

33.1 Introduction

Copper, zinc and iron are essential cationic trace elements. They are transferred and utilized as inorganic ions and transported by specific carriers across membranes. They also require carriers to maintain their solubility within the intra- and extracellular compartments. Their homeostasis is controlled primarily by the gastrointestinal tract and the liver. Each of these elements has its own specific function and metabolic control [1]. The diagnosis of deficiencies or excesses of copper and zinc can be difficult, since no single test reliably indicates whether an individual is at risk. The clinical state, homeostatic mechanisms, metabolism and tissue distribution all have to be considered for the interpretation of data [1].

Copper is a component of many biologically important enzymes, such as cytochrome oxidase, superoxide dismutase, tyrosinase, dopamine-beta-hydroxylase, lysyl oxidase and ceruloplasmin.

Zinc plays a key role in biological functions. It stabilizes organic polymers, participates in hormone binding to nuclear and cell membrane receptors, gene transcription factors and has a regulatory and catalytic role in enzyme function. Numerous zinc metalloproteins have been identified: alkaline phosphatase, superoxide dismutase, aminopeptidases, angiotensin converting enzymes, endopeptidase, collagenase, carboxyl-peptidases and others [1].

Iron is essential for oxygen transport and utilization and for many oxidation-reduction reactions within the cell, especially for electron transfer in mitochondria. Hemoglobin, myoglobin, cytochromes, catalase or hydroxylases are iron-containing proteins involved in oxygen binding, transport or detoxification. Transferrin and lactoferrin are iron-transporting proteins and ferritin is the iron storage protein. Both, excess or deficiency of iron may be harmful [1].

The inborn errors of copper, zinc and iron metabolism are related to their transport across membranes and within the cells.

■ Wilson's Disease

Wilson's disease (WD, hepatolenticular degeneration) is a systemic copper intoxication with a defective copper binding P-type ATPase (WND, ATP 7B). It is an autosomal recessive disorder. The gene for ATP 7B is on chromosome 13q 14.1. A large number of mutations (>60) are known [2]. Copper accumulates initially in the liver due to a reduction in biliary excretion and subsequently becomes dispersed throughout the body, especially in brain. The onset of symptoms is variable, generally between 6 and 50 years of age. In childhood the hepatic form of the disease is prevalent. Later, the disease may initially present clinically with neuropsychological manifestations; however, there is always liver involvement. A Kayser-Fleischer ring is a clue for diagnosing Wilson's disease in addition to pathological liver function [3–5]. Biochemically, WD is defined by a low ceruloplasmin and a low serum copper (<1.2 $\mu\text{mol/l}$, <10 $\mu\text{mol/l}$ respectively). In some patients the levels may be in the lower reference range [5]. The differential diagnosis includes familial hypoceruloplasminemia and other hypoceruloplasminemic neurological syndromes [4, 6]. The distinction from Menkes disease is made by age of onset and different clinical signs and symptoms.

Treatment is directed towards reducing the amount of copper in the body [4, 5]. Measuring urinary copper and zinc can monitor the efficacy of chelation and/or zinc therapy. With adequate treatment the prognosis is good. In severe liver damage, liver transplantation might be necessary [5].

■ Menkes Disease

Menkes disease (MD), an X-linked recessive disorder (Xq13.3), is caused by a mutation in the copper transporting P-type ATPase (MNK, ATP 7A). More than 150 different mutations have been detected [7]. Biochemically, MD is characterized by a maldistribution of copper among organs and within cells, with low copper values in liver and brain and normal or high values in intestine, kidney, muscle and pancreas [4]. At birth plasma copper and ceruloplasmin may be normal or even elevated. Their levels decline or remain within normal limits until about 14 days of life and then decrease [6]. The classical form of MD may start in the neonatal period with hypothermia and hyperbilirubinemia. A diagnosis is usually made after 2 months of age because of progressive neurological deterioration, peculiar facies, hair abnormalities (kinky hair), hypopigmentation, bone changes and cutis laxa. Convulsions, apnea, infection and failure to thrive are also common features. Death generally occurs before the age of 3 years [6, 8] although some patients have survived longer. A milder form with slight mental retardation, ataxia and speech problems has been described [8]. Heterozygous females may also manifest mild features of this disorder, e.g. abnormal scalp hair [6, 9]. A diagnosis is confirmed by increased copper

incorporation in fibroblasts or by molecular genetic methods [4, 7]. There is no effective treatment – with the possible exception of copper-histidinate if substituted early (before the age of 6 weeks) [6, 10, 11]. It remains to be proven whether such treated patients have milder forms of MD, since two of them developed features of the occipital horn syndrome in adolescence [11].

■ Occipital Horn Syndrome

Occipital horn syndrome (OHS), formerly called X-linked cutis laxa, is an allelic variant of MD with mutations in the same ATP 7A gene [12]. The presence of barely detectable amounts (2–5%) of correctly spliced ATP 7A transcripts is sufficient to permit the development of the milder OHS-phenotype [13]. Serum copper and ceruloplasmin are usually below the normal range, but may be normal. Copper accumulation in fibroblasts may be as high as in MD. The clinical features include borderline intelligence, craniofacial abnormalities, skeletal dysplasia, connective tissue abnormalities, chronic diarrhea and orthostatic hypotension. Occipital horns, short clavicles, pectus excavatum, deformation of the bones of the upper extremities and genu valgum can be detected clinically, together with osteoporosis, which is most impressive in radiographs. The skin and joints are lax and hyperextensible, bladder diverticulae and obstructive uropathy are common findings [8, 14]. The clinical picture may first present in infancy with hypothermia, or in childhood (6 years) [12, 15], but usually in adolescence, and progresses slowly into adulthood. A comparison with mild MD is difficult because early clinical findings are not well documented. Brittle hair has been described in one patient and ataxia in another [15].

However, in fibroblasts there were no biochemical differences between MD and OHS [8]. Clinically, OHS must be differentiated from X-linked recessive Ehlers/Danlos syndrome type V. The latter is a milder disease with mild laxity of the skin and joints [12, 15].

Recently a new copper transport defect was described by Buchmann et al. [16] in a patient with symptoms of a demyelinating neuropathy, chronic intestinal pseudo-obstruction, osteoporosis, testicular failure, retinal degeneration and a cardiomyopathy with a tortuous aorta. In this patient, copper could not be incorporated into ceruloplasmin. The symptoms started at age 10 and the patient died at 21 years. These findings were clearly different from Wilson's and Menkes diseases.

■ Acrodermatitis Enteropathica

The clinical symptoms of acrodermatitis enteropathica (AE) are those of a zinc deficiency and include anorexia, failure to thrive, growth retardation, tremors; vesiculobullous, pustular and hyperkeratotic dermatitis; especially periorally, perianally and on the extensor sites of extremities; fine brittle hair with alopecia, and loose, frequent stools. Also the CNS may be affected with depression, irritability, visual disturbances and hypogeusia [17]. Increased susceptibility to infections due to impaired immune function is a common finding. Symptoms usually develop after the neonatal period, in exclusively breast-fed infants only after weaning, because a zinc transport ligand is supposed to be present in breast milk [18]. A low plasma zinc level confirms the diagnosis. Treatment consists of zinc substitution (50–150 mg/day) and usually leads to a rapid improvement of the clinical features. Cessation of treatment is followed by a rapid recurrence of symptoms. Long-term treatment requires one to consider the influence of zinc on the absorption of other trace elements, especially copper [19]. Inheritance of AE is autosomal recessive. The basic defect is still unknown. Impaired cellular zinc processing appears to be the primary cause leading ultimately to reduced zinc absorption [19]. Recently, the gene has been placed to the chromosomal region 8q24.3 [19a].

■ Hemochromatosis

Hereditary hemochromatosis (HH) is an inherited disorder of iron metabolism characterised by excessive iron absorption and toxic accumulation of iron in the parenchymal cells of liver, heart, pancreas and other endocrine organs. There are four types of HH known:

● *Hereditary Hemochromatosis Type 1 (HLA related)*

Hereditary hemochromatosis type 1 (HLA related) is the most frequent hereditary disorder in middle and northern Europe. The gene frequency is 1:10, the disease frequency about 1 in 400 and there is a male preponderance. The HFE-gene is located on chromosome 6p21.3. The C282Y gene mutation is present in 60–100% of patients, mostly in a homozygous form. A second mutation (H63D) seems to be of clinical relevance only in combination with the C282Y mutation [2].

Symptoms appear in the 4th or 5th decade of life and include hepatosplenomegaly, liver cirrhosis, diabetes mellitus, cardiomyopathy, hyperpigmentation and hypogonadism. Hepatocellular carcinoma develops in approximately one third of the patients and leads to death [20, 21].

Diagnosis is based on increased levels of ferritin and serum iron with elevated transferrin saturation and is confirmed by increased hepatic iron

content. Today, molecular genetic methods are the easiest way to make a diagnosis once there is clinical suspicion of HH. In families with HH, early detection of members at risk is mandatory since treatment with regular venesection or desferoxamin in individuals with anemia can reduce or prevent iron overload.

The HFE protein is expressed predominantly in the crypt cells of the duodenum. It is associated with the transferrin receptor (TFR) and with beta-2-microglobulin. HFE regulates iron transport by decreasing the iron uptake by transferrin. In instances where there is a non-functional HFE-protein, more iron is absorbed and accumulates in the liver and other organs [22, 23].

● *Juvenile Hemochromatosis (HH Type 2)*

Juvenile hemochromatosis (HH type 2) has clinical features similar to those of type 1, but the clinical course is more severe and characterized by an earlier onset (below 30 years of age). It presents with abdominal pain in the 1st decade of life, and later with cardiac symptoms and endocrine dysfunction. This autosomal recessive disease is rare. The gene has been localised to chromosome 1q and is genetically distinct from HH type 1 [24].

● *Hemochromatosis Type 3*

In patients with HH but no gene defect on the HFE gene (6p21.3), a mutation has been found in the transferrin receptor-2 (TRF2) gene on chromosome 7q22. The clinical, biochemical and histopathological findings are the same as in type 1 [25].

● *Neonatal or Perinatal Hemochromatosis (NH)*

There is a form of hemochromatosis called *neonatal or perinatal hemochromatosis (NH)* or neonatal iron storage disease, which is characterized by severe liver disease with intrauterine onset associated with extrahepatic siderosis which spares the reticuloendothelial elements. About one hundred cases have been reported [26]. It remains unclear whether NH is the result of a fetal liver disease of unknown etiologies or a heritable disorder of iron processing and storage. Cases in siblings have been described suggesting an autosomal recessive pattern [26]. According to OMIM, neonatal hemochromatosis also includes *neonatal giant cell hepatitis* (231100), which may have different causes, e.g. metabolic disorders like such as in bile acid synthesis. These disorders are not included in this chapter. However, special nosologies within NH include the autosomal recessive tricho-hepato-enteric syndrome (THES) which, to the present time, cannot be distinguished at the molecular genetic level [27]. NH combined with renal tubular dysgen-

esis (RTD) [28], the Finnish lethal neonatal metabolic syndrome (FLNMS), is characterised by severe intrauterine growth retardation, fulminant lactic acidosis perinatally, a Fanconi type aminoaciduria and abnormalities in iron metabolism including liver hemosiderosis and early death. This disorder has been localised to chromosome 2q33-q37 [29].

■ Aceruloplasminemia

Aceruloplasminemia (AC) is a rare autosomal recessive disorder of iron metabolism characterized by a defect in the gene coding for ceruloplasmin (3q23-25) [30]. The gene frequency is 70/100 000 in Japan and the incidence is approximately 1 in 2 million in the case of non consanguineous marriages. Six families have been reported in Japan and one family in England [31]. Homozygous individuals are symptomless until the fourth decade of life. Clinical symptoms include: mental confusion, memory loss, dementia, cerebellar ataxia, altered motor function, retinal degeneration and diabetes mellitus. Biochemical signs are a decreased serum copper and absent or non functioning ceruloplasmin, however, copper homeostasis is only minimally affected, if at all. There are, however, significant alterations in iron metabolism. Serum iron is low, ferritin high and tissue iron deposition is increased, not only in the liver and brain, but also in the pancreas, heart, kidney and endocrine organs. Therapy with intravenous ceruloplasmin alone, or in combination with deferoxamine, can correct iron homeostasis [32].

33.2 Nomenclature

No.	Disorder and affected component	Tissue distribution	Chromosome localisation	MIM
33.1	Wilson's disease	Liver, brain, cornea, kidney, placenta	13q14.3	277900
33.2	Menkes disease	Liver, brain, intestine, muscle, kidney, connective tissue, hair, fibroblasts (for diagnosis)	Xq13.3	309400
	Cu-binding P-type ATPase Cu-dependent enzyme deficiencies Cu-export problem (1) "Classical" form (2) "Mild" form (atypical)		Allelic mutation	
33.3	Occipital Horn syndrome	Connective tissue, bone fibroblasts (for diagnosis), intestine, nervous system	Xq13.3	304150
	Cu-binding P-type ATPase Lysyl oxidase deficiency		Allelic mutation	
33.4	Acrodermatitis Enteropathica	Muscle, bone, liver, pancreas, skin and hair, fibroblasts (for diagnosis)	8q24.3	201100
33.5	Deficiency of zinc-dependent enzymes Hemochromatosis			
33.5.1	(1) Classical form, HFE 1 defective HFE-protein in enterocytes leading to increased absorption of dietary iron and iron accumulation	Liver, skin, joints, heart, endocrine organs, immune system	6p21.3	235200
33.5.2	(2) Juvenile form, HFE 2 similar mechanism, early iron accumulation	Similar HFE 1, heart and endocrine organs more pronounced	1q	602390
33.5.3	(3) HFE 3 transferrin receptor-2-deficiency	Similar HFE 1	7q22	604250
33.5.4	(4) Neonatal hemochromatosis (NH, heterogeneous group) – Finnish lethal neonatal metabolic syndrome, hypotransferrinemia – Renal tubular dysgenesis (RTD) and severe neonatal hemosiderosis – Tricho-hepato-enteric syndrome	Liver, other organs depending on different disorders liver, kidney Liver, pancreas, thyroid, kidney, spleen Liver, pancreas, adrenal, thyroid, pituitary	2q33-q37 Not known Not known	603358 267430 –
33.6	Aceruloplasminemia Ceruloplasmin (ferroxidase-) deficiency Accumulation of iron	Liver, brain, pancreas, eye	3q23-q25	604290

33.3 Metabolic Pathways

■ Metabolic Pathway for Copper

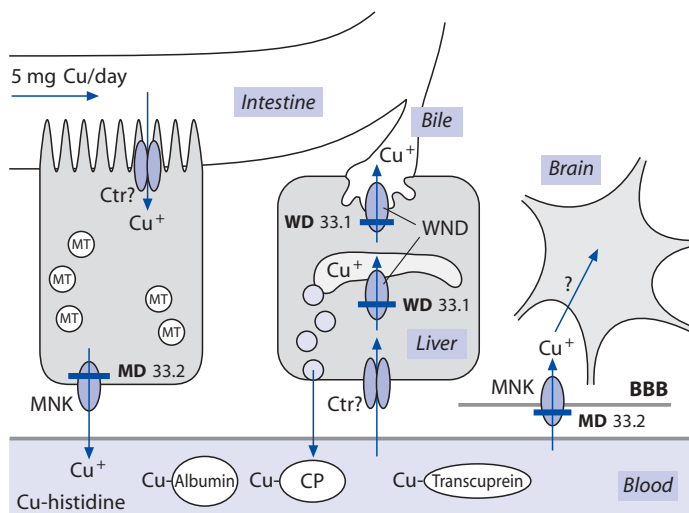


Fig. 33.1. Schematic drawing of the key elements of copper homeostasis and defects in Wilson's and Menkes disease. Dietary copper in the intestine is taken up by intestinal cells *via* an unidentified transporter, possibly hCtrl [1]. Metallothioneins (MT) can buffer excess copper inside these and other cells. The Menkes copper ATPase (MNK, ATP7A) exports copper from intestinal cells into the circulation. In the blood, copper can form complexes with small molecules like histidine and is bound to proteins such as albumin or transcuprein. The liver takes up circulating copper by a first pass process, possibly again involving hCtrl. In hepatocytes, which express the Wilson (WND, ATP7B) rather than the Menkes copper ATPase, copper is transported into the *trans*-Golgi network for incorporation into ceruloplasmin [2]. Holo-ceruloplasmin, a multi-copper ferroxidase with a role in iron homeostasis [3], is secreted into the circulation *via* the secretory pathway. Excess copper is excreted into the bile by hepatocytes. This process requires the activity of the Wilson ATPase, which undergoes copper-induced trafficking from a *trans*-Golgi to a periplasmic location [4]. The transport of copper across the blood brain-barrier (BBB) appears to be catalyzed by the Menkes ATPase expressed in cerebrovascular endothelial cells [5]. The lack of function of the Menkes copper ATPase in Menkes disease ($-MD\ 33.2$) leads to copper accumulation in the intestine and in other tissues. Systemically administered copper can hardly be transported to the brain. A defect in the Wilson copper ATPase in Wilson's disease ($-WD\ 33.1$) results in copper accumulation in hepatocytes, causing liver damage. The lack of holo-ceruloplasmin synthesis also affects iron homeostasis in patients with Wilson's disease

■ Zinc

A transport defect at the luminal level of the enterocytes has been suggested for zinc in acrodermatitis enteropathica.

■ Metabolic Pathway for Iron

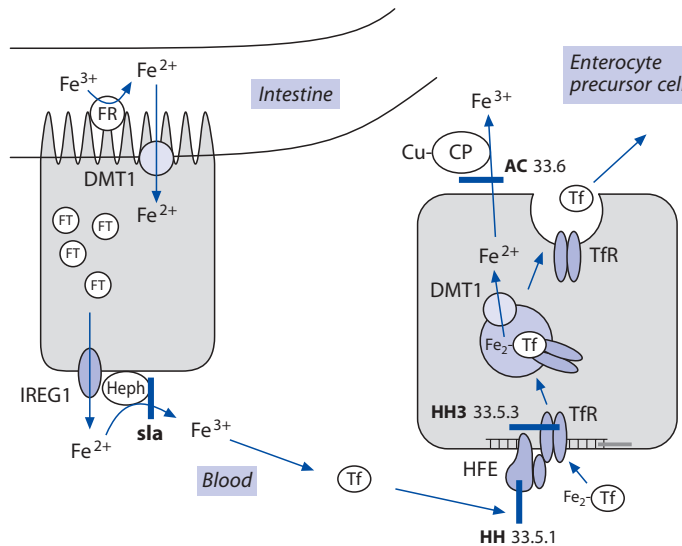


Fig. 33.2. Schematic drawing of the key elements of iron homeostasis and defects in aceruloplasminemia and hemochromatosis. Intestinal iron is reduced by an unknown ferric reductase (*FR*) and transported into intestinal cells by the divalent metal transporter DMT1 (formerly called Nramp2 or DCT1), and also by other routes. Inside cells, iron is stored as ferritin (*FT*) and hemosiderin. (In erythroid cells, most of the iron moves to mitochondria, where it is incorporated into protoporphyrin to make heme.) On the basolateral side, iron leaves the epithelium via a basolateral transporter, IREG1 [6], followed by oxidation through the action of hephaestin, a membrane-bound ceruloplasmin-like multicopper ferroxidase [23]. Iron-loaded transferrin ($\text{Fe}_2\text{-Tf}$) binds to the transferrin receptor (*TfR*) on the surface of cells. The receptor-transferrin complex, localized in clathrin-coated pits (*TTTT*), is invaginated and forms endosomes. These specialized endosomes acquire a low internal pH due to the action of a proton pump (not shown). This leads to the dissociation of the iron from transferrin. Iron leaves the endosomes via DMT1. Apo-transferrin and transferrin receptors recycle to the plasma membrane for re-use. This iron uptake mechanism is found in most cell types, including enterocyte precursor cells. Excess iron can leave at least some cell types by an unknown mechanism involving ceruloplasmin (*CP*), a non-membrane multicopper ferroxidase (see Fig. 31.1). Aceruloplasminemia ($-AC$ 33.6) leads to accumulation of iron in neural cells, hepatocytes and pancreatic islets cells. Sex-linked hemochromatosis ($-sla$) in mice is due to a defect in hephaestin, resulting in iron accumulation in enterocytes due to reduced egress. Hereditary hemochromatosis ($-HH$ 33.5.1) results from mutations in HFE (originally called HLA-H), a protein with sequence similarity to major histocompatibility complex class I molecules [2]. HFE forms a heterodimer with β_2 -microglobulin, and some mutations that lead to hemochromatosis interrupt this interaction and thus lead to excess iron accumulation. Defects in a second transferrin receptor, *TfR2*, have recently been implicated in type 3 hemochromatosis ($-HH3$ 33.5.3) [11]

33.4 Signs and Symptoms with Each of the Signs/Symptoms Tables

Table 33.1. Wilson's disease (many hundreds of patients)

System	Signs/symptoms	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Jaundice		+	±	±
	Hepatosplenomegaly		+	+	+
	Kayser-Fleischer-ring		+	±	±
	Ascites		+	+	+
Routine laboratory	Hemolytic anemia		+	±	±
	ASAT (S)		↑	n-↑	n-↑
	ALAT (S)		↑	n-↑	n-↑
	Albumin (S)		↓	↓-n	↓-n
Special laboratory	Bilirubin (S)		↑	n-↑	n-↑
	Copper (S)	↓	↓	↓	↓
	Copper (liver)	n-↑	↑	↑	↑
	Copper (U)		↑	↑	↑
CNS	Ceruloplasmin (S)	↓	↓	↓	↓
	Incorporation of Cu in ceruloplasmin (liver tissue biopsy)		↓	↓	↓
	Fanconi syndrome (4-hydroxyphenylpyruvic acid, U)	+	+	+	+
	Coma		±	+	±
	Encephalopathy		±	±	+
	Epilepsy			+	+
	Lethargy		±	+	+
	Dystonia			+	+
	Ataxia			+	+
	Athetosis			±	±
	Irritability, tremor		±	±	±
	Handwriting			±	±
Eye	Dysarthria			+	+
	Dysphagia		±	+	+
	Peripheral neuropathy			+	+
	Pseudo-bulbar paralysis			±	±
	Muscle spasmus		±	±	±
	Clumsiness		±	+	+
	Speech difficulties			+	+
	Schizophrenia			±	±
	Cataracts		±	±	±
	Strabismus		±	±	±
	Xerophthalmia		±	±	±
	Night blindness		±	±	±
Hematological	Acute hemolysis		±	±	±
	Thrombocytopenia		±	±	±
	Leucopenia		±	±	±
	Pancytopenia		±	±	±
	Coagulopathies		±	±	±
	Hemorrhages		±	±	±
	Epistaxis		±	±	±
	Intravascular coagulation		±	±	±

Table 33.1 (continued)

System	Signs/symptoms	Infancy	Childhood	Adolescence	Adulthood
Liver	Hepatomegaly		±	±	±
	Atypical hepatitis		±	±	±
	Hepatic coma		±	±	±
	Liver failure		±	±	±
	Esophageal varices		±	±	±
Renal	Renal tubular acidosis		±	±	±
	Aminoaciduria		±	±	±
	Hypercalciuria		+	+	±
	Uricosuria		±	±	±
	Acute renal failure		±	±	±
	Renal stones		±	±	±
	Hematuria		±	±	±
Skeletal	Osteoporosis		±	±	±
	Patchy osteosclerosis		±	±	±
	Osteomalacia		±	±	±
	Chondrocalcinosis		±	±	±
	Osteoarthritis		±	±	±
	Chondromalacia		±	±	±
	Morning stiffness		±	±	±
Endocrine	Amenorrhea			±	±
	Gynaecomastia			±	±
Others	Abdominal pain			±	±
	Peritonitis			±	±
	Blue lanulae of finger nails			±	±

Table 33.2.1. Menkes disease (classical form) (~ 400 patients)

System	Signs/symptoms	Neonatal	Infancy	Childhood	Adolescence
Characteristic clinical findings	Psychomotor retardation	±	+	+	+
	Convulsions	±	+	+	+
	Hypothermia	+	+	+	+
	Connective tissue abnormalities	±	+	+	+
	Feeding difficulties	±	±	±	
	Peculiar facies	+	+	+	+
	Hair abnormality	±	+	+	+
Routine laboratory	Neutropenia	±	±	±	±
	Anemia	±	±	±	±
	Bilirubin (P)	n-↑	n-↑	n-↑	
	EEG – pathological	±	±	±	+
Special laboratory	Copper (S)	↓	↓	↓	↓
	Ceruloplasmin	↓	↓	↓	↓
	Copper (liver)	↓	↓	↓	↓
	Copper (duodenal)	↑	↑	↑	↑
	Copper (FB)	↑	↑	↑	↑
	Copper (CSF)	↓	↓	↓	↓
	Catecholamines (CSF)	↓	↓	↓	↓
	Lactic acid (S)		n-↑	n-↑	
	Organic acids (U)		n-↑	n-↑	
CNS	Mental retardation		+	+	+
	Progressive cerebral degeneration		±	+	+
	Spasticity		±	+	+
	Subdural hematoma		±	±	
Connective tissue	Lax skin		+	+	+
	Bladder diverticula		±	±	±
	Hernia		±	±	
	Tortuous arteries		±	±	±
	Arterial ruptures		±	±	
	Undescended testis		±	±	
Bone	Osteoporosis		+	+	
	Wormian bone	±	+	+	
	Skeletal abnormalities & fractures	±	±	±	
Hair	Kinky hair (pili torti)	±	+	++	
Others	Prematurity	±			
	Failure to thrive		±	+	
	Dysmorphic features		+	±	
	Pudgy cheeks		+	+	
	Cupid – bow lips		+	+	
	Micrognathia		+	+	
	Thrombosis		±	±	
	Gingiva hyperplasia		±	±	
	Dolichomicrocephaly		±	±	
	Hydronephrosis		±	±	

Table 33.2.2. Menkes disease (mild form) (~ 5 patients)

System	Signs/symptoms	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Hair abnormalities			+	+	
	Joint laxity	±	±	+	+	
	Skin laxity	±	±	+	+	
Routine laboratory	Neutropenia			±		
Special laboratory	Ceruloplasmin (S)			↓-n	↓	
	Copper (S)			↓-n	↓	
	Copper (liver)			↓	↓	
	Copper (duodenal)			↑	↑	
	Copper (FB)			↑	↑	
CNS	Mental retardation			±	±	
	Ataxia			±	±	
Others	Facial appearance			±	±	
	Bladder diverticulae			±	±	
	Recurrent urinary tract infection			±	±	
	Vomiting (pyloric stenosis)	±	±	±		
	Recurrent fever			±		

Table 33.3. Occipital horn syndrome (~ 30 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Laxity of skin and soft tissues			+	+	+
	Exostosis (occipital horn)			+	+	+
	Diarrhea			+	+	+
	Orthostatic hypotension				+	+
Special laboratory	Ceruloplasmin (S)			↓-n	↓-n	↓-n
	Copper (S)			↓-n	↓-n	↓-n
	Copper (FB)			↑	↑	↑
	X-ray of long bones, clavicles			±	+	+
	Sonography of kidney and bladder			±	+	+
CNS	Mild psychomotor retardation				±	±
	Muscular hypotonia				±	±
Connective tissue	Tortuous vessels				±	±
	Bladder diverticula		+	+	+	+
Musculoskeletal	X-ray skeletal changes (osteoporosis, occipital horn, short and broad clavicles, deformed long bones)			+	±	±
	Disturbances of joints				±	±
	Chest wall deformity				±	±
	Restricted elbow mobility				±	±
	Hypothermia		+			
Others	Unusual facies				±	±
	Coarse hair				±	±
	Recurrent infection				±	±
	Urinary tract infections			+	+	+

Table 33.4. Acrodermatitis enteropathica (200 patients)

System	Signs/symptoms	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Dermatitis	±	++	+++	++	++
	Failure to thrive		+	++	++	++
	Irritability	±	+	++	±	±
	Anorexia		+	++	±	±
	Diarrhea	±	+	++	++	++
Routine laboratory	Alopecia		+	++	++	++
	Alkaline phosphatase (P)		↓	↓	↓	↓
	Ammonia (B)		n-↑	n-↑	n-↑	n-↑
Special laboratory	Zinc (S)		↓	↓-n	↓-n	↓-n
	Zinc (FB)		↓	↓	↓	↓
	Zinc uptake (duodenal)		↓	↓	↓	↓
	5-Nucleotidase (FB)		↓	↓	↓	↓
CNS	Depression			±	±	±
	Apathy		±	±	±	±
	Tremor			±	±	±
	Ataxia			±	±	±
Eye	Nystagmus			±	±	±
	Photophobia			±	±	±
	Night blindness			±	±	±
Growth	Height-retardation		±	±	±	±
	Delayed puberty				±	±
	Hypogonadismus				±	±
Others	Infections		±	±	±	±
	Pica			±	±	±

Table 33.5. Hereditary hemochromatosis (HH) (HH1: thousands of patients, HH2: about 25 patients, HH3: about 13 patients, NH: 100 patients)

System	Signs/symptoms	Neonatal/infancy				Childhood Juvenile	Adolescence HH (HFE2)	Adulthood		
		NH	+RTD	+FLNMS	+THES			HFE1	HFE2	HFE3
Characteristic clinical findings	Symptoms start in 4 th –6 th decade							+		+
	Symptoms <30 yrs					+	+		+	
	Cirrhosis	+	+	+	++		±	+	+	+
	Diabetes						±	±	±	±
	Hyperpigmentation of skin						+	±	+	±
	Heart failure						++	±	++	±
	Hypogonadotropic hypogonadism						++	±	++	±
	Phenotypic male preponderance							+		
	Intrauterine growth retardation	+	+	±						
	Failure to thrive		+		+					
	Lactic acidosis			+						
	Intractable diarrhea				+					
	Dysmorphic features	+	+	+	+					
	ASAT (S)	↑		↑		n-↑	n-↑	↑	↑	↑
	ALAT (S)	↑		↑		n-↑	n-↑	↑	↑	↑
Routine laboratory	Bilirubin (S)	↑	↑	↑	↑			n-↑		n-↑
	Albumin (S)	↓	↓	↓	↓					
	Iron (S)	↑	↑	↑	↑	↑	↑	↑	↑	↑
	Ferritin (S)	↑	↑	↑	↑	↑	↑	↑	↑	↑
	Transferrin			↓						
Special laboratory	Transferrin saturation	↑	↑	↑	↑		↑	↑	↑	↑
	α-Fetoprotein							n-↑		n-↑
	Iron (liver)	↑	↑	↑	↑		↑	↑	↑	↑
	MRI (iron in liver, also prenatal)		↑							
	Gonadotropins						↓	↓	↓	↓
	FSH						↓	↓	↓	↓
	LH						↓	↓	↓	↓
	Iron (U)						↑	↑	↑	↑
	Iron (U, after deferoxamine)						↑	↑	↑	↑
	Thyroid hormones (S)						n-↓		n-↓	
	Lactate/pyruvate (S)			↑						
	Methionine (S)				↑					
	Aminoaciduria			↑						
Hematological	Anemia		+							
	Coagulopathy		+							
Cardiac	Cardiomyopathy						+	±	+	±
	Arrhythmia						+	±	+	±
	Congestive heart failure						+	±	++	±

Table 33.5 (continued)

System	Signs/symptoms	Neonatal/infancy				Childhood Juvenile	Adolescence HH (HFE2)	Adulthood		
		NH	+RTD	+FLNMS	+THES			HFE1	HFE2	HFE3
Gastro-intestinal	Abdominal pain					+	+	±	+	±
	Hepatomegaly			+			+	+	+	+
	Splenomegaly						+	±	+	±
	Liver-Fibrosis/Cirrhosis	+	+	+	+		+	+	+	+
	Cholestasis	+	+	+	+					
	Ascites	+	+					±		±
	Risk of hepatocellular carcinoma		±					±		
Connec-tive tissue	Arthritis (proximal interphalangeal, metacarpophalangeal, knees, back,neck)							+		+
Joints										
Bone (X-ray)	Joint space narrowing							±		±
	Periarticular sclerosis							±		±
	Subchondral cysts							±		±
	Chondrocalcinosis							±		±
Hair	Loss of body hair							±		±
	Trichomalacia				+					
Skin	Increased melanin						+	±	+	±
	Atrophy of skin							±		±
	Ichthyosiform skin							±		±
	Koilonychia							±		±
Endo-crine	Diabetes mellitus						±	±	+	±
	Anterior pituitary dysfunction						+	±	+	±
	Gonadal dysfunction						+	±	+	±
Others	Weakness, fatigue							±		±
	Weight loss							±		±
	Lack of interest							±		±
	Placental hyperplasia				+					
	Hydramnios				+					

NH, neonatal hemochromatosis; +RTD, with renal tubular dysgenesis; +FLNMS, with Finnish lethal neonatal metabolic syndrome; +THES, with Tricho-hepato-enteric syndrome

Table 33.6. Aceruloplasminemia

System	Signs/symptoms	Adolescence	Adulthood
Characteristic clinical findings	Dementia		+
	Cerebellar ataxia		+
	Motor dysfunction		+
	Retinal degeneration		+
	Diabetes mellitus	±	+
Routine laboratory	Anemia		±
	Iron (S)		↓
	Ferritin (S)		↑
	Glucose (B)		n-↑
Special laboratory	Ceruloplasmin (S)		↓
	Copper (S)		↓
	Transferrin (S)		↓-n
	Iron (U)		↓
	Iron (liver)		↑
	Copper (liver)		n-↑
	CT (liver iron)		↑
	MRI (brain iron)		↑
	EEG		±
	Impaired memory		+
CNS	Dementia		+
	Clumsiness		+
	Muscular hypotonia		±
	Choreoathetosis		+
	Ataxic gait		+
	Dystonia		+
	Slurred speech		+
	Blepharospasms		±
Eye	Retinal pigment degeneration		+
Skin	Pigmentation		±
Liver	Portal fibrosis (slight)		±

33.5 Reference Values

■ Serum Copper and Ceruloplasmin

	Copper (S) ^a (μmol/l)	Ceruloplasmin (S) (μmol/l)
Newborn	4.57 (2.05–10.9)	0.9 (0.07–2.24)
2 months	11.2 (4.6–21.7)	1.64 (0.52–3.58)
6 months	15.28 (8.03–25.2)	2.54 (1.19–5.97)
12 months	19.7 (10.4–32.9)	3.21 (1.64–5.90)
1–5 years	12.6–23.6	2.42±0.9
6–9 years	13.2–21.4	1.9
10–13 years	12.6–19.0	(1.4–2.7)
>14 years	11–22	2.17±0.67

^a Atomic absorption.

Refs [6, 18].

■ Copper in Urine and CSF

	Cu (urine) ^a		Cu (CSF) ^a nmol/l
	μmol/24 h	μmol/mol creat.	
1–2 years	0.15±0.04		40.8–190
Schoolchildren	0.1–0.27 (<0.48)	41.5 ^b (6–119)	
Adults	0.29±0.12 (0.065–0.48)		200 (100–300)

^a Atomic absorption

^b Morning urine

Refs [6, 33, 34]

■ Zinc in Serum and Urine

	Zn (S) ^a μmol/l	Zn (Urine) ^a		
		μmol/24 h	μmol/mol creat	μmol/kg/day
Newborns	11.6 (7.9–15.3)			
1–2 years		1.2±0.3		0.12±0.01
Schoolchildren	12.6 (9.8–16.8)	4.6±2.6	9.1 ^b (2.4–22.6)	
Adults	14.5±2 (11–19)	6.5±2.5		

^a Atomic absorption.

^b Morning urine [17, 19].

■ Hemoglobin, Iron, Ferritin and Transferrin in Serum, Iron in CSF

	Hb g/l (±2SD)	Iron (μmol/l)	Ferritin (μg/l)	Transferrin (g/l, range)
Serum				
Newborn	185±30	6.4–33	110–503	1.8 (1.4–2.29)
3–6 months	115±20		4–405	2.03 (1.58–2.57)
6–12 months	120±15			–
2–6 years	125±10		2–63	2.39 (1.86–3.03)
6–12 years	135±20			2.17 (1.97–3.19)
12–18 years				
Women	140±20		9–79	
Men	145±15		9–59	
Adults				
Women	140±20	6.6–26.0	6–81	2.0–3.4
Men	155±20	10.6–28.0	30–233	
CSF		0.4 (0.2–0.6)		14.4 mg/l

Refs [34, 35].

33.6 Pathological Values/Differential Diagnosis

	Cu (S) μmol/l	Cu (U) μg/24 h	Cu (CSF) μg/100 ml	Cpl (S) μmol/l	Zn (S) μmol/l	Zn (urine) μmol/d	Fe (S) μmol/l	Fe (U) μmol/l	Ferritin μg/l	Transferrin + -saturation
Wilson's disease										
– presymptomatic	<10	>100								
– symptomatic	<10	>100	↑	0–1.3	–	–	–	–	–	–
Menkes disease										
– classical form	2–6 ↓	↓, n-↑	↓	<1 ↓	n-↑	n-↑	–	–	–	–
– mild form	9.5 ↓	↓	↓	n-↓						
Occipital Horn syndrome	n-↓	↓		n-↓	–	–	–	–	–	–
Acrodermatitis enteropathica	–	–	–	–	7.1±5.0	1.5±0.9	–	–	–	–
Hemochromatosis	n	n	–	n	–	–	>30 ↑	↑	>300 ↑ up to 5000	↑ >70
Aceruloplasminemia	0.16–0.7 ↓	–	0.2 (n)	<1 ↓	–	–	<7 n-↓	↓	>300 ↑ up to 5000	n-↓

Refs [14, 27, 29, 31, 32, 33–35].

■ Reference and Pathological Values for Copper in Liver, Duodenum and Fibroblasts, and for Iron in Liver

	Cu (liver) (μg/g dry weight)	Cu (duodenal) (μg/g dry weight)	⁶⁴ Cu (FB) (ng/mg protein/20 h)	Iron (liver) (μg/g dry weight)	HIC ^a μg/g w.w.	AAS ^b
Controls	15–50 (50–120 first year)	7–30	11±3	103–760	<1	
Wilson's disease	200–3000	–	14±4	–	–	
MD classical Form	10–20	50–90	70±12	–	–	
MD mild form	18	98		–	–	
OHS	–	–	51.8 (control 4–9.6)	–	–	
Hemochromatosis	–	–	–	1000–5000	2–10	
Aceruloplasminemia	n↑	–	–	1000–4000	↑	3290

^a HIC, Hepatic iron index (hepatic iron concentration divided by age).

^b AAS, Atom absorption spectroscopy (μg/g wet weight).

Refs [2, 4, 6, 32, 34, 35].

33.7 Loading Test

In Wilson's disease a penicillamine loading test has been used for heterozygote detection. Copper excretion is greater in heterozygotes than in normal persons after loading.

In hemochromatosis a deferoxamine and penicillamine loading test has been tried in a few patients as diagnostic tool. Urinary iron excretion is increased in iron storage disorders after deferoxamine.

33.8 Diagnostic Flow Chart

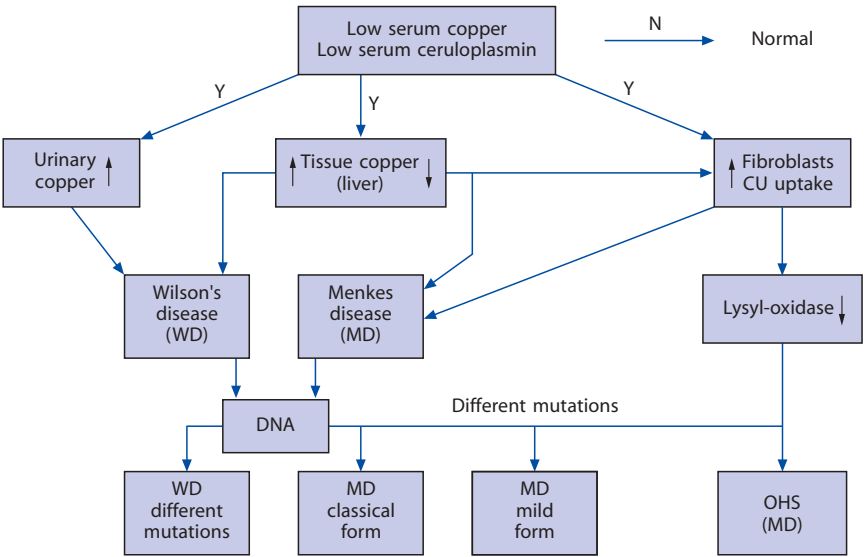


Fig. 33.3. Diagnostic flow chart for inborn errors of copper metabolism. Urinary copper studies should be performed even if serum studies are normal

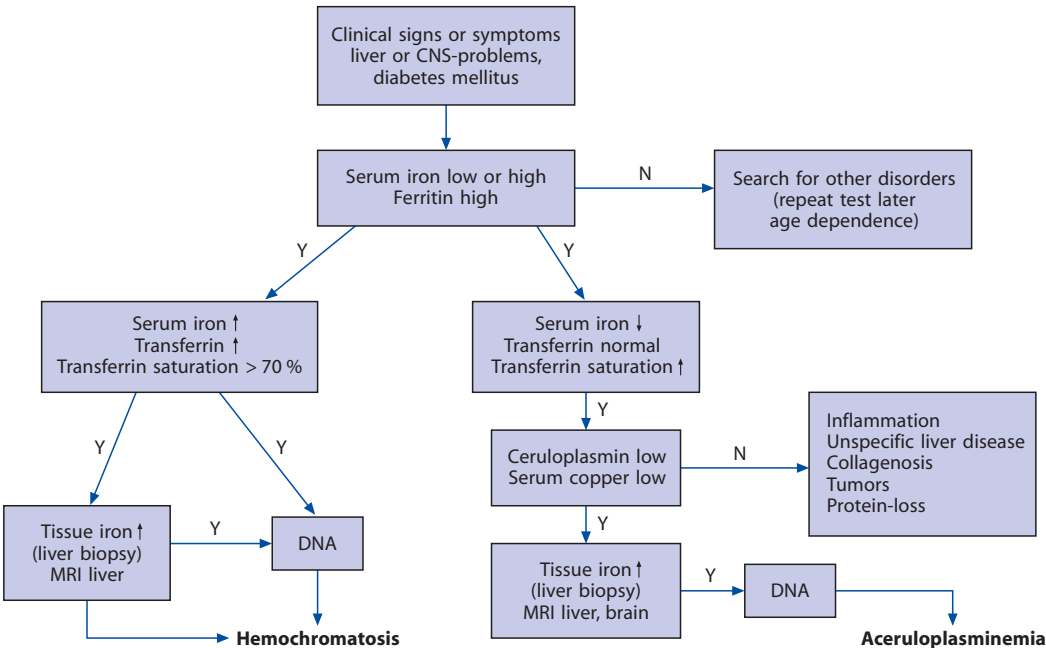


Fig. 33.4. Diagnostic flow chart for inborn errors of iron metabolism

For inborn errors of copper and iron metabolism these flow charts summarize the biochemical procedures for diagnosing Wilson's disease, Menkes disease, the occipital horn syndrome, hereditary hemochromatosis and aceruloplasminemia. However, biochemical diagnosis is only supplemental to the clinical findings, since these are quite unique for the different diseases.

For zinc, one has to exclude nutritional zinc deficiency. A trial of zinc therapy will indicate whether a patient is deficient or zinc dependent, e.g. if he has acrodermatitis enteropathica.

33.9 Specimen Collection

Disorder	Test	Preconditions	Material	Handling	Pitfalls
33.1 Wilson disease	Cu	Fasting	Serum	Room temperature	Contamination
33.2 Menkes disease			Tissue biopsy		Infection
33.3 Occipital horn syndrome			Liver biopsy Fibroblasts		Pregnancy Contraception Cirrhosis Malnutrition
33.4 Acrodermatitis enteropathica	Urine Cu	Morning urine or 24-h urine	Urine	Room temperature	
	Ceruloplasmin Zn	Fasting	Serum	Centrifuge within 30 min; room temperature	Contamination
			Blood		Malnutrition
33.5 Hemochromatosis	Fe	Morning urine or 24-h urine	Urine		
		Fasting	Hair Serum Liver biopsy	Room temperature	Age-dependent values
33.6 Aceruloplasminemia	Ferritin Transferrin Transferrin saturation	Fasting			Infection Malnutrition Non-specific liver disorders (alcohol) Tumors
	Urine Fe	Morning urine or 24-h urine	Urine		

33.10 Prenatal Diagnosis

Disorder	Material	Method	Timing, Trimester
33.1 Wilson disease	Chorionic villi Cultured amniocytes	DNA	I–II
30.2 Menkes disease	Chorionic villi Cultured amniocytes	Cu concentration Cu uptake	I–II
30.3 Occipital horn syndrome	Chorionic villi	Cu concentration Cu uptake	I–II
30.4 Acrodermatitis enteropathica	–	–	–
30.5 Hemochromatosis	Liver (neonatal hemochromatosis)	MRI	II–III
30.6 Aceruloplasminemia	–	–	–

33.11 DNA Analysis

Disorder	Specimen	Method	Results
33.1 Wilson disease	B (EDTA)	DNA isolation	More than 80 mutations
33.2 Menkes disease	B (EDTA)	PCR amplification Fluorescence in situ hybridisation (FISH)	More than 160 different mutations
33.3 Occipital horn syndrome	FB	Southern blot hybridis PCR	
33.4 Acrodermatitis enteropathica	FB	Fastlink	Recognition of gene region, no mutations so far
33.5 Hemochromatosis (HH)			
33.5.1 HH 1	B (EDTA)	DNA isolation	65–100% homozygosity for 1 mutation: C282Y
33.5.2 HH 2	B (EDTA)	PCR, DANN sequences Restriction analysis	
33.5.3 HH 3		DNA isolation PCR amplification DNA sequencer	
33.6 Aceruloplasminemia	B (EDTA)	PCR amplification cDNA	Different mutations

33.12 Initial Treatment

■ General Intervention

- 33.1 Initial treatment is only symptomatic, e.g. for liver failure or psychiatric disorders.
- 33.2 Only symptomatic approach, e.g. antiepileptics.
- 33.3 No acute problems, therefore no treatment.
- 33.4 Specific treatment with zinc can be started after blood samples obtained.
- 33.5 Symptomatic treatment, e.g. liver failure (NH) or diabetes in HH.
- 33.6 Symptomatic treatment for diabetes and psychiatric symptoms.

■ Specific Intervention (by Disorder)

No.		Therapy	Application	Dose	Duration
33.1	Wilson disease	Chelating agents (D-penicillamine, trientine)	Orally	Children: 25 mg/kg/d Adults: 1–2 g in 4 doses	Lifelong individually adapted dose Side effects in pa- tients with neuro- logical symptoms
		Tetrathiomolybdate	Orally	6×20 mg/d (2 mg/kg b.w.)	In patients with neurological symp- toms
		Zinc salts (zinc acetate)	Orally	1–5 y 2×25 mg 6–16 y 3×25 mg >16 y 3×50 mg 1 h before meals	
33.2	Menkes disease	Liver transplantation Cu-histidinate, even- tually followed 12 h later by D-penicillamine	IM or SC	50–150 µg/kg b.w./d (elemental Cu) 150–250 mg/d	Indefinite; individu- ally adapted dose Lifelong
33.3	Occipital horn syndrome	Cu-histidinate	IM or SC	600 µg Cu ²⁺ /d	Lifelong
33.4	Acrodermatitis enteropathica	Zinc sulphate Zinc acetate	Orally	35–150 mg Zn ²⁺ /d	Indefinite; individu- ally adapted dose
33.5	Hemochromatosis	Phlebotomy		500 ml 1–2×weekly 500 ml once every 1–3 months 1000 mg/d	Until normalisation of Transferrin satu- ration (<50%), thereafter
		Deferoxamine Liver transplantation (NH)	IV		
33.6	Aceruloplasminemia	Fresh frozen plasma (FFP) and deferoxamine	IV	450 ml once a week 1000 mg/d 1000 mg/d	6–12 weeks under clinical control and MRI and EEG
		Deferoxamine alone	IV		10 months or longer

33.13 Summary/Comments

Inherited disorders of copper and zinc metabolism are rare disorders and not easily diagnosed. Currently, screening tests are unwarranted. A diagnosis depends on a good clinical evaluation and the specific determination of specific trace elements in plasma or serum followed by more sophisticated diagnostic tests, e.g. tissue concentrations of trace elements (liver biopsy) or molecular genetic analyses. The same is true for the disorders of iron metabolism. Hereditary hemochromatosis, however, is a relatively common disorder in man with a long asymptomatic period and early diagnosis is important to prevent organ and tissue damage with early treatment.

The six diseases mentioned in this chapter have different clinical presentation and also present differently at different ages. Classical MD presents in the neonatal period, with variable expression, whereas the milder form of MD and the OHS are seen only in childhood or adolescence. The recognition of Acrodermatitis enteropathica is prevented by breast milk and develops only after weaning. Although hereditary hemochromatosis is a genetically heterogenous disease most of the patients are homozygous for one mutation and that makes DNA testing an important diagnostic tool. Aceruloplasminemia, still a rare disorder, has many features in common with HH and Wilson's disease.

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