Inherited Disorders of Manganese Metabolism

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Abstract While the neurotoxic effects of manganese were recognized in 1837, the first genetic disorder of manganese metabolism was described only in 2012 when homozygous mutations in *SLC30A10* were reported to cause manganese-induced neurotoxicity. Two other genetic disorders of manganese metabolism have now been described – mutations in *SLC39A14* cause manganese toxicity, while mutations in *SLC39A8* cause manganese and zinc deficiency. Study of rare genetic disorders often provides unique insights into disease pathobiology, and the discoveries of these three inherited disorders of manganese metabolism are already transforming our understanding of manganese homeostasis, detoxification, and neurotoxicity. Here, we review the mechanisms by which mutations in *SLC30A10*, *SLC39A14*, and *SLC39A8* impact manganese homeostasis to cause human disease.

Keywords Manganese • SLC30A10 • SLC39A14 • SLC39A8 • Homeostasis • Transporter

Introduction

Manganese (Mn) is an essential element required for the activities of numerous enzymes (Aschner et al. 2009). In adults, adequate intake is 1.8 mg/day in females and 2.3 mg/day in males (Freeland-Graves et al. 2016). As Mn is essential, its deficiency is linked to a number of health conditions including impaired cognitive function, asthma, osteoporosis, and dyslipidemia (Freeland-Graves et al. 2016). However, the causal role of Mn in these diseases is not clear. In contrast, more information is available about Mn toxicity. When systemic levels of Mn increase, the metal accumulates in the brain, primarily in the basal ganglia, and leads to the onset of a parkinsonian-like movement disorder (Aschner et al. 2009; Olanow 2004; Perl and Olanow 2007). Historically, Mn-induced neurotoxicity was reported in

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individuals exposed to elevated Mn from occupational sources (e.g., welding, manufacture of dry batteries and steel, and mining) (Aschner et al. 2009). Recent studies suggest that Mn-induced neurotoxicity may also occur due to environmental exposure to elevated Mn (Lucchini et al. 2014, 2012). Furthermore, individuals with defective liver function, due to diseases such as cirrhosis, fail to excrete Mn and may develop Mn-induced neurotoxicity in the absence of exposure to elevated Mn (Butterworth 2013). Calculations show that, under physiologic conditions, brain Mn levels are ~5–14 ng Mn/mg protein (corresponding to 20–53 μM Mn) (Bowman and Aschner 2014). In mammalian systems, neurotoxicity occurs when there is ~3-fold elevation in brain Mn levels, which corresponds to 16–42 ng Mn/mg protein or 60–158 μM Mn (Bowman and Aschner 2014). Thus, intracellular levels of Mn need to be maintained within a narrow physiologic range. Earlier studies on Mn homeostasis used yeast as a model organism and led to the discovery of an elegant regulatory system in which the Mn influx transporters, Smf1p and Smf2p, are degraded when intracellular Mn levels increase (Culotta et al. 2005; Jensen et al. 2009). However, in mammalian systems, Mn-induced downregulation of DMT1, the homolog of Smf1 proteins, has not been observed, suggesting that there may be fundamental differences between the regulatory systems that control cellular Mn in yeast and mammals. A major breakthrough in understanding the mechanisms that regulate Mn homeostasis in humans came in 2012 with the discovery that individuals harboring homozygous mutations in *SLC30A10* suffer from familial Mn-induced neurotoxicity (Quadri et al. 2012; Tuschl et al. 2008, 2012). Remarkably, soon after this, two other genetic diseases of Mn metabolism were discovered. In 2016, mutations in *SLC39A14* were also reported to induce Mn neurotoxicity (Tuschl et al. 2016), and in 2015, mutations in *SLC39A8* were identified to cause Mn and zinc (Zn) deficiency (Boycott et al. 2015; Park et al. 2015). *SLC30A10*, *SLC39A14*, and *SLC39A8* all code for Mn transporters and induce disease by directly altering cellular and tissue Mn (and Zn, in case of *SLC39A8*) levels. Below, we describe the mechanisms by which loss-of-function mutations in these genes impact Mn metabolism in greater detail.

SLC30A10

The first detailed clinical study of a patient later shown to harbor homozygous mutations in *SLC30A10* was published in 2008 (Tuschl et al. 2008). In this report, the authors described findings from a 12-year-old female patient who was born to consanguineous parents and developed difficulty in walking and in conducting fine movements with her hands. Clinical analyses revealed that the patient had a characteristic “cock-walk” gait and signs of dystonia along with ~10-fold increase in blood Mn (Tuschl et al. 2008). Magnetic resonance imaging provided evidence of Mn deposition in the basal ganglia, anterior pituitary, and the dentate nucleus and white matter of the cerebellum (Tuschl et al. 2008). Liver biopsy revealed that the patient had cirrhosis and that liver Mn levels were elevated (Tuschl et al. 2008). The

patient also had polycythemia and hyperbilirubinemia (Tuschl et al. 2008). Importantly, there was no history of exposure to elevated Mn from environmental sources, and levels of other essential metals in plasma [copper (Cu) and Zn] were normal (Tuschl et al. 2008). These findings raised the possibility that the patient may have a defect in Mn metabolism, perhaps of genetic origin, which led to Mn retention and subsequent neurotoxicity.

In 2012, analyses from additional patients who presented with the above-described clinical picture were published (Quadri et al. 2012; Tuschl et al. 2012). Whole-genome homozygosity mapping and exome sequencing revealed that affected patients carried homozygous mutations in the *SLC30A10* gene (Quadri et al. 2012; Tuschl et al. 2012). The disease exhibited autosomal recessive inheritance, and unaffected siblings and parents were heterozygous for mutations in *SLC30A10* (Quadri et al. 2012; Tuschl et al. 2012). Interestingly, the clinical presentation described in 2008 and 2012 recapitulated findings from a prior case report published in 2000 (Gospe et al. 2000). This patient underwent follow-up examinations and was included in the 2012 studies, and genetic analyses identified homozygous mutations in *SLC30A10* (Lechpammer et al. 2014; Tuschl et al. 2012). He recently died and findings obtained from a complete autopsy were published in 2014 (Lechpammer et al. 2014). Features observed included hepatomegaly and micronodular cirrhosis with portal hypertension in the liver and severe neuronal loss in the globus pallidus (Lechpammer et al. 2014). There was a 16-fold increase in Mn levels in the basal ganglia and a 9-fold increase in the liver, but levels of Zn and iron (Fe) were normal in the brain (there was a 2–3-fold increase in liver Zn and Cu, but it was likely due to cirrhosis and compromised hepatic function) (Lechpammer et al. 2014). Neuronal loss in the globus pallidus is consistent with changes seen in humans patients exposed to elevated Mn from occupational sources (Olanow 2004; Perl and Olanow 2007).

The clinical and genetic studies described above suggest that mutations in SLC30A10 affect Mn metabolism in a manner that leads to Mn retention, likely due to decreased biliary excretion of Mn, and the observed neurotoxicity develops as a consequence of secondary Mn accumulation in the brain. The hepatic injury seen in affected patients is an important aspect of the disease, may be life-threatening, and can be explained by the observed deposition of Mn in the liver (Quadri et al. 2012; Tuschl et al. 2012). Polycythemia, another hallmark of the disease, may be a direct consequence of increased Mn levels as Mn and other transition metals, such as cobalt and nickel, have the ability to mimic the effects of hypoxia on erythropoietin gene expression by stabilizing hypoxia-inducible factor 1α (Ebert and Bunn 1999).

Some insights into the molecular mechanisms that lead to Mn retention when SLC30A10 function is compromised are now available. SLC30A10 belongs to the SLC30 family of metal transporters, which has ten members, SLC30A1–SLC30A10 (Huang and Tepaamorndech 2013; Kambe et al. 2015; Kolaj-Robin et al. 2015). SLC30A1–SLC30A8 transport Zn from the cytosol to the cell exterior or into the lumen of cellular organelles (i.e., mediate Zn efflux; the classification of SLC30A9 as a transporter is likely incorrect, and recent evidence suggests that it functions as a nuclear receptor coactivator) (Huang and Tepaamorndech 2013; Kambe et al.

2015; Kolaj-Robin et al. 2015). While SLC30A10 was initially thought to act as a Zn efflux transporter (Bosomworth et al. 2012), the fact that patients harboring mutations in this gene had elevated Mn levels suggested that it may mediate efflux of Mn, instead of Zn, and that disease-causing mutations may interfere with its Mn efflux function. To test this hypothesis, we performed a set of mechanistic experiments in cell and neuronal culture and in *C. elegans* (Leyva-Illades et al. 2014). Localization assays revealed that $SLC30A10_{WT}$ trafficked to the cell surface; in contrast, disease-causing mutants tested were trapped in the endoplasmic reticulum (Leyva-Illades et al. 2014). Mn measurement assays in cell culture revealed that $SLC30A10_{WT}$, but not a disease-causing mutant, reduced intracellular Mn levels. We performed a pulse-chase assay and confirmed that the reduction in intracellular Mn on $SLC30A10_{WT}$ expression was due to an increase in Mn efflux and not a reduction in Mn influx (Leyva-Illades et al. 2014). Additional studies revealed that expression of $SLC30A10_{WT}$, but not a disease-causing mutant, protected HeLa and GABAergic AF5 cells and primary midbrain neurons against Mn toxicity (Leyva-Illades et al. 2014). In contrast, siRNA-mediated knockdown of SLC30A10 in GABAergic AF5 cells led to Mn accumulation and heightened sensitivity to Mn toxicity (Leyva-Illades et al. 2014). In *C. elegans*, expression of $SLC30A10_{WT}$ protected dopaminergic neurons against Mn-induced neurodegeneration, rescued a Mn-induced locomotor defect, and enhanced viability when worms were exposed to high Mn; these effects were not evident when a disease-causing mutant was expressed (Leyva-Illades et al. 2014). These results suggest that SLC30A10 functions as a cell surface-localized Mn efflux transporter that enhances manganese efflux and protects against Mn toxicity. Mutations that induce human disease block the Mn efflux activity of the transporter, leading to increased Mn accumulation within cells.

The fact that SLC30A10 mediates Mn efflux implies that there is an important difference between its substrate specificity and that of other SLC30 family transporters, which mediate Zn efflux. Additionally, available evidence suggests that SLC30A10 lacks observable Zn efflux activity in cells and organisms. To summarize here, in cell culture, expression of $SLC30A10_{WT}$ reduced intracellular Mn and protected against Mn toxicity, but did not reduce Zn levels or alter viability in response to Zn toxicity (Leyva-Illades et al. 2014; Zogzas et al. 2016). Similarly, in *C. elegans*, expression of $SLC30A10_{WT}$ protected against Mn, but not Zn, toxicity (Chen et al. 2015; Leyva-Illades et al. 2014). Finally, in humans, loss-of-function mutations in SLC30A10 increased Mn levels in the liver, blood and brain, but increases in plasma or brain Zn levels have not been reported (as described earlier, an increase in liver Zn was reported in one patient, but this was likely due to hepatic decompensation) (Lechpammer et al. 2014; Quadri et al. 2012; Tuschl et al. 2008, 2012). The mechanisms underlying the difference in metal specificity of SLC30A10 and other SLC30 proteins are largely unknown, but two recent papers have begun to provide some understanding (Nishito et al. 2016; Zogzas et al. 2016). We generated a predicted structure of SLC30A10, based on the solved crystal structure of the related bacterial Zn transporter YiiP, and performed structure-function assays (Zogzas et al. 2016). YiiP has a transmembrane domain with six membrane spanning

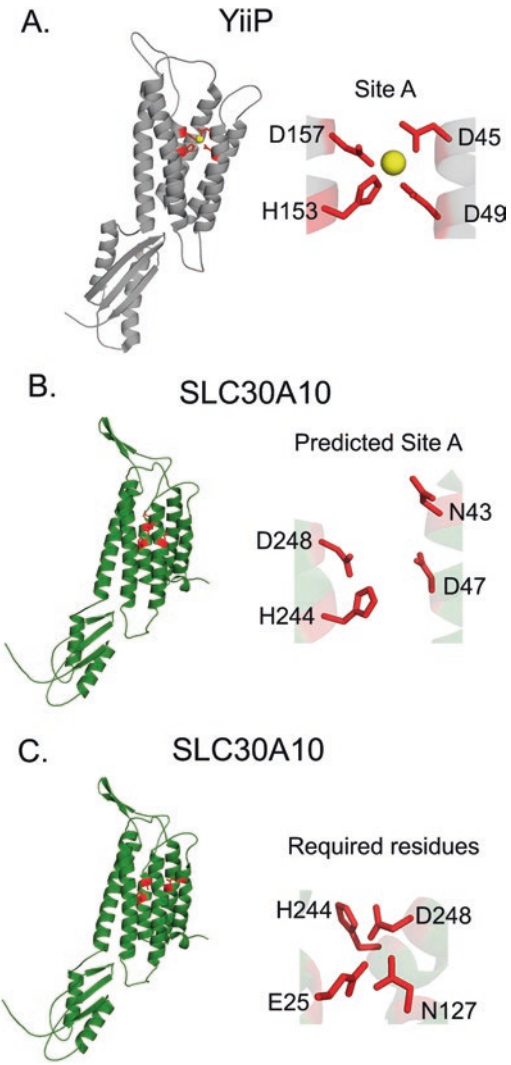

Fig. 1 Comparison of the solved crystal structure of YiiP with the predicted structure of SLC30A10. (**a**) Crystal structure of YiiP (Protein Data Bank code 3H90) is depicted in the cartoon format in *gray*; residues corresponding to Site A are shown as *red* sticks; Zn ion is shown as *yellow* sphere. (**b**) Predicted structure of SLC30A10 is shown in the cartoon format in *green*; residues corresponding to Site A of YiiP, which form the putative Site A of SLC30A10, are shown as *red* sticks. (**c**) Predicted structure of SLC30A10 is shown in the cartoon format in *green*; residues in the transmembrane segments that are required for Mn efflux activity are shown as *red* sticks. Note that not all required residues are depicted here and that residue H244 is not required by itself, but acts cooperatively with N127. Further details are provided in our ref. (Zogzas et al. 2016)

segments and a C-terminal domain (Lu et al. 2009, Lu and Fu 2007; Fig. 1a). In YiiP, side chains of amino acid residues Asp-45 and Asp-49 in the second and His-153 and Asp-157 in the fifth transmembrane segments form a metal coordination site, referred to as Site A, which coordinates Zn and is required for transport (Lu et al. 2009, Lu and Fu 2007; Fig. 1a). Prior studies show that, in other transporters of this superfamily (named cation diffusion facilitator), including in other SLC30 family proteins studied, residues that correspond to Site A of YiiP are crucial for metal coordination, specificity, and transport (Hoch et al. 2012; Huang and Tepaamorndech 2013; Kambe et al. 2015; Kolaj-Robin et al. 2015; Lu et al. 2009; Lu and Fu 2007; Martin and Giedroc 2016; Montanini et al. 2007; Ohana et al.

2009; Shusterman et al. 2014). Sequence analyses revealed that, in SLC30A10, residues corresponding to Site A of YiiP are Asn-43, Asp-47, His-244, and Asp-248 (Zogzas et al. 2016; Fig. 1b). The presence of Asn-43 was interesting because asparagine residues have a higher propensity to coordinate with Mn than with Zn (Dokmanic et al. 2008). Indeed, in Zn-transporting SLC30 proteins, this asparagine residue is replaced with histidine (Zogzas et al. 2016), which has a higher propensity to coordinate with Zn than asparagine (Dokmanic et al. 2008). Based on this, we hypothesized that perhaps a single amino acid change, from histidine in Zn-transporting SLC30 proteins to asparagine in SLC30A10, conferred Mn-transport capability to SLC30A10. To test this idea, first, we analyzed the obtained predicted structure of SLC30A10. Intriguingly, we discovered that, in SLC30A10, the side chain of Asn-43 pointed away from the putative ion binding pocket, located in the space between the second and fifth transmembrane domains, reducing the likelihood that it was involved in ion coordination and transport (Zogzas et al. 2016; Fig. 1b). Consistent with this, Mn transport assays in cells transfected with $SLC30A10_{WT}$ or mutants revealed that, among putative Site A residues, only Asp-248 was required for transport and side chains of Asn-43 and Asp-47 were not required (Zogzas et al. 2016; Fig. 1b and c). Instead, side chains of Glu-25 and Asn-127, located in the first and fourth transmembrane segment respectively and facing Asp-248, were required (Zogzas et al. 2016; Fig. 1c). His-244, a putative Site A residue, was not required by itself, but acted cooperatively with Asn-127 (Zogzas et al. 2016; Fig. 1c). Thus, in SLC30A10, only one of four residues that form its putative Site A is required for transport activity, suggesting that the mechanism of ion coordination in this transporter may be substantially different from that of Zn-transporting SLC30 proteins. Further biochemical and structural assays are now required to elucidate the nature of the difference in ion coordination and transport between SLC30A10 and other SLC30 proteins.

In the above discussion, it is important to note that, so far, our structure-function assays have been performed in HeLa cells, which do not express endogenous SLC30A10 and are amenable to genetic manipulation. However, substantial differences exist between immortal cell lines and neuronal and hepatic systems, where SLC30A10 functions under physiologic conditions. Transport kinetics of SLC30A10 mutants may differ between cell types due to changes in intracellular localization or differences in available interacting partners. The relevance of cell type-specific effects is underlined by findings in another recent paper on SLC30A10 function in which the authors expressed human $SLC30A10_{WT}$ or mutants in a chicken cell line (Nishito et al. 2016). They discovered that, in the avian system, SLC30A10 was largely trapped in the Golgi [the wild-type protein traffics to the plasma membrane in mammalian systems (Leyva-Illades et al. 2014; Zogzas et al. 2016)] and the side chain of the Asn-43 residue was required to protect against Mn-induced cell death (Nishito et al. 2016). Thus, in addition to biochemical and structural studies in minimal systems, it will be important to validate transport activities of SLC30A10 mutants in physiologically relevant primary neuronal and hepatocyte cultures.

How can the loss of the Mn efflux function of SLC30A10 at the cellular level lead to the phenotype observed in patients? SLC30A10 is expressed in the liver

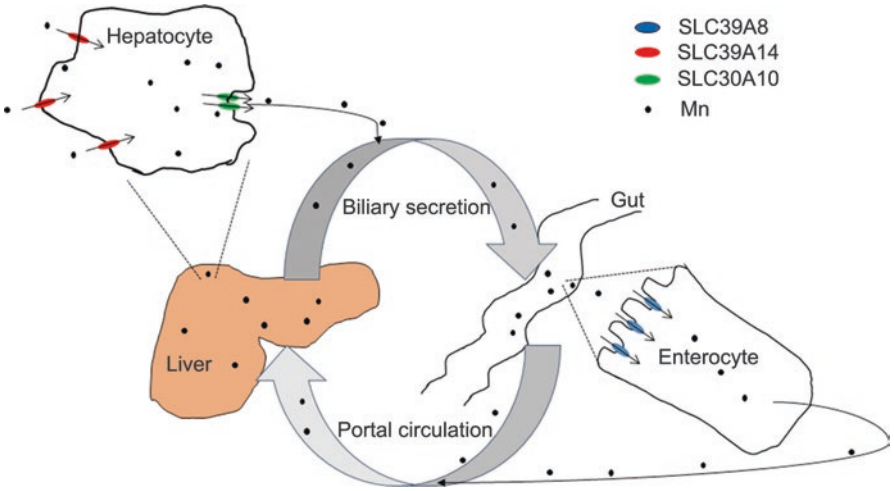

Fig. 2 Proposed model for the role of SLC39A8, SLC39A14, and SLC30A10 in regulating Mn homeostasis and detoxification. SLC39A8 may localize to the apical domain of enterocytes and mediate Mn (and Zn) influx into enterocytes. Metal ions would then be exported from enterocytes into blood by other transporters. SLC39A14 may localize to the basolateral aspect of hepatocytes and mediate influx of Mn from blood into hepatocytes. Finally, SLC30A10 may localize to the canalicular domain of hepatocytes and mediate efflux of Mn into bile. Consistent with the clinical data, this model predicts that loss of function of SLC39A8 should reduce Mn levels in blood; loss of function of SLC39A14 should increase Mn in blood, but not in the liver; and loss of function of SLC30A10 should increase Mn in the blood and liver

(Lechpammer et al. 2014; Quadri et al. 2012). Presumably, the efflux activity in the liver mediates biliary Mn excretion (Fig. 2). Loss of SLC30A10 function should decrease Mn excretion, and the retained Mn should accumulate in the liver leading to hepatic injury. Decreased Mn excretion should also lead to Mn accumulation in the blood and brain. Additionally, SLC30A10 is expressed in neurons of the basal ganglia, including in the globus pallidus (Lechpammer et al. 2014; Quadri et al. 2012). Loss of SLC30A10 function in the basal ganglia may further enhance Mn accumulation and induced damage in regional neurons. This sequence of events may culminate with the development of Mn-induced neurotoxicity. The above model remains to be experimentally tested, but is consistent with the available clinical data. To obtain more concrete evidence, we and others are generating global and tissue-specific SLC30A10 knockout mice to determine whether neurotoxicity is due to loss of efflux activity in the liver, brain, or both organs.

SLC39A14

In 2016, mutations in *SLC39A14* were reported to lead to the onset of Mn-induced neurotoxicity in humans (Tuschl et al. 2016). SLC39A14 belongs to the SLC39 family of metal transporters (Jeong and Eide 2013). While most members of this family mediate Zn influx into cells, SLC39A14 is known to mediate influx of Mn, Fe, and cadmium (Cd), in addition to Zn (Jeong and Eide 2013; Liuzzi et al. 2006; Girijashanker et al. 2008; Jenkitkasemwong et al. 2012; Pinilla-Tenas et al. 2011; Taylor et al. 2005). Patients harboring homozygous mutations in this gene were born to consanguineous parents (Tuschl et al. 2016). Clinical signs were evident early in life and included loss of developmental milestones, progressive dystonia, and bulbar dysfunction. Around age 10 years, patients developed severe, generalized dystonia that was resistant to treatment, spasticity, limb contractures, and scoliosis and lost the ability to move about by themselves. Some patients showed features of parkinsonism, such as hypomimia, tremor, and bradykinesia (Tuschl et al. 2016). Levels of Mn in blood were ~3–25-fold greater than normal; in contrast, blood levels of Fe, Zn, and Cd in tested patients were normal (Tuschl et al. 2016). Magnetic resonance imaging showed evidence of Mn deposition in the globus pallidus, striatum (lesser than the pallidus), and anterior pituitary and extensive involvement of the white matter (cerebellum, spinal cord, and dorsal pons). In some patients, evidence of cerebral and cerebellar atrophy was present (Tuschl et al. 2016). Importantly, however, there was no evidence of Mn deposition in the liver (Tuschl et al. 2016), suggesting that the transport activity of SLC39A14 may be required for import of Mn into hepatocytes. Consistent with the absence of Mn deposition in the liver, affected patients did not develop liver disease seen in individuals carrying SLC30A10 mutations (Tuschl et al. 2016). Polycythemia was also absent (Tuschl et al. 2016). Autopsy findings were available from one individual and revealed severe neuronal loss in the globus pallidus and the dentate nucleus of the cerebellum (Tuschl et al. 2016). Neurons in the caudate, putamen, thalamus, and cerebral cortex were largely preserved (Tuschl et al. 2016).

What are the molecular mechanisms that lead to Mn retention and neurotoxicity in patients harboring *SLC39A14* mutations? In HEK-293 cells, $SLC39A14_{WT}$, as well as disease-causing mutants tested, appeared to traffic to the cell surface (Tuschl et al. 2016). However, in this system, Mn influx was greater in cells expressing $SLC39A14_{WT}$ compared to those expressing disease-causing mutants, suggesting that the mutations inhibited the Mn transport activity of the protein (Tuschl et al. 2016). The mechanism by which loss-of-function mutations in a Mn importer lead to Mn toxicity may rely on the localization of the transporter in cells and tissues. SLC39A14 is expressed in the liver as well as in neurons, including in the globus pallidus (Tuschl et al. 2016). SLC39A14 may function to transport Mn from blood into hepatocytes, while SLC30A10 may function to transport Mn from within hepatocytes to bile (Fig. 2; also see Tuschl et al. 2016). In such a scenario, the influx transporter SLC39A14 and efflux transporter SLC30A10 would function synergistically to mediate biliary Mn excretion (Fig. 2). This model remains to be

experimentally tested, but is consistent with available data. The model predicts that loss of SLC30A10 function should increase liver, blood, and brain Mn, while loss of SLC39A14 function should increase Mn in blood, but not in the liver. These features are seen in patients who harbor loss-of-function mutations of the respective genes (Lechpammer et al. 2014; Quadri et al. 2012; Tuschl et al. 2008, 2012, 2016). Notably, patients carrying SLC39A14 mutations accumulate Mn in the brain (Tuschl et al. 2016). Similar findings were reported when SLC39A14 was depleted in zebrafish (Tuschl et al. 2016). These results imply that while the transport activity of SLC39A14 may be crucial for hepatic Mn influx, it is not required for the uptake of Mn into neuronal cells. Overall, the neurotoxicity seen in patients harboring SLC39A14 mutations is probably a consequence of a decrease in the biliary excretion of Mn, which leads to Mn accumulation in the brain and subsequent neuronal injury. Finally, the reason why patients with mutations in SLC30A10, but not SLC39A14, develop polycythemia remains to be clarified. As described earlier, a possible mechanism for polycythemia in patients with SLC30A10 mutations is Mn-induced chemical hypoxia followed by increased erythropoietin production. It may be that SLC39A14 is required for Mn influx into erythropoietin-producing cells, and when this transporter is mutated, the chemical hypoxia leading to erythropoietin production does not occur.

SLC39A8

In 2015, mutations in *SLC39A8* were reported to cause an inherited disorder of Mn and Zn deficiency (Boycott et al. 2015; Park et al. 2015). SLC39A8 belongs to the SLC39 family of transporters, similar to SLC39A14, and is known to mediate influx of Zn, Mn, Fe, Cd, and cobalt (Jenkitkasemwong et al. 2012; Jeong and Eide 2013; Wang et al. 2012). One of the reports described clinical findings from a German child born to unrelated parents who was initially referred at 4 months of age (Park et al. 2015). The patient presented with infantile spasms, dwarfism, cranial asymmetry, and hearing loss. Radiology demonstrated craniosynostosis of the coronary and lambdoid sutures with asymmetrical brain atrophy; the cerebellum was normal (Park et al. 2015). The second report identified six children from the Hutterite ethno-religious group, which is genetically isolated due to sociocultural practices, and two children of Egyptian descent born to consanguineous parents (Boycott et al. 2015). Patients from the Hutterite group presented with profound intellectual disability, developmental delay, hypotonia, strabismus, and cerebellar atrophy. Signs were evident soon after birth and head control was achieved only in early childhood. Additional features included short stature, osteopenia, recurrent infections, and, in most cases, an inability to walk (Boycott et al. 2015). The Egyptian children were siblings who presented at age 2 and 8 years with intellectual disability, developmental delay, hypotonia, and strabismus. One of the siblings had myoclonic seizures (Boycott et al. 2015). Genetic analyses revealed that affected patients carried missense mutations in both copies of *SLC39A8* (Boycott et al. 2015; Park

et al. 2015). The Hutterite and Egyptian patients had homozygous mutations that changed glycine at amino acid 38 to arginine; the German patient carried the above glycine-to-arginine mutation and an isoleucine-to-asparagine mutation at amino acid 340 (Boycott et al. 2015; Park et al. 2015). In all cases tested, parents were heterozygous for these mutations (Boycott et al. 2015; Park et al. 2015). Importantly, plasma Mn levels were below detectable limits in the German patient, while serum Zn levels were normal (Park et al. 2015). However, in the Hutterite and Egyptian groups, blood or erythrocyte Mn levels were low only in four out of seven patients (Mn values were not available for one patient) and within the normal range in the other three; plasma or serum Zn values were mildly decreased in five patients (Boycott et al. 2015). The reason for the difference in metal ion levels in the patients is unknown. It may be reflective of the difference in the mutations present. Immunoblot analyses from fibroblasts isolated from a control subject and a Hutterite patient who was homozygous for the glycine-to-arginine mutation demonstrated comparable SLC39A8 protein levels (Boycott et al. 2015), suggesting that this mutation, by itself, did not affect protein expression. One possibility is that the isoleucine-to-asparagine mutation at amino acid 340 may mislocalize, degrade, and/or profoundly inhibit Mn transport activity of SLC39A14, and this may be the underlying reason that Mn levels are undetectable in the patient harboring this mutation. Differences in Zn levels in patients reported in the two papers may also be related to differential effects of the mutations. It may be that when SLC39A8 function is completely abolished, activities of other Zn transporters are altered to compensate; this compensatory effect may not be evident when SLC39A8 activity is only partially inhibited. Further understanding of the cellular changes leading to the disease phenotype requires in-depth knowledge of the effects of the disease-causing mutations on SLC39A8 transport activity, which is not yet available. Biochemical and cell biological approaches used to study functional consequences of SLC30A10 and SLC39A14 mutations should provide necessary insights.

While the mechanisms by which mutations in SLC39A8 lead to human disease are not yet clear, changes in glycosylation may play a role. The German patient described above exhibited a defect in *N*-linked glycosylation, with a primary problem in galactosylation (Park et al. 2015). The phenotypic presentation of this patient had similarities to that seen in a congenital disorder of glycosylation when *SLC35A2*, a UDP-galactose transporter, is mutated (Park et al. 2015). SLC35A2 imports UDP-galactose from the cytosol into the Golgi (Ng et al. 2013). Within the Golgi, galactose is transferred to acceptor proteins by galactosyltransferase enzymes (Hennet 2002), several of which require Mn for activity (Wagner and Cynkin 1971; Schachter et al. 1971). Conceivably, when intracellular Mn is low, activities of galactosyltransferases may be inhibited, leading to the observed glycosylation defect. In support of this idea, prior studies have demonstrated that glycosylation defects occur when SPCA1, the Golgi localized P-type pump that transports Mn from the cytosol into the Golgi, is depleted (Ramos-Castaneda et al. 2005). Thus, it is not surprising that there are phenotypic similarities between patients with *SLC35A2* and *SLC39A8* mutations. Indeed, after the discovery of the glycosylation defect, Park et al. went on to screen for *SLC39A8* mutations in patients who had impaired glycosylation of

unknown origin and identified another patient with mutations in the *SLC39A8* gene; Mn levels in blood were below the detectable limit in this patient (Park et al. 2015). However, the extent to which a defect in glycosylation contributes to the development of the disease and the severity of the phenotype in all patients who have mutations in SLC39A8 is not clear. Patients who were homozygous for the glycine-to-arginine mutation did not have a severe Mn deficiency and, compared to patients without detectable Mn in blood or plasma, exhibited milder defects in glycosylation; yet, they had extensive neurologic damage (Boycott et al. 2015; Park et al. 2015). These results imply that while there may be a relationship between Mn levels and glycosylation efficiency, the contribution of the glycosylation defect to disease development and severity needs further assessment. The relative contribution of Mn versus Zn deficiency to disease pathobiology is also unclear and needs to be elucidated. Overall, mutations in SLC39A8 lead to severe neurological disease that is related to Mn and Zn deficiency (Fig. 2) and a defect in *N*-linked glycosylation. Future studies need to determine the effect of disease-causing mutations on transporter activity and to elucidate the mechanisms by which altered transport activity changes metal levels; affects cellular processes, such as glycosylation; and induces disease.

Concluding Perspectives

The discoveries of the above-described genetic diseases are poised to revolutionize our understanding of Mn homeostasis, detoxification, and induced toxicity at the cellular and organismal level. All three disorders were identified within the last 5 years; therefore, detailed understanding of disease pathobiology cannot be expected. However, rapid progress is anticipated as multiple laboratories are now working on elucidating the mechanisms by which these proteins mediate Mn transport and regulate Mn homeostasis and detoxification, by which the transporters themselves are regulated in response to changing Mn levels and by which changes in transporter activities alter brain Mn to induce disease. While these genetic diseases are rare, studying these disorders is expected to improve our understanding of Mn biology in general and the mechanisms of Mn-induced neurotoxicity in particular. Indeed, *SLC39A14* knockout mice are available (Aydemir et al. 2012), and *SLC30A10* knockout mice are being generated. It is likely that these genetic models will make invaluable contributions to the neurotoxicity field. It also noteworthy that recent population-based studies identified a common single nucleotide polymorphism in *SLC30A10* that was associated with increased Mn levels in blood, altered neurological function, and decreased *SLC30A10* expression (Wahlberg et al. 2016). Similarly, single nucleotide polymorphisms in *SLC39A8* and *SLC39A14* have been associated with increased Cd levels in humans (Rentschler et al. 2014). These discoveries raise the intriguing possibility that single nucleotide polymorphisms in these genes may alter the risk for the development of toxicity from Mn and other metals in the general population. Finally, increasing Mn efflux may be an effective

strategy for protection against or treatment of Mn-induced neurotoxicity (Leyva-Illades et al. 2014; Mukhopadhyay and Linstedt 2011). As SLC30A10, SLC39A14, and SLC39A8 appear to be the primary transporters responsible for maintaining homeostatic control of Mn in humans, understanding the function and regulation of these proteins may directly augment the ability to generate therapeutically viable and effective efflux enhancing drugs.

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