemia. *Gut* 47:858–60
39. Holmberg CG, Laurell CB. 1948. Investigations in serum copper. II. Isolation of the copper-containing protein and a description of some of its properties. *Acta Chem. Scand.* 2:550–56
40. Holtzman NA, Elliott DA, Heller RH. 1966. Copper intoxication. Report of a case with observations on ceruloplasmin. *N. Engl. J. Med.* 275:347–52
41. Holtzman NA, Gaumnitz BM. 1970. Identification of an apoceruloplasmin-like substance in the plasma of copper-deficient rats. *J. Biol. Chem.* 245:2350–53
42. Holtzman NA, Gaumnitz BM. 1970. Studies on the rate of release and turnover of ceruloplasmin and apoceruloplasmin in rat plasma. *J. Biol. Chem.* 245:2354–58
43. Hung IH, Suzuki M, Yamaguchi Y, Yuan DS, Klausner RD, Gitlin JD. 1997. Biochemical characterization of the Wilson disease protein and functional expression

in the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* 272:21461–66
44. Klomp LWJ, Farhangrazi ZS, Dugan LL, Gitlin JD. 1996. Ceruloplasmin gene expression in the murine central nervous system. *J. Clin. Invest.* 98:207–15
45. Klomp LWJ, Gitlin JD. 1996. Expression of the ceruloplasmin gene in the human retina and brain: implications for a pathogenic model in aceruloplasminemia. *Hum. Mol. Genet.* 5:1989–96
46. Kohno S, Miyajima H, Takahashi Y, Inoue Y. 2000. Aceruloplasminemia with a novel mutation associated with parkinsonism. *Neurogenetics* 2:237–48
47. Koschinsky ML, Chow BK-C, Schwartz J, Hamerton JL, MacGillivray RTA. 1987. Isolation and characterization of a processed gene for human ceruloplasmin. *Biochemistry* 26:7760–67
48. Koschinsky ML, Funk WD, VanOost BA, MacGillivray RT. 1986. Complete cDNA sequence of human preceruloplasmin. *Proc. Natl. Acad. Sci. USA* 83:5086–90
49. Larin D, Mekios C, Das K, Ross B, Yang AS, et al. 1999. Characterization of the interaction between the Wilson and Menkes disease proteins and the cytoplasmic copper chaperone, HAH1p. *J. Biol. Chem.* 274:28497–504
50. Laurell CB, Kullander S, Thorell J. 1968. Effect of administration of a combined estrogen-progestin contraceptive on the level of individual plasma proteins. *Scand. J. Clin. Lab. Invest.* 21:337–43
51. Lee GR, Nacht S, Lukens JN, Cartwright GE. 1968. Iron metabolism in copper-deficient swine. *J. Clin. Invest.* 47:2058–69
52. Libby P. 2001. Current concepts of the pathogenesis of the acute coronary syndromes. *Circulation* 104:365–72
53. Logan JL, Harveyson KB, Wisdom GB, Hughes AE, Archibold GP. 1994. Hereditary caeruloplasmin deficiency, dementia and diabetes mellitus. *Q. J. Med.* 87:663–70.
54. Loudianos G, Gitlin JD. 2000. Wilson disease. *Semin. Liver Dis.* 20:353–64
55. Machonkin TE, Quintanar L, Palmer AE, Hassett R, Severance S, et al. 2001. Spectroscopy and reactivity of the type 1 copper site in Fet3p from *Saccharomyces cerevisiae*: correlation of structure with reactivity in the multicopper oxidases. *J. Am. Chem. Soc.* 123:5507–17
56. Mackiewicz A, Ganapathi MK, Schultz D, Kushner I. 1987. Monokines regulate glycosylation of acute-phase proteins. *J. Exp. Med.* 166:253–58
57. Matsuda I, Pearson T, Holtzman NA. 1974. Determination of apoceruloplasmin by radioimmunoassay in nutritional copper deficiency, Menkes' kinky hair syndrome, Wilson's disease, and umbilical cord blood. *Pediatr. Res.* 8:821–24
58. McKie AT, Marciani P, Rolfs A, Brennan K, Wehr K, et al. 2000. A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. *Mol. Cell* 5:299–309
59. Messerschmidt A, Huber R. 1990. The blue oxidases, ascorbate oxidase, laccase and ceruloplasmin. Modeling and structural relationships. *Eur. J. Biochem.* 187: 341–52
60. Messerschmidt A, Rossi A, Ladenstein R, Huber R, Bolognesi M, et al. 1989. X-ray crystal structure of the blue oxidase ascorbate oxidase from zucchini. Analysis of the polypeptide fold and a model of the copper sites and ligands. *J. Mol. Biol.* 206:513–29
61. Meyer LA, Durley AP, Prohaska JR, Harris ZL. 2001. Copper transport and metabolism are normal in aceruloplasminemic mice. *J. Biol. Chem.* 276:36857–61
62. Miyajima H, Adachi J, Kohno S, Takahashi Y, Ueno Y. 2001. Increased oxysterols associated with iron accumulation in the brains and visceral organs of acaeruloplasminaemia patients. *Q. J. Med.* 94: 417–22
63. Miyajima H, Adachi J, Tatsuno Y, Takahashi Y, Fujimoto M, et al. 1998. Increased

very long-chain fatty acids in erythrocyte membranes of patients with aceruloplasminemia. *Neurology* 50:130–36

64. Miyajima H, Fujimoto M, Kohno S, Kaneko E, Gitlin JD. 1998. CSF abnormalities in patients with aceruloplasminemia. *Neurology* 51:1188–90
65. Miyajima H, Kono S, Takahashi Y, Sugimoto M, Sakamoto M, et al. 2001. Cerebellar ataxia associated with heteroallelic ceruloplasmin gene mutation. *Neurology* 57:2205–10
66. Miyajima H, Kohno S, Takahashi Y, Yonekawa O, Kanno T. 1999. Estimation of the gene frequency of aceruloplasminemia in Japan. *Neurology* 53:617–29
67. Miyajima H, Nishimura Y, Mizoguchi K, Sakanoto M, Shimizu T, et al. 1987. Familial apoceruloplasmin deficiency associated with blepharospasm and retinal degeneration. *Neurology* 37:761–67
68. Miyajima H, Takahashi Y, Kamata T, Shimizu H, Sakai N, et al. 1997. Use of desferrioxamine in the treatment of aceruloplasminemia. *Ann. Neurol.* 41:404–47
69. Miyajima H, Takahashi Y, Serizawa M, Kaneko E, Gitlin JD. 1996. Increased plasma lipid peroxidation in patients with aceruloplasminemia. *Free Radic. Biol. Med.* 20:757–60
70. Mohrenweiser HW, Decker RS. 1982. Identification of several electrophoretic variants of human ceruloplasmin including CpMichigan, a new polymorphism. *Hum. Hered.* 32:369–73
71. Mollgard K, Dziegielewska KM, Saunders NR, Zakut H, Soreq H. 1998. Synthesis and localization of plasma proteins in the developing human brain. Integrity of the fetal blood-brain barrier to endogenous proteins of hepatic origin. *Dev. Biol.* 128:207–21
72. Montosi G, Donovan A, Totaro A, Garuti C, Pignatti E, et al. 2001. Autosomal-dominant hemochromatosis is associated with a mutation in the ferroportin (SLC11A3) gene. *J. Clin. Invest.* 108: 619–23
73. Morita H, Ikeda S, Yamamoto K, Morita S, Yoshida K, et al. 1995. Hereditary ceruloplasmin deficiency with hemosiderosis: a clinicopathological study of a Japanese family. *Ann. Neurol.* 37:646–56
74. Murata Y, Yamakawa E, Iizuka T, Kodama H, Abe T, et al. 1995. Failure of copper incorporation into ceruloplasmin in the Golgi apparatus of LEC rat hepatocytes. *Biochem. Biophys. Res. Commun.* 209:349–55
75. Njajou OT, Vaessen N, Joosse M, Berghuis B, van Dongen JWA, et al. 2001. Mutation in SLC11A3 is associated with autosomal dominant hemochromatosis. *Nat. Genet.* 28:213–14
76. Okamoto N, Wada S, Oga T, Kawabata Y, Baba Y, et al. 1996. Hereditary ceruloplasmin deficiency with hemosiderosis. *Hum. Genet.* 97:755–58
77. Olivares M, Uauy R. 1996. Copper as an essential nutrient. *Am. J. Clin. Nutr.* 63: 791S–96S
78. Osaki S, Johnson D, Frieden E. 1966. The possible significance of the ferrous oxidase activity of ceruloplasmin in normal human serum. *J. Biol. Chem.* 241:2746–57
79. Osaki S, Johnson DA, Frieden E. 1971. The mobilization of iron from the perfused mammalian liver by a serum copper enzyme, ferroxidase. *J. Biol. Chem.* 246: 3018–23
80. Patel BN, David S. 1997. A novel glycosylphosphatidylinositol-anchored form of ceruloplasmin is expressed by mammalian astrocytes. *J. Biol. Chem.* 272:20185–90
81. Patel BN, Dunn RJ, David S. 2000. Alternative RNA splicing generates a glycosylphosphatidylinositol-anchored form of ceruloplasmin in mammalian brain. *J. Biol. Chem.* 275:4305–10
82. Pollitt E. 1993. Iron deficiency and cognitive function. *Annu. Rev. Nutr.* 13:521–37
83. Rae TD, Schmidt PJ, Pufahl RA, Culotta VC, O'Halloran TV. 1999. Undetectable

intracellular free copper: the requirement of a copper chaperone for superoxide dismutase. *Science* 284:805–8

84. Roeser HP, Lee GR, Nacht S, Cartwright GE. 1970. The role of ceruloplasmin in iron metabolism. *J. Clin. Invest.* 49:2408–17
85. Rosenzweig AC. 2000. Copper delivery by metallochaperone proteins. *Acc. Chem. Res.* 34:119–28
86. Salzer JL, Lovejoy L, Linder MC, Rosen C. 1998. Ran-2, a glial lineage marker, is a GPI-anchored form of ceruloplasmin. *J. Neurosci. Res.* 54:147–57
87. Sato M, Gitlin JD. 1991. Mechanisms of copper incorporation during the biosynthesis of human ceruloplasmin. *J. Biol. Chem.* 266:5128–34
88. Schaefer M, Gitlin JD. 1999. Genetic disorders of copper transport. *Am. J. Physiol.* 276:G311–14
89. Schaefer M, Hopkins RG, Failla ML, Gitlin JD. 1999. Hepatocyte-specific localization, copper-dependent trafficking and developmental expression of the Wilson disease protein in the liver. *Am. J. Physiol. Gastrointest. Liver Physiol.* 276:G639–G46
90. Scheinberg IH, Gitlin D. 1952. Deficiency of ceruloplasmin in patients with hepatolenticular degeneration (Wilson's disease). *Science* 116:484–89
91. Spizzo T, Byersdorfer C, Duesterhoeft S, Eide D. 1997. The yeast FET5 gene encodes a FET3-related multicopper oxidase implicated in iron transport. *Mol. Gen. Genet.* 256:547–56
92. Stearman R, Yuan DS, Yamaguchi-Iwa Y, Klausner RD, Dancis A. 1996. A permease-oxidase complex involved in high-affinity iron uptake in yeast. *Science* 271:1552–57
93. Sternlieb I, Morell AG, Tucker WD, Greene MW, Scheinberg IH. 1961. The incorporation of copper into ceruloplasmin *in vivo*: studies with copper^{64} and copper^{67}. *J. Clin. Invest.* 40:1834–40
94. Takahashi N, Ortel TL, Putnam FW. 1984. Single-chain structure of human ceruloplasmin: the complete amino acid sequence of the whole molecule. *Proc. Natl. Acad. Sci.USA* 81:390–94
95. Takahashi Y, Miyajima H, Shirabe S, Nagataki S, Suenaga A, et al. 1995. Characterization of a nonsense mutation in the ceruloplasmin gene resulting in diabetes and neurodegenerative disease. *Hum. Mol. Genet.* 51:81–84
96. Terada K, Kawarada Y, Miura N, Yasui O, Koyama K, et al. 1995. Copper incorporation into ceruloplasmin in rat livers. *Biochim. Biophys. Acta.* 1270:58–62
97. Thompson KJ, Shoham S, Connor JR. 2001. Iron and neurodegenerative disorders. *Brain Res. Bull.* 55:155–64
98. Urbanowski JL, Piper RC. 1999. The iron transporter Fth1p forms a complex with the Fet5 iron oxidase and resides on the vacuolar membrane. *J. Biol. Chem.* 274:38061–70
99. Vulpe CD, Kuo YM, Murphy TL, Cowley L, Askwith C, et al. 1999. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. *Nat. Genet.* 21:195–99
100. Yang F, Friedrichs WE, deGraffenried L, Herbert DC, Weaker FJ, et al. 1996. Cellular expression of ceruloplasmin in baboon and mouse lung during development and inflammation. *Am. J. Respir. Cell. Mol. Biol.* 14:161–69
101. Yang F, Naylor SL, Lum JB, Cutshaw S, McCombs JL, et al. 1986. Characterization, mapping and expression of the human ceruloplasmin gene. *Proc. Natl. Acad. Sci. USA* 83:3257–61
102. Yang FM, Friedrichs WE, Cupples RL, Bonifacio MJ, Sanford JA, et al. 1990. Human ceruloplasmin. Tissue-specific expression of transcripts produced by alternative splicing. *J. Biol. Chem.* 26510780–85
103. Yazaki M, Yoshida K, Nakamura A, Furihata K, Yonekawa M, et al. 1998. A novel

splicing mutation in the ceruloplasmin gene responsible for hereditary ceruloplasmin deficiency with hemosiderosis. *J. Neurol. Sci.* 156:30–34

104. Yonekawa M, Okabe T, Asamoto Y, Ohta M. 1999. 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.* 42:157–62
105. Yoshida K, Furihata K, Takeda S, Nakamura A, Yamamoto K, et al. 1995. A mutation in the ceruloplasmin gene is associated with systemic hemosiderosis in humans. *Nat. Genet.* 9:267–72
106. Yoshida K, Kaneko K, Miyajima H, Tokuda T, Nakamura A, et al. 2000. Increased lipid peroxidation in the brains of aceruloplasminemia patients. *J. Neurol. Sci.* 175:91–95
107. Zaitseva I, Zaitsev V, Card G, Moshkov K, Bax B, et al. 1996. The X-ray structure of human serum ceruloplasmin at 3.1 Å: nature of the copper centres. *J. Biol. Inorg. Chem.* 1:15–23