

Manganese homeostasis: from rare single-gene disorders to complex phenotypes and diseases

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Manganese (Mn) participates in a variety of distinct physiological processes, including acting as a cofactor for several enzymes and metalloenzymes, in addition to playing a role in immune function, endocrine function, hematopoiesis, and oxidative stress regulation. Mn homeostasis is tightly regulated via intestinal absorption and hepatobiliary and intestinal excretion. In this issue of the *JCI*, Mercadante and colleagues explored the role of the metal transporter *Slc30a10* in vivo using a mouse model system. The authors used whole-body and tissue-specific gene knockouts to show that *Slc30a10* is paramount for Mn excretion in the liver and small intestines. These findings provide further insights into mechanisms for Mn homeostasis as well as potential targets for addressing Mn-associated disorders or environmental exposures.

Manganese homeostasis

Manganese (Mn) is an essential metal typically acquired from the diet and water supply (1, 2). Over the past decade, discoveries in human genetics have placed a greater focus on the importance of understanding Mn homeostasis. First, several single-gene Mendelian disorders of Mn homeostasis have been described (Table 1). In 2012, the first report of a disease now referred to as hypermanganesemia with dystonia 1 (HMNDYT1) (OMIM #613280) detailed two consanguineous families with autosomal-recessive mutations in the Mn exporter *SLC30A10* (encoding the protein ZNT10) (3, 4). Subsequently, a report describing a similar phenotype, hypermanganesemia with dystonia 2 (HMNDYT2) (OMIM #617013), described autosomal-recessive mutations in the Mn importer *SLC39A14* (encoding the protein ZIP14) (5). Furthermore, severe hypomanganesemia was attributed to autosomal-recessive mutations in the Mn importer *SLC39A8*

(encoding the protein ZIP8). In this condition, chronically low serum Mn is associated with seizures, developmental delays, deafness, and liver disease and is characterized by impaired activity of Mn-dependent glycosylation enzymes (congenital disorder of glycosylation type II [CDG2N] [OMIM #616721]) (6).

In addition to these rare single-gene disorders, which have been highly informative regarding key roles of Mn transporters, Mn homeostasis has been implicated in complex diseases and traits through GWAS. In particular, the coding variant rs13107325 (Ala391Thr) in *SLC39A8* has been associated with reduced blood pressure and hypertension risk (7), lower HDL cholesterol levels (8), higher BMI (9), increased schizophrenia risk (10, 11), and increased alcohol intake and alcohol use disorder risk (12), among many other traits (13). This variant has an 8% minor allele frequency in individuals of European ancestry. The correspond-

ing protein was reported to have modestly reduced function (14) and reduced expression in the human liver. Notably, in a GWAS of blood Mn levels (15), the only two genome-wide significant loci were *SLC39A8* (the lead SNP was the same Ala391Thr coding variant and was associated with lower blood Mn) and noncoding SNPs on chromosome 1 near *SLC30A10*. Thus, genetic variation influences Mn homeostasis over a wide range, from subtle effects that influence pleiotropic phenotypic variation and risk of disease, to more dramatic effects that cause major disease due to Mn excess or deficiency.

Intestinal absorption is one point of regulation of Mn homeostasis, and less than 5% of ingested Mn is absorbed. The divalent metal transporter 1 (DMT1), encoded by the gene *SLC11A2*, likely facilitates this intestinal absorption (1, 16). Conversely, Mn is eliminated through biliary excretion (16). In fact, hepatic regulation of biliary Mn excretion may regulate whole-body Mn homeostasis. We previously showed that *SLC39A8*, an importer of Mn, is expressed on the canalicular membrane of hepatocytes and serves to “reclaim” Mn from the bile back into the hepatocyte, thereby defending against Mn depletion (17). Indeed, although *SLC39A8* is expressed in multiple tissues, mice with a liver-specific *Slc39a8* knockout had decreased blood and tissue Mn levels that were similar to those seen with whole-body *Slc39a8* knockout and consistent with the key role of hepatic *SLC39A8* in Mn homeostasis (Figure 1) (17).

SLC30A10 is essential for manganese excretion

In the current issue of the *JCI*, Mercadante et al. provide data revealing the critical role of the Mn exporter *SLC30A10*, thus advancing our understanding of biliary Mn excretion (18). Mice deficient in whole-body *Slc30a10* showed elevated blood and tissue Mn levels and, because of impaired biliary excretion of Mn, had

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Table 1. Single-gene Mendelian disorders of Mn homeostasis

Gene	Protein	Function	Mendelian disorder	Blood Mn level	GWAS associations
<i>SLC30A10</i>	SLC30A10 (ZNT10)	Apical biliary and intestinal Mn efflux (excretion)	HMNDYT1 (dystonia, parkinsonism, liver disease, polycythemia)	Increased	Blood Mn, heel bone mineral density, reticulocyte count
<i>SLC39A8</i>	SLC39A8 (ZIP8)	Apical reuptake of Mn from bile (reclamation)	CDG2N (seizures, developmental delay, short stature, deafness)	Decreased	Blood Mn, blood pressure, BMI, HDL cholesterol, schizophrenia, alcohol use disorder, cognitive performance
<i>SLC39A14</i>	SLC39A14 (ZIP14)	Basolateral uptake of Mn into hepatocytes and enterocytes	HMNDYT2 (dystonia, parkinsonism)	Increased	Memory performance

Based on refs. 3–15.

biliary Mn levels that were inappropriately low for the tissue Mn excess. The authors showed that *Slc30a10* was localized to the canalicular membrane of hepatocytes and that hepatocyte-specific deletion of *Slc30a10* resulted in the virtual absence of biliary excretion following Mn injection, confirming that hepatocyte *Slc30a10* is essential for biliary Mn excretion. Interestingly, although liver-specific deletion of *Slc30a10* also virtually eliminated biliary Mn excretion, it resulted in only a modest increase in tissue Mn, indicating that *Slc30a10* expression in other tissues, or other factors, may compensate for the reduced biliary Mn excretion. They went on to show that intestinal enterocyte *Slc30a10* also contributes to Mn excretion; however, even the double-knockout (hepatocyte/enterocyte) mice failed to show the same degree of tissue Mn excess seen in the whole-body knockout mice. This report establishes the key physiologic role of *SLC30A10* in vivo in mediating biliary and intestinal excretion of Mn and defending against whole-body Mn excess (Figure 1) (18).

SLC39A14 (ZIP14) is another Mn importer expressed in both hepatocytes and enterocytes (Figure 1). In both humans and mice, deficiency of *SLC39A14* is associated with hypermagnesemia and Mn toxicity (5, 19). In the liver, *SLC39A14* is localized to the basolateral membrane of hepatocytes and is the major transporter responsible for importing Mn into the liver; hepatocyte-specific *Slc39a14* ablation in mice reduced liver Mn but had no effect on blood Mn levels (20). *SLC39A14* is also expressed on the basolateral membrane of enterocytes, where it imports Mn from blood to enterocytes and facilitates its fecal excretion. Enterocyte-specific deletion increased blood Mn and

caused tissue Mn overload, indicating that intestinal *SLC39A14* is quantitatively more important for Mn homeostasis (20). Notably, *SLC39A14* was not identified as

a significant locus in the GWAS for blood Mn, suggesting that modest genetic variation in its expression or function does not substantially influence Mn homeostasis

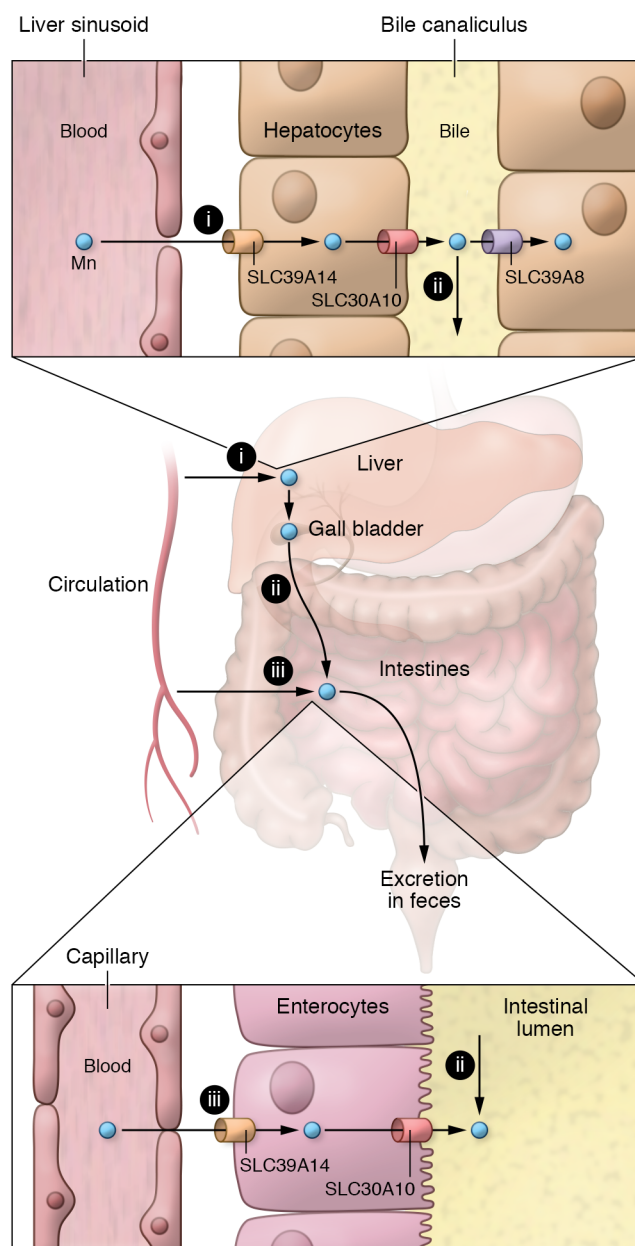


Figure 1. Model for Mn homeostasis with a focus on excretion pathways.

Systemic Mn homeostasis is largely regulated by pathways of Mn excretion in hepatocytes and enterocytes. (i) Mn uptake from blood by hepatocytes is mediated by *SLC39A14* at the basolateral membrane. Mn excretion from hepatocytes into bile is mediated by *SLC30A10* at the canalicular membrane. (ii) Mn reuptake from bile into hepatocytes is mediated by *SLC39A8* also at the canalicular membrane. Biliary Mn ultimately arrives in the intestinal lumen and, if not reabsorbed (not shown), is excreted in the feces. (iii) In addition, Mn is taken up from blood by enterocytes via *SLC39A14* at the basolateral membrane and effluxed into the lumen by *SLC30A10* at the apical surface. This figure does not show absorption of Mn via uptake pathways from the gut lumen into enterocytes and blood.

in humans, in contrast to *SLC39A8* and *SLC30A10*.

Thus, *SLC30A10* and *SLC39A8* appear to act as the primary regulators of biliary Mn excretion and systemic Mn homeostasis, with *SLC30A10* exporting Mn into the bile and *SLC39A8* reclaiming it from the bile (Figure 1). In this view, it is perhaps no surprise that these two genes were the top genetic loci associated with blood Mn levels on GWAS (15). These common variant studies indicate that subtle changes in expression or function of these two transporters can influence Mn homeostasis and blood concentrations. In the case of the highly pleiotropic *SLC39A8*, reduced Mn concentrations due to the Ala391Thr allele appear to directly influence a wide variety of phenotypic traits and disease risks. Altered Mn homeostasis may influence phenotypes through modulation of the activity of certain Mn-dependent enzymes including transferases, hydrolases, lyases, isomerases, and oxidoreductases (2). For example, *Slc39a8* deletion in mice resulted in a significant decrease in the tissue activity of arginase, a well-recognized Mn-dependent enzyme, which could explain the association of this variant with reduced blood pressure (17). Furthermore, both humans and mice with genetically reduced *SLC39A8* activity have lower levels of protein N-glycosylation due to reduced activity of certain Mn-dependent glycosyltransferases (6, 17), which could affect a variety of phenotypic traits. A hypothesis — not yet confirmed — is that the pleiotropic effects of modestly genetically reduced *SLC39A8* activity lead to reduced blood and tissue Mn concentrations, which in turn cause reduced activity of specific Mn-dependent enzymes in specific tissues. It remains a mystery why genetic variants at the *SLC30A10* locus that are also associated with blood Mn levels lack the pleiotropic associations with phenotypes and disease risk that *SLC39A8* variants show. The common variants at *SLC30A10* may have a quantitatively reduced effect on Mn concentrations and thus less of an effect on phenotype, or there could be tissue-specific effects of genetic variation on these transporters.

One of the important frontiers in the area of Mn-related phenotypic traits and disease is understanding the relationship

between systemic Mn homeostasis and the role of Mn in the brain. The Mendelian conditions of deficiency in *SLC30A10* or *SLC39A14* (Mn excess) or *SLC39A8* (Mn deficiency) have major neurobehavioral and brain phenotypes (Table 1). Furthermore, the *SLC39A8* Ala391Thr coding variant associated with lower blood Mn is associated with schizophrenia, alcohol use disorder, and reduced cognitive performance. In addition to their effects on Mn excretion and systemic Mn homeostasis, all three of these genes are expressed in the brain as well. As Mn overload occurs in the brain as a result of loss of *SLC30A10* or *SLC39A14*, neither of these proteins can be responsible for Mn uptake into the brain. Conversely, the systemic Mn deficiency associated with loss of *SLC39A8* may be exacerbated in the brain if *SLC39A8* also plays a key role in Mn uptake into the brain. Many questions remain: How is Mn transported across the blood-brain barrier? Do blood Mn concentrations directly influence Mn concentrations in the brain? What cell types in the brain express these Mn transporters and what is their function in the brain? Does variation in brain Mn concentrations influence specific Mn-dependent enzymes in the brain, thereby influencing phenotypic traits and risk of disease? Notably, in the Mendelian condition of *SLC39A8* deficiency, which is characterized by severely reduced Mn concentrations, clinical improvement was noted with Mn supplementation (21). Could Mn supplementation ameliorate some of the disease risk associated with genetically reduced *SLC39A8* activity, such as the risk for schizophrenia or alcohol use disorder? Substantial work is required to understand the role of systemic and local Mn homeostasis in brain function, cognition, and neurobehavioral and psychiatric disorders.

In summary, the seemingly esoteric topic of Mn homeostasis has turned out to be of critical importance to human biology and disease, as revealed by rare single-gene disorders that result in markedly dysregulated Mn homeostasis, and through genetic variation in Mn homeostasis that leads to a variety of phenotypes and risk of disease. Continued investigation into the regulation of Mn homeostasis is likely to lead directly to therapeutic hypotheses around the prevention and

treatment of a number of human conditions associated with altered Mn homeostasis.

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