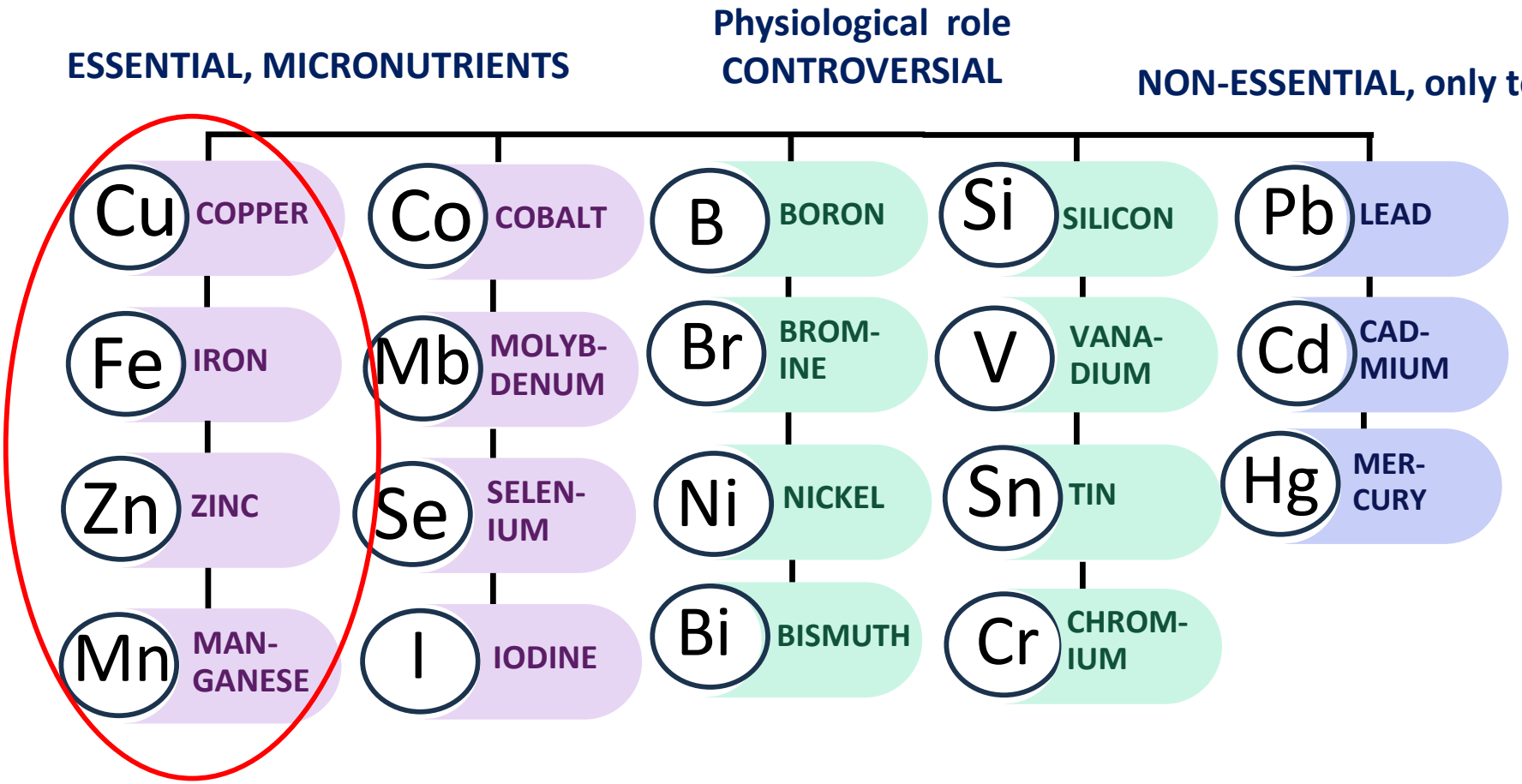
Trace Elements and Metals Disorders

Sarah C. Grünert/ Jörn Oliver Sass

Trace Elements in the Human



Nutritional Deficiencies and Intoxications

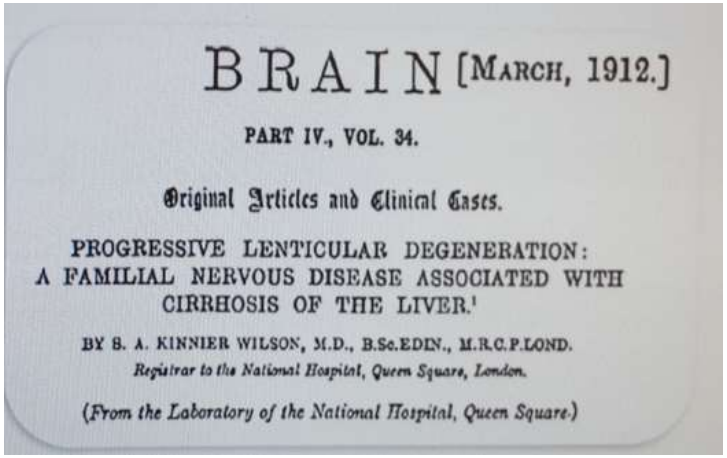
SSIEM ACADEMY 2024

Cu COPPER	↓↓ very rare; e.g., after excessive Zn intake; impaired liver function and iron uptake ↑↑ in healthy individuals excreted via bile + feces
Fe IRON	↓↓ impaired physical and cognitive performance, anemia; common in developmental countries ↑↑ accidental overdosing is rare; growth impairment
Zn ZINC	↓↓ usually not in industrialized countries (skin, wound healing, immune system/ inflammations) ↑↑ Ataxia, lethargy, impaired uptake of Cu + Fe
Mn MAN-GANESE	↓↓ not of concern in Europe ↑↑ may affect fetal development; neurotoxicity

Examples for Physiological Roles of Trace Elements

- Metals ions may have vital biological effects
- Cofactors of enzymes (Zn^{2+} is a very common cofactor)
- Important structural elements that may be essential for molecule and tissue structures
- Involvement in the transport of oxygen (Fe^{2+} in heme)
- …

Wilson Disease



BRAIN [MARCH, 1912.]

PART IV., VOL. 34.

Original Articles and Clinical Cases.

PROGRESSIVE LENTICULAR DEGENERATION: A FAMILIAL NERVOUS DISEASE ASSOCIATED WITH CIRRHOSIS OF THE LIVER.[1]

BY S. A. KINNIER WILSON, M.D., B.Sc.EDIN., M.R.C.P.LOND.

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(From the Laboratory of the National Hospital, Queen Square.)

- First described in 1912 by Samuel A.K. Wilson
- Autosomal recessive defect of the Cu-ATPase encoded by *ATP7B*
- Worldwide prevalence 1:33,000; Europe 1:50,000 to 1:83,000
- Impaired copper excretion into bile $\Rightarrow$ accumulation of unbound Cu in liver, and secondarily in brain, kidneys and cornea

Wilson Disease

Clinical presentation

Copper accumulation results in oxidative damage and cellular apoptosis

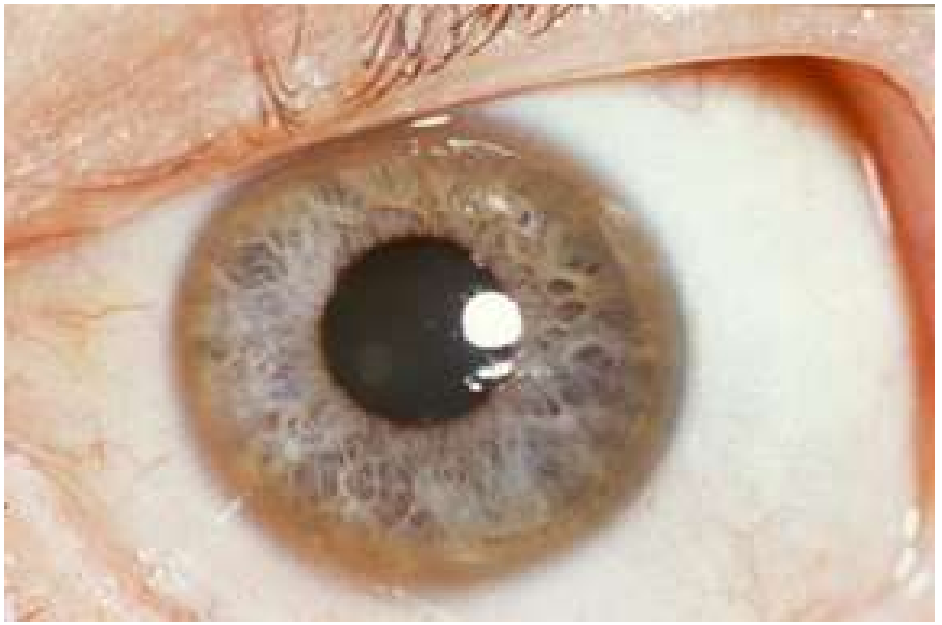
Usually presentation during childhood or teenage years

- Liver: hepatitis, liver failure, cirrhosis (starting at school age)
- CNS: neurodegeneration, dysarthria, tremor, drooling, dysphagia, rigidity, impulsivity or psychosis (onset often at 12-30 years)
- Renal: Fanconi syndrome
- Blood: hemolytic anemia (without evidence for an immunologic cause)
- Eye: Kayser-Fleischer ring

Wilson Disease: Kayser-Fleischer Ring

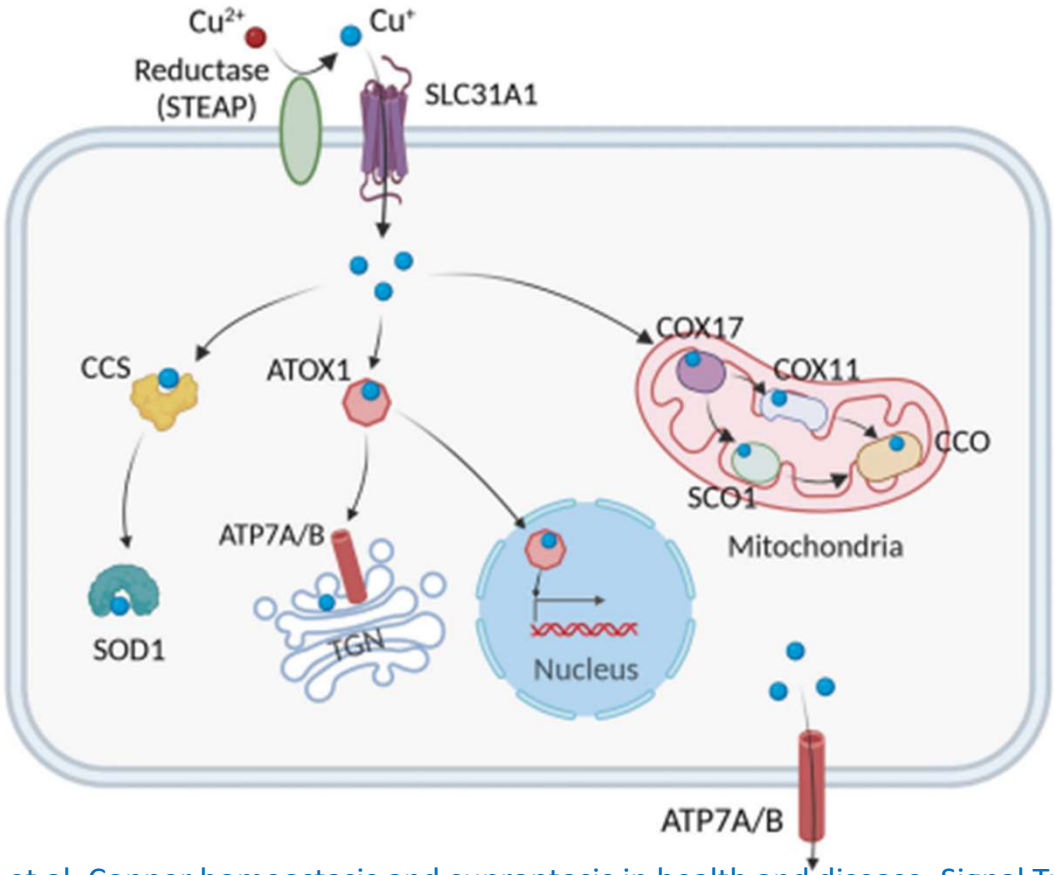
Corneal deposition of copper classically detected by slit-lamp biomicroscopy

Only seen from adolescence



Copper Transport

Cu-ATPases **ATP7A** and **ATP7B**:
major transporters for exporting cellular Cu^{2+} towards blood or bile.



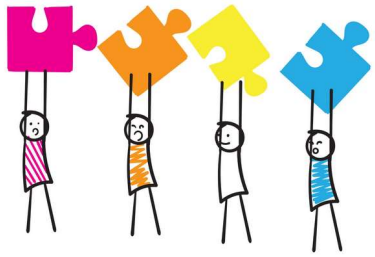
- Expression of **ATP7B**:
  predominantly in the liver:
  mutations $\Rightarrow$ Cu^{2+} not released from enterocytes to bile
  Wilson disease

- Expression of **ATP7A**: in most tissues/ organs, but NOT in the liver:
  mutations $\Rightarrow$ Cu^{2+} not released from enterocytes to blood
  Menkes disease,
  Occipital Horn syndrome

Chen et al. Copper homeostasis and cuproptosis in health and disease. Signal Transduct Targeted Ther 2022;7:378

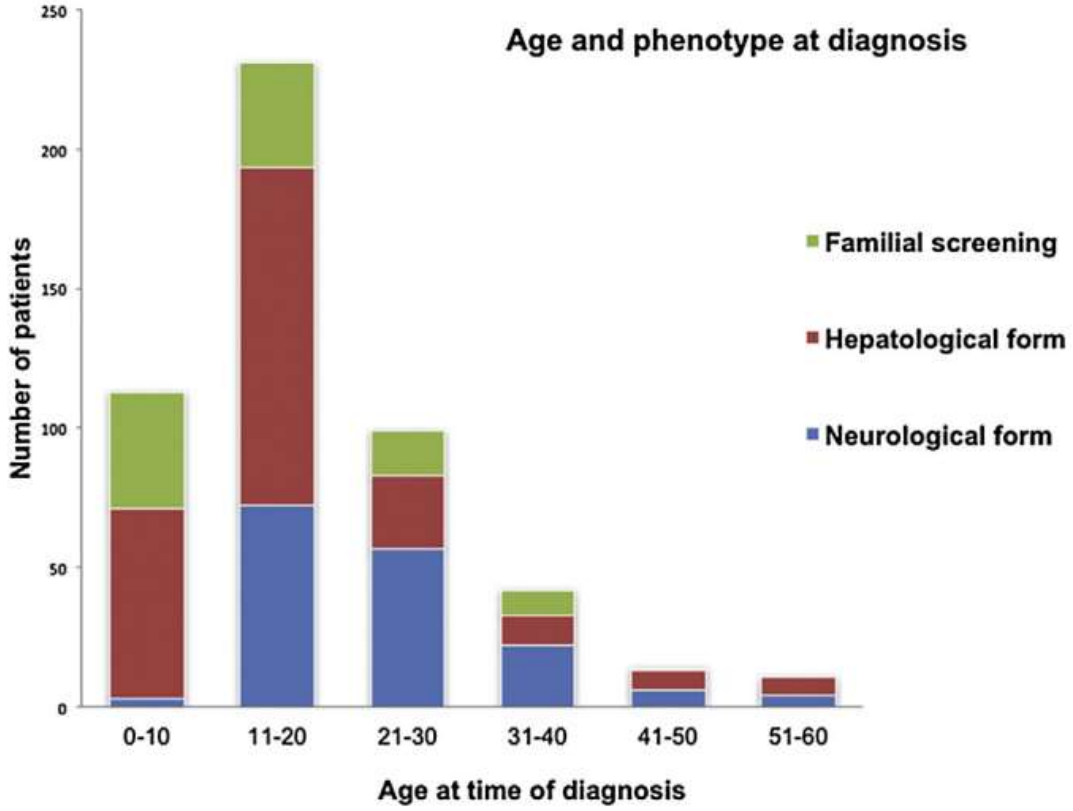
Wilson Disease: Diagnostics

- Low serum Cu (particularly free Cu) & ceruloplasmin
- Raised 24 h urine copper pre & post penicillamine
- Raised liver copper
- *ATP7B* sequencing (more than 600 pathogenic variants described, common mutation in Europe: p.His1069Gln)
- Liver and brain imaging
- Diagnostic delay: interval between symptom onset and diagnosis 3x longer in patients with mainly neurological symptoms than in those with rather hepatic symptoms



Poujois & Woimant. Wilson's disease: A 2017 update. Clin Res Hepatol Gastroenterol. 2018;42:512-20

Wilson disease: Clinical Presentation



Age and phenotype of patients from the French Wilson disease registry covering 604 patients.

Poujois A, Woimant F. Wilson's disease: A 2017 update. Clin Res Hepatol Gastroenterol. 2018 Dec;42(6):512-520.

Wilson Disease: Brain Imaging

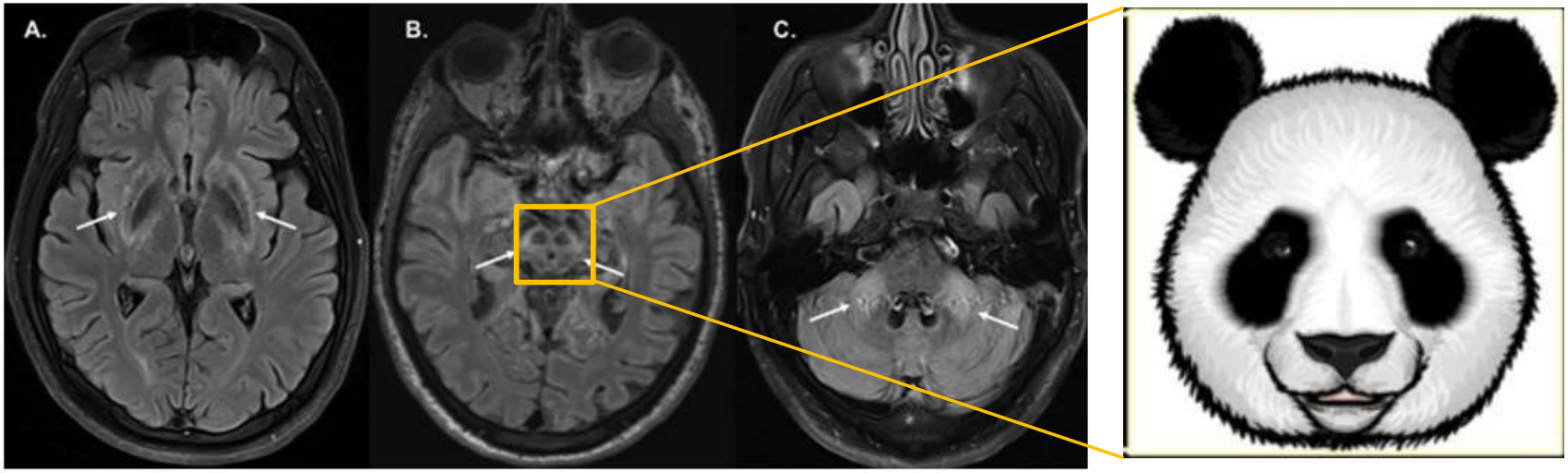


Figure 2 Classical brain MRI findings in Wilson's disease patients. Bilateral high signal intensities on Flair-weighted images in the basal ganglia (A), the mesencephalon (B) (with the sign of the ''giant Panda face''), and the cerebellum (C).

Poujois & Woimant. Wilson's disease: A 2017 update. Clin Res Hepatol Gastroenterol. 2018;42:512-20.

Wilson Disease: Treatment

- Lifetime use of copper chelating agents or zinc salts
- Chelators: induction of urinary copper excretion
  Recommended for liver and neurological disease
  - D-penicillamine (Side effects include proteinuria, thrombocytopenia, rash)
  - Trientine (fewer side effects)
- Zinc salts: inhibition of the intestinal absorption of copper
  Recommended for asymptomatic patients or mild neurologic disease
- Two phases:
  – initial active chelation phase
  – maintenance phase (moderate chelation or Zn to prevent copper deficiency)
- Cu-restricted diet at least in the beginning, later only avoidance of shellfish, cocoa products and liver

Wilson Disease: Prognosis

- Frequent and careful biochemical monitoring after initiation of treatment necessary to prevent over-/undertreatment
- Excellent in asymptomatic cases
- Liver disease: most recover, few need transplant due to liver failure or carcinoma
- CNS disease: initial deterioration possible & residual handicap

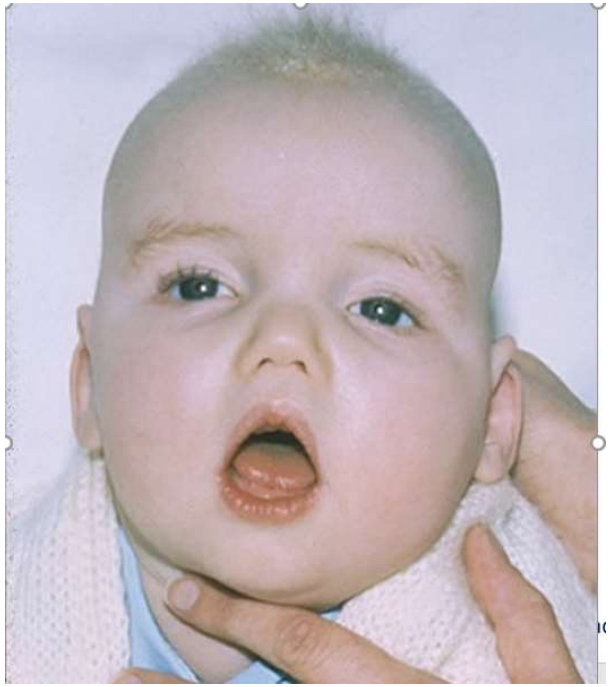
Menkes Disease

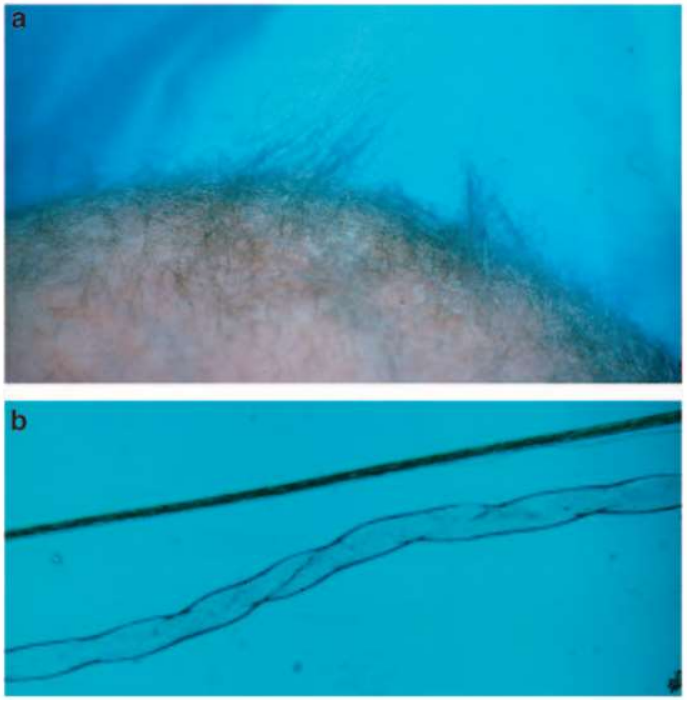
- lethal multisystemic disorder of copper metabolism
- X-linked recessive defect of the Cu-ATPase encoded by *ATP7A*

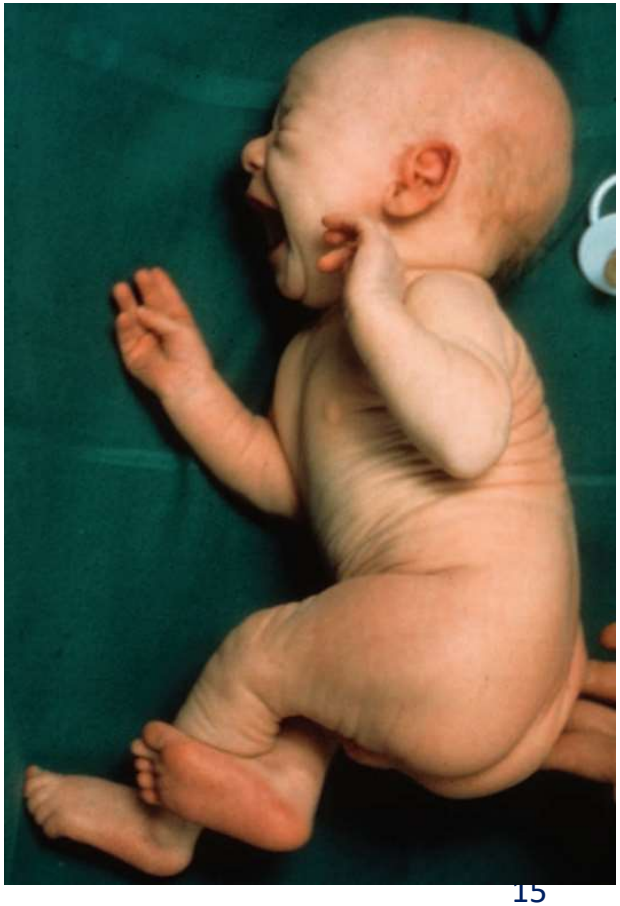
Clinical presentation:

- Progressive neurodegeneration and connective tissue disturbances
- peculiar ‘kinky’ hair; unusual sparse and lusterless scalp hair that becomes tangled on the top of the head at the age of 1–2 months
- neonatal period: prolonged jaundice, hypothermia, hypoglycemia and feeding difficulties, spontaneous fractures
- Numerous vascular, urogenital and skeletal abnormalities

Menkes Disease: Clinical Presentation







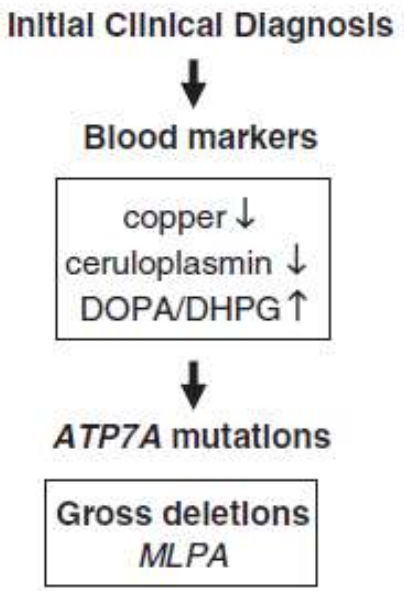
Tümer & Møller. Menkes disease. Eur J Hum Genet. 2010;18:511-8

Menkes Disease: Clinical Presentation

- Therapy-resistant seizures in most patients from about 2 to 3 months of age
- Developmental regression becomes obvious around 5–6 months of age
- Failure to thrive, poor eating, vomiting, and diarrhea
- Muscular tone is initially decreased, later spasticity and weakness of the extremities
- Motor dysfunction progresses ⇒ spontaneous movements become limited; drowsiness and lethargy emerge
- Diagnosis typically made at 3–6 months of age, often due to the abnormal hair

Menkes Disease: Diagnosis

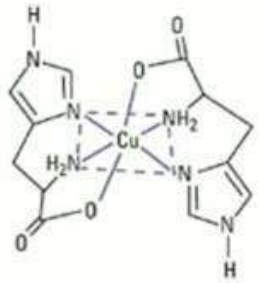
- Low serum copper (<11 µmol/L) & ceruloplasmin (unreliable in neonates, overlap with healthy babies)
- Abnormal catecholamines (due to dopamine hydroxylase deficiency):
  Elevated plasma dopamine/norepinephrine or dihydroxyphenylacetic acid/dihydroxyphenylglycol ratios
- *ATP7A* sequencing
- Hair microscopy (may also be normal initially)



Vairo et al. A systematic review and evidence-based guideline for diagnosis and treatment of Menkes disease. Mol Genet Metab. 2019;126:6-13
Tümer Z, Møller LB. Menkes disease. Eur J Hum Genet. 2010 May;18:511-8

Menkes Disease: Treatment and Prognosis

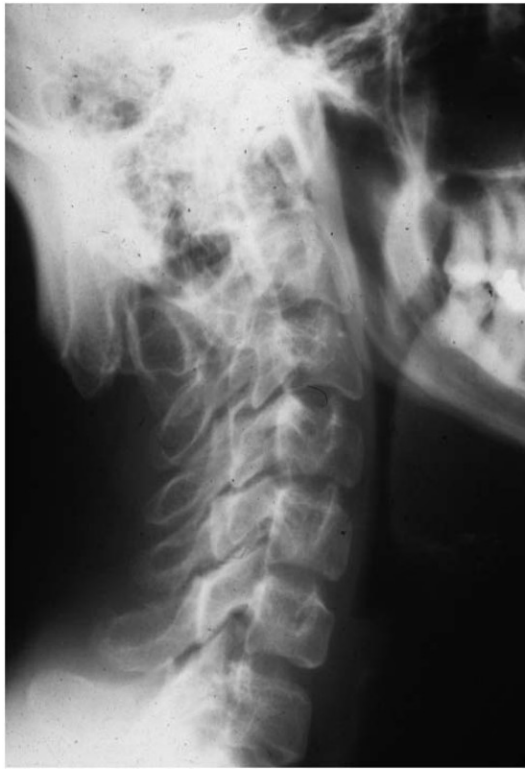
- Subcutaneous copper histidine treatment only recommended for asymptomatic patients < 30 days of life (250 µg twice daily)



- Early onset of treatment rather than the genotype seems to be the most important factor for the outcome
- Copper histidine increases the survival and reduces the neurological burden in neonatally treated patients
- Severely affected patients die usually before the third year of life

Occipital Horn Syndrome

- Mild *ATP7A* mutations
- Principal clinical features are related to connective tissue
- Occipital exostoses
- Wrinkled skin
- Umbilical or inguinal hernias
- Hypermobile joints
- Intractable diarrhea
- Urinary tract infections, bladder diverticulae
- Vascular anomalies
- Cognition: low-borderline to normal
- Normal hair!



Tümer & Møller. Menkes disease. Eur J Hum Genet. 2010;18:511-8

Hepcidin and Regulation of Iron Homeostasis

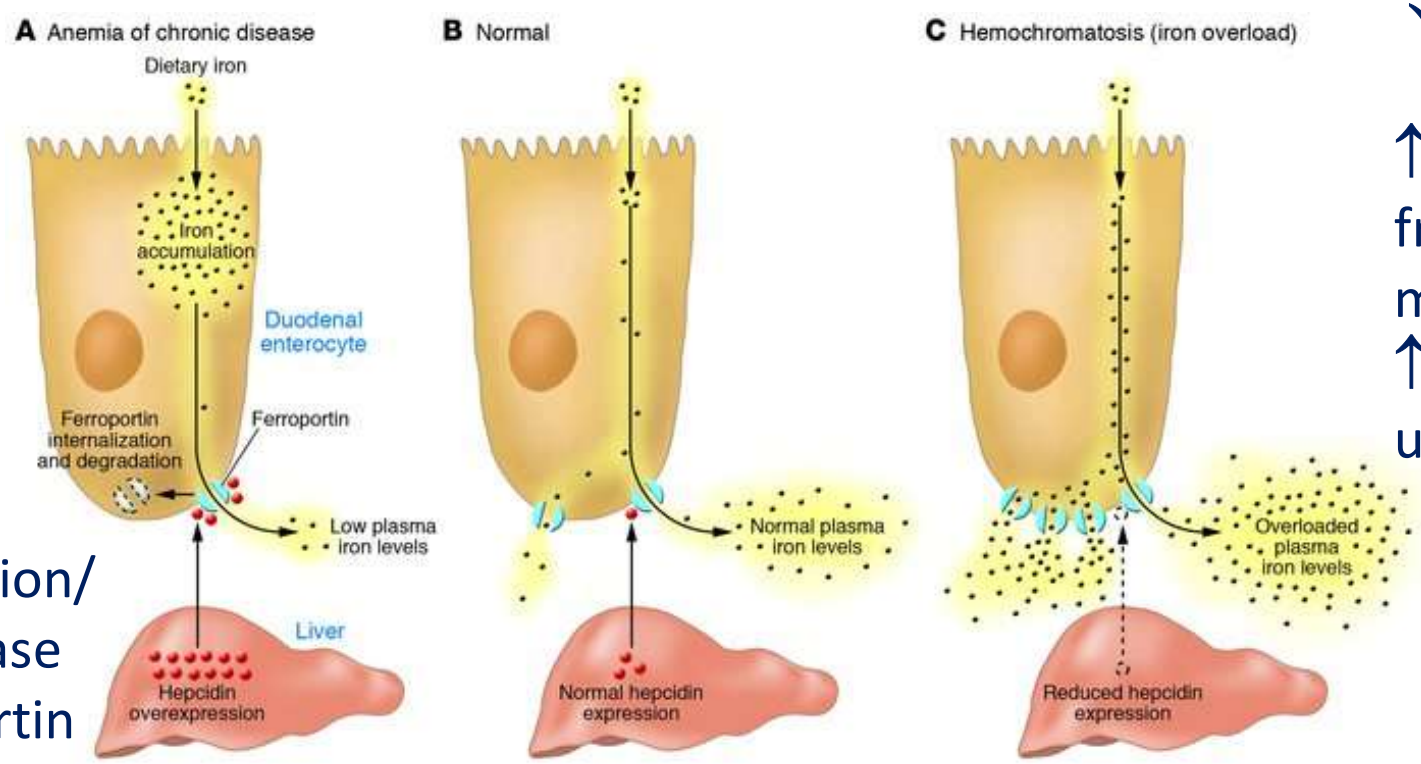
Hepcidin degrades ferroportin and thus terminates the iron entry to plasma from duodenal mucosa cells and iron-recycling macrophages.

↓ Hepcidin

⇒

↑ iron release from the macrophages

↑ duodenal iron uptake



↑ Hepatic hepcidin production in inflammation/ chronic disease

⇒ ↓Ferroportin

⇒ ↓ Plasma iron

De Domenico et al. Hepcidin regulation: ironing out the details. J Clin Invest. 2007;117:1755-8

Hereditary Hemochromatosis

- Inherited iron overload disorder characterized by excessive absorption of iron, due to deficiency of hepcidin
- Hepcidin is the main regulator of iron homeostasis
- One of the most common genetic diseases in Europe, USA and Australia (1:200-400)
- Deposition of excess iron into parenchymal cells leads to tissue damage and ultimately organ failure
- The liver, pancreas, joints, heart, skin, and pituitary glands are most commonly involved

Hereditary Hemochromatosis: Classification

Novel classification	Molecular pattern	Note
HFE-related	*p.Cys282Tyr* homozygosity or compound heterozygosity of *p.Cys282Tyr* with other rare *HFE* pathogenic variants[106–109] or *HFE* deletion[110]	Low penetrance; consider presence of host-related or environmental cofactors for IO In subjects with other HFE genotypes (eg, *p.Cys282Tyr/His63Asp* compound heterozygosity or *p.His63Asp* homozygosity) consider second-line genetic testing for rarer variants
Non-*HFE*-related	Rare pathogenic variants in "non-*HFE*" genes: • *HJV*-related • *HAMP*-related • *TFR2*-related • *SLC40A1* (GOF)-related	Potentially, mutations in any hepcidin-regulatory gene may be causative (the effects of novel mutations should be confirmed through functional and epidemiological studies) Molecular subtypes characterization only at specialized centers, but the diagnosis of non-*HFE* related HC is sufficient to start phlebotomies at nonspecialized centers*
Digenic†	Double heterozygosity and/or double homozygosity/heterozygosity for mutations in 2 different genes involved in iron metabolism (*HFE* and/or non-*HFE*)	More commonly, *p.Cys282Tyr* mutation in *HFE* gene might coexist with mutation in other genes; rarely, both mutations involve non-*HFE* genes
Molecularly undefined	Molecular characterization (still) not available after sequencing of known genes (provisional diagnosis)	Patients should be referred (or DNA should be sent) to specialized centers

95% of HH cases

Girelli et al. Hemochromatosis classification: update and recommendations by the BIOIRON Society. Blood. 2022;139:3018-29

Hereditary Hemochromatosis: Diagnostics

- Transferrin saturation > 45%
- Ferritin raised
- *HFE* sequencing
- Liver biopsy reserved for staging of hepatic fibrosis
- Determining the hepatic iron concentration via MRI can help predict the long-term risk of developing cirrhosis

Accuracy of Diagnostic Tests for Hereditary Hemochromatosis

Diagnostic test	Sensitivity	Specificity
Hepatic iron index ≥ 1.9[12]	93%	100%
Transferrin saturation > 45%[13]	98%	99%
Ferritin > 200 ng per mL (200 mcg per L) in women[5,14]	66%	85%
Ferritin > 300 ng per mL (300 mcg per L) in men[5,14]	66%	85%
Serum iron > 168 ng per mL[15]	68%	83%
C282Y genetic testing[16]	91.3% to 92.4%	98.8% to 100%

Hepatic Iron Index =
Hepatic Iron Concentration [µmol per gram dry weight]/age [years])

Kane et al. Hereditary Hemochromatosis: Rapid Evidence Review. Am Fam Physician. 2021;104:263-70

Hereditary Hemochromatosis: Clinical presentation

- Low clinical penetrance with up to 25% of individuals with p.C282Y homozygosity clinically asymptomatic
- Clinical manifestations of iron overload typically between 40 and 60 years of age
- Women typically present later than men (postmenopausally), likely because of menstrual blood loss

Hereditary Hemochromatosis: Treatment

- Repeated phlebotomy in patients with elevated ferritin (>300 µg/L in men and > 200 µg/L in women, along with a transferrin saturation >45%)
- Initially, weekly removal of 500 mL of blood
- When ferritin is 50-100 µg/L ⇒ phlebotomy 3–4 times per year
- Goal: Ferritin levels near 50 µg/L
- Ascorbic acid increases iron absorption
  ⇒ Avoidance of vitamin C supplements
- Liver transplantation for patients with decompensated cirrhosis or hepatocellular carcinoma

Kowdley et al. ACG Clinical Guideline: Hereditary Hemochromatosis. Am J Gastroenterol. 2019;114:1202-18

Hereditary Hemochromatosis: Prognosis

- The presence of cirrhosis or a ferritin level greater than 2,000 µg/L at the time of diagnosis or in treated patients is associated with higher mortality (mostly liver-related)
- Treatment with phlebotomy will reduce risk of developing complications related to liver disease or liver cancer
- Improvement of abnormal liver tests, heart dysfunction, fatigue
- No improvement of diabetes, arthralgias, hypogonadism

Other Disorders of Iron Metabolism

Neurodegeneration with Iron Accumulation in the Brain (NBIA)

- Clinically and genetically heterogeneous group of disorders affecting children and adults
- Increased basal ganglia iron on brain MRI
- Treatment mostly symptomatic

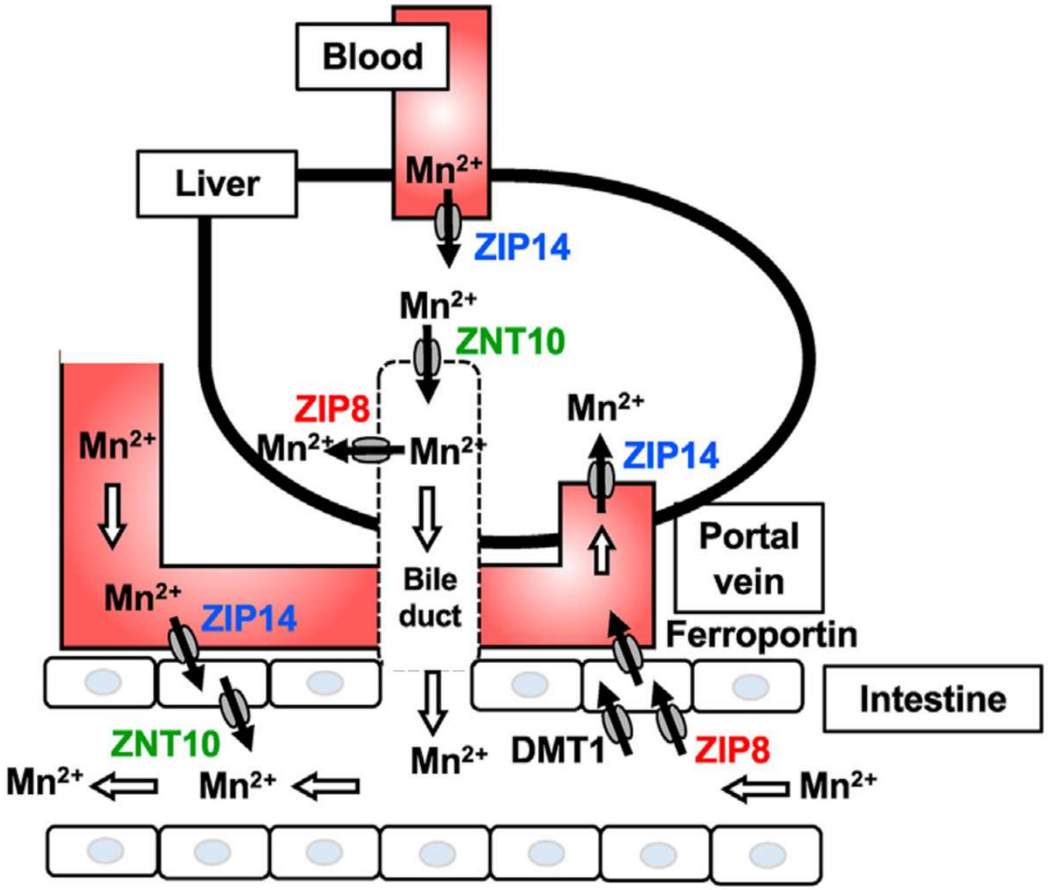
Aceruloplasminaemia

- Iron accumulation in the brain and viscera
- Clinical triad of retinal degeneration, diabetes mellitus, and neurologic disease starting at 30 years of age
- Complete absence of ceruloplasmin ferroxidase activity
- Biallelic variants in the *CP* gene

Hayflick et al. Neurodegeneration with brain iron accumulation. Handb Clin Neurol. 2018;147:293-305
Miyajima et al. Aceruloplasminemia. 2003 [updated 2018]. In: Adam et al., eds. GeneReviews® [Internet]

Zinc and Manganese Transport

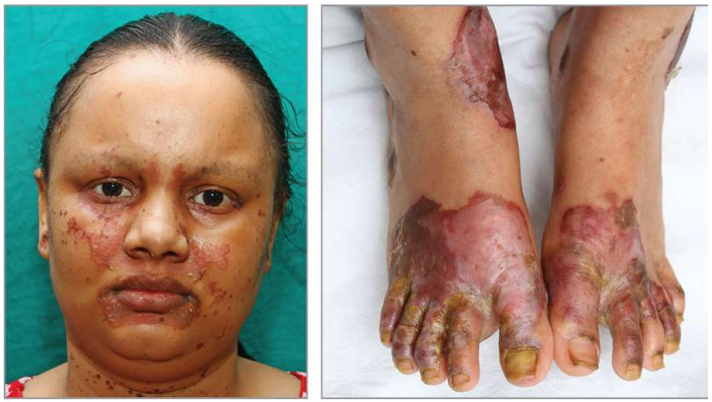
- **SLC30A4 (ZIP4)**: transports Zn^{2+} from the duodenum→ enterocyte
- **SLC30A2 (ZnT2)**: transports zinc into secretory vesicles in mammary epithelial cells
- **SLC30A10 (ZnT10)**: transports Mn from the cytosol to the cell exterior (and protects against Mn toxicity)
- **SLC39A8 (ZIP8) and SLC39A14 (ZIP14)**: provide the cell with Mn, Zn, Fe, Co(, Cd); ZIP8 is also important in intracellular membrane transport, including to the Golgi apparatus, ZIP14 mainly in hepatic Mn uptake



Fujishiro & Kambe. Manganese transport in mammals by zinc transporter family proteins, ZNT and ZIP. J Pharmacol Sci. 2022;148;125-33

Acrodermatitis Enterohepatica

- Autosomal recessive defect of zinc uptake by intestine; prevalence 1:500,000
- Biallelic variants in the *SLC39A4* gene, encoding the zinc transporter ZIP4
- First symptoms usually while weaning from breastfeeding
- Triad of alopecia, diarrhoea, and dermatitis (perioral & perianal rash, later pustules, crusting)
- Secondary infections
- Growth impairment



Sivakumar A, Vageshappa RK, Kumari R. Acrodermatitis Enteropathica. JAMA Dermatol. 2024 Jan 1;160(1):102

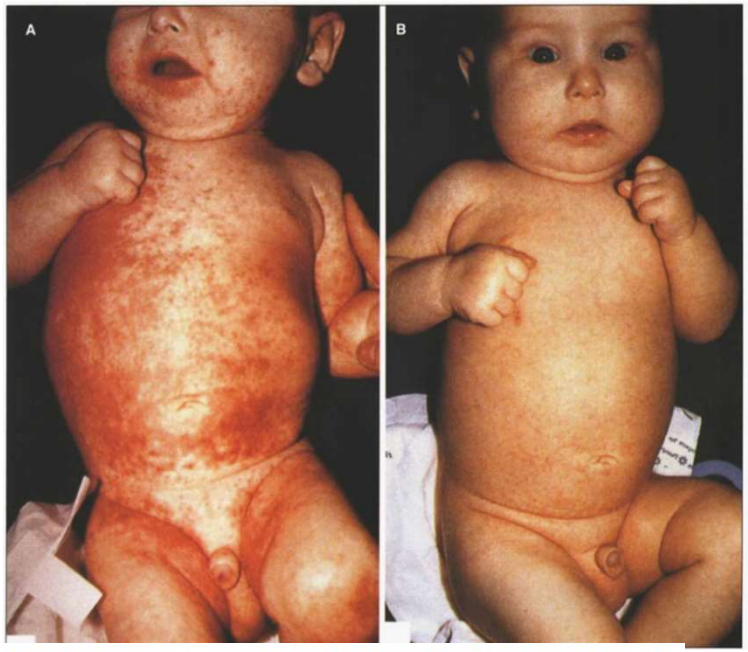
Stiles et al. Role of zinc in health and disease. Clin Exp Med. 2024;24:38
Ferreira & Gahl. Disorders of metal metabolism. Transl Sci Rare Dis. 2017;2:101-39

Acrodermatitis Enterohepatica: Diagnostics

- Low serum zinc level < 700 µg/L in fasting state
- Low alkaline phosphatase (zinc-dependent enzyme)
- Response to trial of Zn^{2+}
- *SLC39A4* sequencing

Stiles et al. Role of zinc in health and disease. Clin Exp Med. 2024;24:38

Acrodermatitis enterohepatica: Treatment & Prognosis



Acrodermatitis enteropathica showing skin rash.
(A) Before treatment. (B) After zinc treatment.

- Left untreated, acrodermatitis enteropathica is fatal within the first few years of life
- Life-long zinc supplementation, 30-50 mg zinc/ day (oral and i.v. formulations available)
- Zinc supplementation results in the disappearance of skin lesions within a week
- Monitor Cu levels (risk of deficiency)

Ferreira & Gahl. Disorders of metal metabolism. Transl Sci Rare Dis. 2017;2:101-39

Transient Neonatal Zinc Deficiency

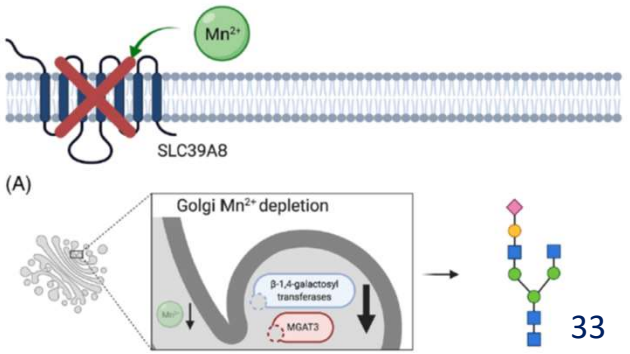
- Heterozygous mutation in the *SLC30A2* gene of the breastfeeding mother
- *SLC30A2* encodes ZnT2, a zinc transporter in mammary epithelial cells
- Low maternal milk zinc concentrations
- Infants have a clinical phenotype similar to acrodermatitis enteropathica while receiving breastmilk
- Symptoms resolve after weaning

Stiles et al. Role of zinc in health and disease. Clin Exp Med. 2024;24:38
Ferreira & Gahl. Disorders of metal metabolism. Transl Sci Rare Dis. 2017;2:101-39

Congenital Disorder of Glycosylation Type IIn (CDG2N), SLC39A8 Deficiency

- Developmental delay/ intellectual disability, truncal postural hypotonia, dyskinetic movements, feeding difficulties, strabismus, cortical blindness
- Abnormal *N*-glycome (MALDI-ToF MS), which may present with a type II pattern of dysglycosylation
- Manganese deficiency:
  Manganese supplementation ($MnSO_4$); Galactose following Mn dose finding only (!)

⇒ Improvement of glycosylation and of the activities of other affected manganese-dependent enzymes, associated with clinical improvement



Park et al. N-glycome analysis detects dysglycosylation missed by conventional methods in SLC39A8 deficiency. J Inherit Metab Dis 2020;43:1370-81

Park. SLC39A8-CDG. 2023. In: Adam et al., eds. GeneReviews® [Internet]

Hypermanganesemia with Dystonia 1 (HMNDYT1) due to mutations in SLC30A10 (ZnT10)

- Dystonia, dysarthria, and parkinsonism
- Hypermanganesemia (whole blood Mn often > 2,000 nmol/L (ref. <320)
- Polycythemia
- Liver Disease due to impaired biliary excretion

⇒ intravenous disodium calcium EDTA lowers blood manganese levels and neurological findings and halts liver disease; iron supplementation

DD: Hypermanganesemia with Dystonia 2 (HMNDYT2) due to mutations in SLC30A14 (ZIP14)

- Dystonia, without liver disease (Mn uptake into liver impaired), no polycythemia
- Treatment with disodium calcium EDTA

Tuschl et al. Hypermanganesemia with Dystonia 1. 2012 [Updated 2021]. In: Adam et al., eds. GeneReviews® [Internet].
Tuschl et al. SLC39A14 Deficiency. 2017 [Updated 2022]. In: Adam et al., eds. GeneReviews® [Internet].

Summary:
Trace Elements and Metals Disorders

- Nutritional deficiencies and intoxications
- Genetic deficiencies affecting Cu-ATPases encoded by *ATP7A* (intestinal, not hepatic) and *ATP7B* (e.g. hepatic): Menkes and Wilson Disease
- Genetic hepcidin deficiency as a cause of impaired regulation of iron homeostasis and hereditary hemochromatosis
- Zinc and manganese transport - important cofactors/ glycosylation