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Review

Manganese transport in mammals by zinc transporter family proteins, ZNT and ZIP

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ABSTRACT

Manganese (Mn) is an essential trace element required for various biological processes. However, excess Mn causes serious side effects in humans, including parkinsonism. Thus, elucidation of Mn homeostasis at the systemic, cellular, and molecular levels is important. Many metal transporters and channels can be involved in the transport and homeostasis of Mn, and an increasing body of evidence shows that several zinc (Zn) transporters belonging to the ZIP and ZNT families, specifically, ZNT10, ZIP8, and ZIP14, play pivotal roles in Mn metabolism. Mutations in the genes encoding these transporter proteins are associated with congenital disorders related to dysregulated Mn homeostasis in humans. Moreover, single nucleotide polymorphisms of ZIP8 are associated with multiple clinical phenotypes. In this review, we discuss the recent literature on the structural and biochemical features of ZNT10, ZIP8, and ZIP14, including transport mechanisms, regulation of expression, and pathophysiological functions. Because a disturbance in Mn homeostasis is closely associated with a variety of phenotypes and risk of human diseases, these transporters constitute a significant target for drug development. An understanding of the roles of these key transporters in Mn metabolism should provide new insights into pharmacological applications of their inhibitors and enhancers in human diseases.

1. Introduction

Manganese (Mn) is a metal widely distributed in rocks, soil, and many plants and animals. Mn exists in the 0–7 valence states, but mostly as Mn^{2+} or Mn^{3+}.[1] In the environment, it exists mainly in oxidized chemical forms, such as MnO_2 and Mn_3O_4, as a metallic Mn, and does not exist in free form.[2] It is a component of various enzymes that are essential for life. Mn-superoxide dismutase, pyruvate carboxylase, arginase, and many other metalloenzymes contain Mn at their active sites.[3,4] Thus, Mn is an essential element for living organisms. However, occupational Mn intoxication occurs in workers exposed to Mn in mines and metal refineries, the first clinical description of which dates back to 1837.[5] Exposure to excess amounts of Mn causes neurological symptoms, including dystonia and parkinsonism.[6] Alterations in the quantity and metabolism of dopamine in the brain by Mn are believed to cause symptoms similar to those of Parkinson's disease.[7,8] Thus, Mn is a dual-faced metal, being essential and toxic depending on its amount in the body. Organisms must tightly regulate the absorption, retention, and excretion of Mn in the body to maintain Mn homeostasis.

The total amount of Mn in the human body is approximately 10–20 mg.[9] Humans intake Mn primarily from diets, such as cereals and seeds. After digestion, Mn is absorbed by duodenal epithelial cells in a divalent form. It has been suggested that Mn is transported via the same pathway as used for iron (Fe) because excess amounts of Fe in the diet suppress intestinal Mn absorption.[10,11] In 1997, divalent metal transporter 1 (DMT1) was isolated as a divalent Fe transporter in the intestine, and was reported to have the ability to transport divalent Mn (Mn^{2+}) into intestinal epithelial cells.[12] Mn is also known to exist in a trivalent form (Mn^{3+}) in the body. It is believed that Mn^{3+} bound to transferrin, an iron-carrier protein, is transported into cells via endocytosis as a transferrin–transferrin receptor complex. It has been suggested that the Fe transport system is the major player in Mn transport in the body.

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Recently, it was shown by us, as well as by other groups, that zinc (Zn) transporters, ZIP8 and ZIP14, encoded by *SLC39A8* and *SLC39A14*, transport Mn^{2+} and cadmium (Cd^{2+}) in addition to Zn^{2+} into cells.[13–17] It was also found that ZNT10, a Zn exporter encoded by *SLC30A10*, is involved in the excretion of Mn^{2+} from cells.[18–21] These studies have indicated that the transporters for Zn, in addition to those for Fe, are involved in Mn transport pathways. However, it remained unclear whether these Zn transporters involved in Mn^{2+} transport play essential roles in the body because both Mn^{2+} and Mn^{3+} exist in the body. Recent findings of genetic defects in ZIP8, ZIP14, and ZNT10 have led to the understanding that these Zn transporters do play essential roles in Mn metabolism in the body.[22–25] Mutations in *ZIP14/SLC39A14* and *ZNT10/SLC30A10* cause parkinsonism-like neurological symptoms accompanying hyperaccumulation of Mn in the brain, whereas a mutation in *ZIP8/SLC39A8* causes a whole-body glycosylation disorder symptom with very low Mn levels in the blood. The results of genome-wide association studies have suggested much broader roles of ZIP8 than expected in a variety of diseases. Thus, a loss of function of these transporters results in aberrant Mn metabolism and systemic symptoms. However, detailed mechanisms underlying Mn transport by these Zn transporters remain to be elucidated.

In this review, we describe the structural and biochemical features, including transport mechanisms, regulation of expression, and pathophysiological functions, of ZNT10, ZIP8, and ZIP14, involved in Mn transport. Because transporters constitute valuable targets in drug development, understanding the roles of Mn transporters and their relation to human diseases should provide new insights into pharmacological applications of their inhibitors and enhancers in human diseases related to the dysfunction of Mn homeostasis.

1.1. Biochemical features of ZNT10, ZIP8, and ZIP14

1) Structural features and Mn transport mechanism of ZNT10

ZNT10 is a member of the ZNT/SLC30A transporter family. However, it is unique as an Mn transporter, whereas other members act as Zn transporters. In this section, properties of ZNT transporters are first reviewed with regard to the structural and biochemical features of bacterial ZNT homologs and subsequently of mammalian ZNT members, which is followed by a discussion on the properties of ZNT10.

X-ray and cryo-electron microscopy (cryo-EM) structures of a bacterial ZNT homolog, YiiP (*Escherichia coli* and *Shewanella oneidensis*), reveal that YiiP forms homodimers, each protomer of which possesses six transmembrane (TM) helices with N- and C-termini in the cytoplasm.[26–30] These structures also provide evidence for the alternating-access mechanism mediated by conformational changes between an inward- and outward-facing conformation.[28,30] A change in conformation of a four-helix bundle consisting of TM1, TM2, TM4, and TM5 relative to a two-helix pair outside the bundle consisting of TM3 and TM6 provides accessibility to Zn on either side of the membrane. The pH gradient drives a vectorial Zn transport in a 1:1 exchange stoichiometry across biological membranes and, thus, YiiP operates as a Zn^{2+}/H^{+} exchanger.[29,30] Four hydrophilic amino acid residues (three Asp and one His; DDHD) in anti-parallel TM2 and TM5 in the four-helix bundle constitute an intramembranous tetrahedral Zn-binding site.[26–30] On the basis of these observations on YiiP, the topology of ZNT transporters has been predicted, which was confirmed recently by cryo-EM of ZNT8.[31] One striking difference is that the His residue in the N-terminal portion of ZNT8 is involved in two adjacent Zn-binding sites formed in the C-terminal cytosolic region, whereas in YiiP, the binuclear Zn center is formed only by its C-terminal cytosolic region. However, the N-terminal site is apparently not essential for the Zn transport activity because the deletion of the homologous portion of ZNT2 did not result in the loss of this activity.[32]

Mammalian ZNT transporters have six TM helices with N- and C-termini in the cytoplasm, and form homodimers[33] (Fig. 1A), except for ZNT5, which has a long N-terminal region and forms heterodimers.[34] ZNT transporters operate as Zn^{2+}/H^{+} exchangers,[35,36] as suggested in studies on YiiP. The intramembranous tetrahedral Zn-binding site of most ZNT transporters, for example, the DDHD motif in YiiP, consists of two Asp and two His residues (HDHD). In several biochemical studies, it has been observed that these intramembranous motifs are essential for Zn transport, because D-A or H-A substitutions result in the loss of both Zn-binding and Zn-transport activities.[35,37–39] Intriguingly, plant ZNT homologs, which are thought to specifically transport Mn, have four Asp residues (DDDD) in the intramembranous binding site,[40–43] although no orthologs have been found in vertebrate genome.

ZNT10 is a cell-surface Mn exporter.[44] In contrast to features conserved in mammalian ZNT family proteins, ZNT10 is unique in that it has a tetrahedral motif consisting of two Asp, one His, and one Asn residues (NDHD) in the intramembranous Zn-binding site, in which the first H is substituted for N (Fig. 2A). Moreover, ZNT10 has a cytosolic loop between TM4 and TM5, and a C-terminal domain rich in Arg (R) and Lys (K) residues, unlike those in other ZNT members, which are rich in His (H) residues. The N-terminal portion of ZNT10 is too short to form two adjacent Zn-binding sites,[45] which is different from the case of ZNT8.[31] These features are believed to be associated with the Mn transporting ability of ZNT10. Indeed, in several studies, it has actually been observed that the NDHD motif is essential for such function. We showed the importance of the first N (Asn-43) in a tetrahedral NDHD motif using a cell system for evaluating resistance to high Mn concentrations.[21] In this study, the critical function of Asn-43 was examined by amino acid swapping mutations between ZNT10 and ZNT1, because both show a high sequence similarity among ZNT members and exhibit similar features, such as cell surface localization and a short cytosolic N-terminal region.[32,46] However, the substrate metal is completely different; ZNT10 is a cell surface Mn exporter,[44] whereas ZNT1 is a cell surface Zn exporter.[47,48] The substitution of Asn-43 with His residue in ZNT10, along with two other amino acid substitutions in TM2 and TM5, conferred Zn transport activity, although these substitutions abolished the Mn transport ability[21] (Fig. 2B). In contrast, a ZNT1 mutant, in which His-43 in the motif was substituted with Asn (alteration from HDHD to NDHD), showed Mn transport ability, however, this substitution abolished Zn transport ability.[21] Loss of Mn transport activity by the alteration of NDHD to HDHD in ZNT10 was confirmed in another study using a different evaluation system.[49] Thus, the Asn residue in the NDHD motif is required for Mn transport, whereas the first His residue in the HDHD motif is required for Zn transport. Generally, Mn is preferably bound directly to oxygen-containing amino acids, such as Asp, Glu, Asn, and Gln, and backbone carbonyl groups,[50] which may explain the ability of the NDHD motif to mobilize Mn and the disability of the HDHD, if the tetrahedral coordination is formed on Mn by the NDHD. This speculation is consistent with the fact that *Streptococcus pneumoniae* ZNT homolog Mn transporter, MntE, has the NDDD motif.[51] However, the Mn transport ability was, interestingly, retained upon substitution of Asn-43 with Ala (ADHD)[49,52] (Fig. 2C), suggesting that tetrahedral formation may not occur in the transport of Mn by ZNT10.

Recently, ZNT10 was suggested to operate in the efflux of Mn, coupled to calcium (Ca) exchange,[49] but not as a proton exchanger as in other ZNT transporters.[35,36] Interestingly, substitution of Asn-

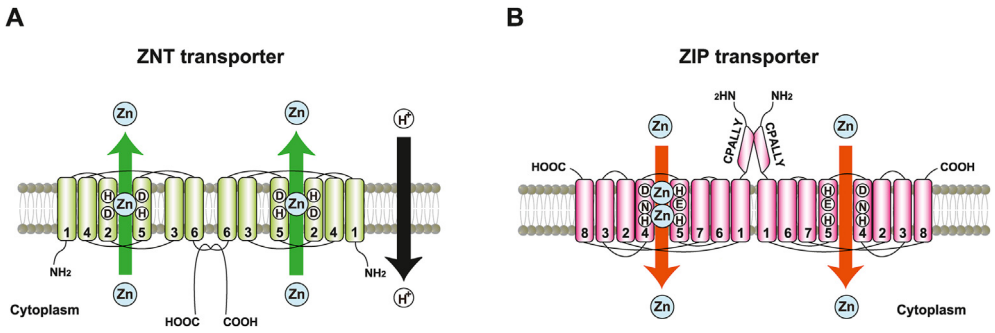

Fig. 1. Diagrammatic representation of the predicted structures of ZNT and ZIP transporters. A. Proposed topology of ZNT is described. ZNT protomers have six transmembrane (TM) domains, and generally form homodimers. A transmembranous zinc binding site exists in the compact four-helix bundle consisting of TMs 1, 2, 4, and 5. The binding site is generally formed by conserved aspartic acid (D) and histidine (H) residues of TM2 and TM5. Proton (H^+) gradient facilitates the antiport of Zn by ZNT proteins. ZNT proteins mobilize Zn (all members, except ZNT10) and Mn (ZNT10) from the cytosol into the intracellular compartments or extracellular space. B. Proposed topology of ZIP is described. ZIP protomers are predicted to have eight TMs and have been experimentally shown to form homodimers. The conserved amphipathic amino acid residues, H, N, and D, in the HNhPDG sequence in TM4 and H, E, and H in the HEhPHG sequence in TM5 would form a binuclear metal center within the TMs. LIV-1 subfamily members have the potential conserved metalloprotease motif (HEXPHEXGD) in TM5 (not shown) and the long extracellular N-terminal region with the CPLAYY motif. ZIP family members mobilize Zn (all members) and Mn (ZIP8 and ZIP14) in directions opposite to the mobilization by the ZNT proteins.

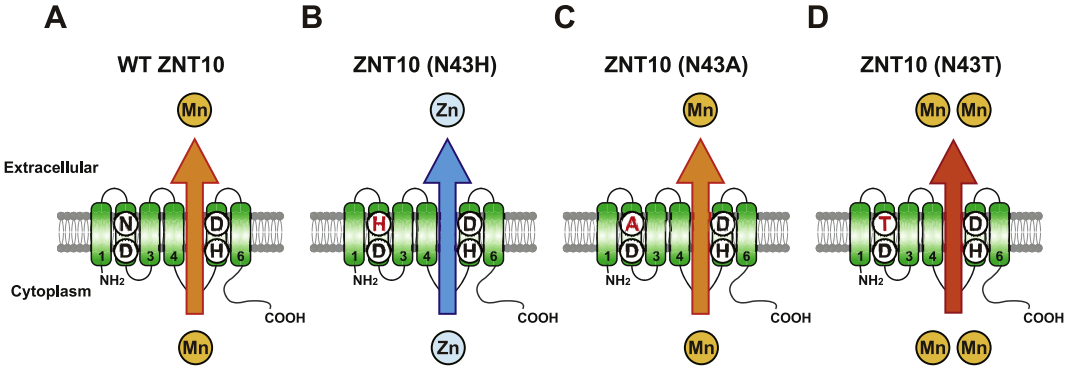

Fig. 2. Illustrations summarizing the Mn and Zn transport property of ZNT10 and its mutants. A. WT ZNT10. B. ZNT10 (N43H) mutant. Other two amino acid substitutions in TM2 and TM5 are required for the mutant to mobilize Zn (21). C. ZNT10 (N43A) mutant. D. ZNT10 (N43T) mutant. ZNT10 (N43T) mutant is operative in an uncoupled channel mode. Metal specificity and transport activity are shown as Mn and Zn circles.

43 with Ala (in ADHD) enhances the coupling between Mn and Ca exchange than in NDHD.[49] Moreover, substitution of Asn-43 with Thr (in TDHD) induces uncoupled channel-like Mn transport activity, and enables ZNT10 to be permeable to both Ca and Mn[49] (Fig. 2D). This may support the notion that ZNT10 transports Mn through a mode different from that employed by other ZNT transporters, wherein the first His residue in the HDHD motif is critical for determining Zn as a substrate metal. The molecular understanding of the mode of operation of ZNT10 has not yet been elucidated, and needs to be clarified in future studies.

2) Structural features and Mn transport mechanism of ZIP8 and ZIP14

ZIP8 and ZIP14 are members of the ZIP/SLC39A transporter family.[33,53] They are unique in that they can mobilize different divalent cations, including Mn and Zn.[54] In this section, we have reviewed the properties of ZIP transporters, first using bacterial ZIP homologs and mammalian ZIP members, and then discussed the properties of ZNT8 and ZIP14.

Compared with the crystal structure of the ZNT homolog, YiiP, the structure of the ZIP homolog, ZIPB (*Bordetella bronchiseptica*), was revealed recently. ZIPB functions as a monomeric transport unit having eight TM helices, with its N- and C-terminal regions located extracellularly, as predicted previously.[55,56] The inner four-helix bundle within the TM helices embraces a central binuclear metal center, in which the metal ion coordination sites are termed M1 and M2.[57] The M1 site is penta-coordinated, whereas M2 is hexa-coordinated, primarily by the amino acid residues located within two conserved hexapeptides, ^{177}HNhPEG^{182} and ^{207}QD/NhPEG^{212} (h refers to a hydrophobic residue), in TM4 and TM5, respectively. In ZIP4, the M1 site is essential for Zn transport, whereas the M2 site is auxiliary, because the M2 site is neither required for Zn transport nor for M1 binding.[57] However, most individual residues in the binuclear metal center are functionally dispensable.[58] Water molecules occupy two coordination sites of M1 and one coordination site of M2.[57] ZIPB mobilizes extracellular Zn through facilitated diffusion into the cytoplasm down an inward Zn concentration gradient,[59] wherein these water molecules contribute to Zn mobility, that is, there is a water-mediated Zn transport.[60]

Mammalian ZIP transporters are believed to have the same topology as ZIPB, with eight TM helices, having extracellular N- and C-terminal regions (Fig. 1B). Based on biochemical analyses, it has been predicted that they form homodimers,[53,61] as shown for ZIPB,[62] as well as heterodimers between some members.[63] Amino acid residues forming two conserved hexapeptide regions, HNhPEG and QD/NhPEG, in TM4 and TM5 of ZIPB are moderately changed to HNhPDG and HEhPHG in mammalian ZIPs, but M1 and M2 sites of an intramembranous binuclear Zn binding site are believed to be

formed, as in the ZIPB structure. The HEhPHG sequence in TM5 is conserved as the potential metalloprotease motif (HEXPHEXGD) in LIV-1 subfamily members, which also has the CPLAYY motif in the extracellular region preceding the first TM1. In mammalian ZIP transporters, based on the results of several Zn or Cd uptake assays, a symport mechanism, whereby Zn is transported alongside bicarbonate (HCO_3^-), is suggested to be operative.[64,65] However, this mechanism needs to be experimentally confirmed[66] because of the discrepancy with that of ZIPB.

Compared with the detailed molecular information on Mn transport by ZNT10, information on the Mn transport by ZIP8 and ZIP14 is less clear. ZIP8 and ZIP14 are primarily localized to the plasma membrane, with some reports suggesting their localization to endosomes and lysosomes[67,68] Both are broad-spectrum metal transporters that take up Mn, Fe, and Cd, in addition to Zn[54,69,70] and can mobilize Cd and Mn with high affinity.[17,65] However, ZIP8 mediates the uptake of Cd, Zn, cobalt (Co), and Fe, and weakly mediates the uptake of Mn in metal uptake assays using *Xenopus* oocytes.[71] This suggests that the Mn transport activity of ZIP8 and ZIP14 may be specifically enhanced in mammalian cells. ZIP8 and ZIP14 are assigned to the LIV-1 subfamily of ZIP transporters. Uniquely, ZIP8 and ZIP14 have a Glu (E) residue in place of the highly conserved His (H) residue in TM5 (Fig. 3). The H-to-E substitution (i.e., a change from HEXPHEXGD to EEXPHELGD) may contribute to a shift in increased metal selectivity to Mn in addition to Zn[55,72] (Fig. 3). The Cd transport activity of ZIP8 and ZIP14 is facilitated by HCO_3^-,[17,65] which suggests that their metal transport activity, including the transport of Mn, is operative in the symport mechanism that drives metals across an HCO_3^- gradient. However, this may need to be further examined, as described above.

As discussed in more detail below, a ZIP8 single nucleotide polymorphism (SNP) (rs13107325 C→T, Ala391Thr) is known to be associated with multiple phenotypes.[73,74] ZIP8 A391T is associated with significant impairment of systemic Mn homeostasis, but not with Zn metabolism,[75,76] suggesting that A391, which is predicted to be present close to TM7 helix (Fig. 3), may affect the determinant of metal specificity in the binuclear metal center within the TM helices of ZIP8. Interestingly, several other ZIP transporters, including ZIP9, also have Ala residues at this position.[45] ZIP9 is homologous to *Saccharomyces cerevisiae* Atx2p (antioxidant 2),[53,77] which is involved in Mn homeostasis,[78] and is essential for efficient O- and N-glycosylation.[79] In contrast, ZIP14 does not possess an Ala residue at this position (Ser residue in ZIP14). Thus, it is worthwhile to clarify how the A391T substitution in ZIP8 contributes to Mn transport.

1.2. Regulation of the expression of ZNT10, ZIP8, and ZIP14

Expression of ZNT10, ZIP8, and ZIP14 is regulated in a tissue- or cell-specific manner, which could be associated with the pathophysiological situation. There are many review articles on zinc pathophysiology, but little opportunity has been described for Mn physiopathology. Here, we provide a simple overview of the regulation of their expression, because it would be useful to focus on Mn transport.

The transcription of *ZNT10* is enhanced by several stimuli. Vitamin D is one such stimulus that upregulates the transcription of *ZNT10* through binding of the vitamin D receptor to vitamin D responsive elements (VDREs) in the 5′-flanking region of its promoter.[80] The upregulation of *ZNT10* transcription by bile acid is also mediated by the vitamin D receptor.[81] The transcription of *ZNT10* is also induced by MPP^+ in an ATF4-dependent manner, which may contribute to its protective role in MPP^+-induced toxicity via the PERK-ATF4 pathway.[82] In contrast, interleukin (IL)-6 stimulates the downregulation of *ZNT10*.[15]

Lipopolysaccharides (LPS) or IL-6 stimulate the transcription of *ZIP14*,[15,83,84] which may be mediated by AP-1.[85] The transcription of *ZIP14* is increased during ER stress, which is mediated by the binding of ATF6 to the ER stress response element (ERSE)-like sequence.[86] At the protein level, ZIP14 is downregulated by Fe deficiency and is upregulated by an Fe overload.[87] Moreover, the expression of ZIP14 is slightly decreased through degradation in the lysosomal pathway in response to relatively high Mn exposure,[88] suggesting that its expression is regulated through a complicated mechanism by its substrate metals at the protein level.

The expression of ZIP8 is upregulated by LPS or tumor necrosis factor (TNF),[89] which is mediated by NF-κB through the physical interaction with the κB-binding site in its 5′-flanking region.[90] The expression of ZIP8 is markedly increased by IL-1β treatment.[91] The expression of *ZIP8* mRNA is also induced by IFN-γ.[92] In contrast, the expression of ZIP8 is suppressed by GSH and Cd exposure through

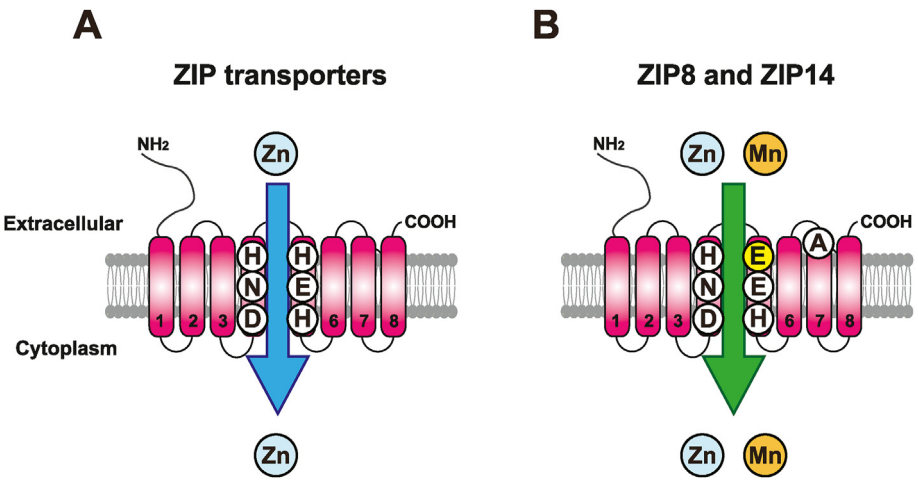

Fig. 3. Illustration of the possibility of increased selectivity for Mn in addition to that for Zn by H-to-E substitution in TM5 of ZIP8 and ZIP14. A. Diagrammatic representation of ZIP transporters (LIV-1 subfamily members), except for ZIP8 and ZIP14. The potential conserved metalloprotease motif (HEXPHEXGD) is present in TM5. B. A diagrammatic representation of ZIP8 and ZIP14. The H residue is substituted with an E residue in the metalloprotease motif. The position of A391 at a location close to TM7 of ZIP8, whose substitution with the T residue because of single nucleotide polymorphism (rs13107325 C→T, Ala391Thr) is known to be associated with multiple clinical phenotypes (see text), is also shown.

the downregulation of Sp1.[93] MicroRNA-488 suppresses the expression of ZIP8.[94]

Zn or Mn metabolism would be closely associated with the regulation of expression of ZNT10, ZIP8, and ZIP14, and their regulation should, therefore, be more closely elucidated based on the expanded insights on Zn and Mn pathophysiology.

1.3. Pathophysiological functions of ZNT10, ZIP8, and ZIP14

Mutations in the *ZIP8*, *ZIP14*, and *ZNT10* genes in humans have been found to cause hereditary abnormalities in Mn metabolism (Fig. 4). In 2012, mutations in *SLC30A10/ZNT10* were found in patients with parkinsonism-like symptoms accompanying hyperaccumulation of Mn in the brain and liver[18,20] (OMIM #613280). In 2016, mutations in *SLC39A14/ZIP14* were also found in patients with excessive Mn accumulation and progressive childhood-onset parkinsonism-dystonia[95] (OMIM #617013). In 2015, patients with type II congenital disorders of glycosylation showing blood Mn deficiency were found to have mutations in *SLC39A8/ZIP8*[96,97] (OMIM #616721). The findings of these hereditary diseases allude to critical roles played by ZIP8, ZIP14, and ZNT10 in maintaining normal Mn metabolism in humans and show that dysfunctions of these transporters could cause serious diseases associated with the disorders of Mn metabolism. In this section, physiological and pathological significance of ZIP8, ZIP14, and ZNT10 is summarized, and the phenotypes of patients and studies on knockout mice are discussed. The phenotypes of knockout mice described in the selected reports are also summarized in Table 1 for easy reference.

1) Pathophysiology of ZNT10

In patients harboring the *SLC30A10/ZNT10* mutation, the frameshift-induced premature termination of the protein occurs in the cytosolic loop between TM4 and TM5 or in the C-terminal region.[18,20] Immunohistochemical analysis revealed that ZNT10 was absent at the plasma membrane facing the lumen of the bile ducts in the liver of these patients. Blood Mn concentrations were markedly increased, leading to hyperaccumulation of Mn in the brain and liver. Because biliary excretion from the liver is believed to be the major route of Mn excretion from the body,[10,98] the loss of ZNT10 functions in the liver was assumed to impair the biliary excretion of Mn, leading to hyperaccumulation of Mn in the brain and liver of these patients. However, the phenotypes of *Znt10*-knockout mice show that the pathways for Mn excretion via Znt10 are more complex than expected. In *Znt10*-knockout mice, which died at 6–8 weeks, the Mn levels in the brain, blood, and liver were markedly higher than those in control mice at 6 weeks. Notably, comparison of whole-body *Znt10*-knockout mice with tissue-specific *Znt10*-knockout mice revealed that Mn levels in the brain were not increased in liver-specific (carrying Alb-*Cre*) or pan-neuronal/glial-specific (carrying Nes-*Cre*) *Znt10*-knockout mice.[99] In contrast, endoderm-specific *Znt10*-knockout mice (carrying Foxa3-*Cre*), in which the expression of Znt10 was lost in the liver and intestine, showed markedly elevated Mn levels in the brain, blood, and liver, which was similar to the phenotype observed in whole-body knockout mice.[99] Similar results were reported in another strain.[100] These results indicate that Znt10 plays a role in Mn excretion in the intestine, in addition to that in the liver (Fig. 4). Using Caco2 cells cultured in a Transwell plate, ZNT10 was localized on the luminal side of Caco2 cells and played a role in transporting Mn from the basolateral to the luminal side,[99] suggesting an important role for ZNT10 in the excretion of Mn from the blood into the intestinal lumen. Consistent with these results, a previous study showed that intestinal excretion of Mn plays a more critical role than biliary excretion in the presence of excess Mn in the body.[101]

2) Pathophysiology of ZIP14 and ZIP8

Mutations in *SLC39A14/ZIP14* also result in the accumulation of Mn in the whole body and brain, leading to childhood-onset parkinsonism-dystonia symptoms.[95] The identified mutations include three missense mutations (F98V, G383R, and N469K located in the N-terminal domain, TM5, and TM8, respectively) and a frameshift mutation generating a truncated protein. These mutations are predicted to impair the transporter activity of ZIP14.[95] Whole-body *Zip14*-knockout mice exhibited the same pathological features as patients with *SLC39A14/ZIP14* mutations.[102] Because ZIP14 functions as an importer of Mn, the introduction of ZIP14

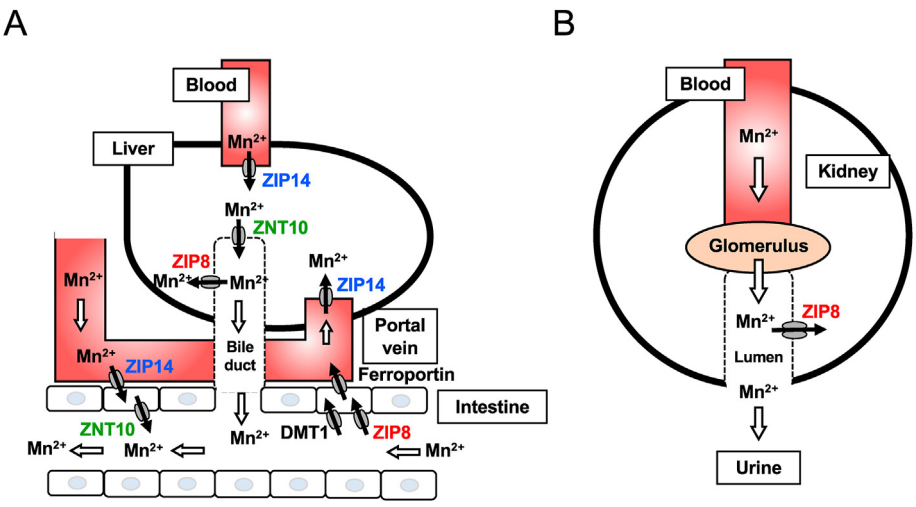

Fig. 4. Diagrammatic representation of the pivotal and proposed roles of ZNT10, ZIP8, and ZIP14 in Mn metabolism in the intestine, liver, and kidney. A. Luminal Mn is captured and taken up into the enterocytes from the apical side via DMT1 and probably ZIP8, and Mn is effluxed into the portal vein across the basolateral border, probably mediated by ferroportin. Mn in the portal vein and serum is taken up into the hepatocytes by ZIP14, which is excreted into the bile, mediated by ZNT10. Biliary Mn is recovered by ZIP8 at the canalicular membrane. Mn taken up from the basolateral side into the intestinal epithelial cells via ZIP14 is excreted into the lumen, mediated by ZNT10. B. Reabsorption into the proximal tubules of the kidney may play an important role in controlling the amount of Mn in the whole body. Mn undergoes glomerular filtration and Mn in the primary urine is presumably reabsorbed into proximal tubular epithelial cells via ZIP8. Modified from ref.[131]

Table 1
Tissue-specific knockout mice and blood Mn levels.

Mn transporter	Gene name	Cre-gene	Tissue	Blood Mn	Reference
Znt10	Slc30a10	–	Whole body	Very high	100
		Nes	Brain-specific	–	99
		Alb	Liver-specific	–	99
		Foxa3	Intestine-specific	–	99
		Alb/Vill	Liver-and intestine	Very high	100
Zip14	Slc39a14	–	Whole body	Very high	102
		Alb	Liver-specific	–	103
		Vill	Intestine-specific	Very high	104
Zip8	Slc39a8	–	Whole body	Very low	109
		Alb	Liver-specific	Very low	109

siRNA reduced Mn uptake in neuronal SH-SY5Y cells[15]; it was puzzling to note that the loss of ZIP14 function results in increased systemic levels of Mn. However, the basolateral localization of ZIP14 in cells excreting Mn[68] provides an explanation because such localization can provide an excretion route for the blood Mn and dysfunction causes an accumulation of Mn in the body. Thus, the generation of tissue-specific *Zip14*-knockout mice provides clearer answers. ZIP14 is highly expressed in the liver and duodenum, which suggests that the dysfunction of ZIP14 prevents hepatic or intestinal Mn uptake from the blood, leading to reduced biliary Mn excretion and elevated Mn accumulation in the blood and brain. While liver-specific *Zip14*-knockout mice (carrying Alb-*Cre*) did not show Mn accumulation in the brain,[103] intestine-specific *Zip14*-knockout mice (carrying Vill-*Cre*) showed increased Mn accumulation in the brain.[104] These results suggest that basolaterally localized ZIP14 in enterocytes provides a primary excretion route for the blood Mn. Because ZNT10 is expressed at the luminal side of the enterocytes, as discussed above, the blood Mn may be eliminated into the intestinal lumen via cooperative functions of ZIP14 at the basolateral membrane and of ZNT10 at the luminal membrane of the enterocytes (Fig. 4). Because ZNT10 is also expressed at the apical membranes of hepatocytes, a similar Mn elimination system may be operative in the liver (Fig. 4). However, the intestinal Mn elimination system may become more critical than the hepatobiliary Mn elimination system when excess Mn exists in the body.

Infants with mutations in *SLC39A8/ZIP8* were reported to show symptoms of congenital disorders of glycosylation and had very low blood Mn levels.[96,97] Because galactosyltransferase, a critical enzyme for adding galactose to sugar chains, is dependent on Mn, Mn deficiency due to *SLC39A8/ZIP8* mutations was believed to lead to whole-body galactosylation disorders. Whole-genome sequencing of these patients revealed mutations at two locations in *SLC39A8/ZIP8*. One patient had double mutations at G38R and I340N, and another patient had double mutations at V33M and S335Y. These mutations are assumed to be located in the extracellular N-terminal domain (V33, G38) and TM5 (S335, I340).[105] Mn uptake by the double-mutated ZIP8 (G38R, I340N) ectopically expressed in HeLa cells was markedly lower than that by the wild-type ZIP8.[106] However, the effect of each mutation remains to be elucidated.

In inherited Zn or copper (Cu) deficiency, a mutation in *SLC39A4/ZIP4* leads to systemic Zn deficiency called acrodermatitis enteropathica, and a mutation in the Cu transporter, ATP7A, leads to systemic Cu deficiency called Menkes disease,[107,108] both of which are attributed to reduced intestinal Zn or Cu absorption. In contrast, the mechanisms underlying ZIP8 mutation-induced Mn deficiency may not be caused by reduced intestinal Mn absorption. In an excellent genetic study, it was clearly shown that Zip8 at the canalicular membrane recovers a part of Mn excreted in the bile, and that the loss of Zip8 causes disordered recovery of Mn from the bile, leading to dyshomeostasis of Mn in the body.[109] In this study, Mn levels were found to be decreased in the liver, kidney, brain, and blood of both tamoxifen-inducible whole-body *Zip8*-knockout mice, and liver-specific *Zip8*-knockout mice (carrying Alb-*Cre*).[109] Moreover, biliary Mn levels were increased and decreased with the liver-specific knockout and overexpression of Zip8, respectively.[109] Thus, a Mn reabsorption mechanism is crucial for maintaining systemic Mn homeostasis (Fig. 4).

In addition to the liver and intestine, kidney is another principal organ that controls the excretion and reabsorption of trace elements. In the kidney, ZIP8 is highly expressed in the boundary region between the cortex and outer medulla where the S3 segment of the proximal tubules is abundant.[14] Using the S1, S2, and S3 segment-derived immortalized cells cultured in the Transwell plates, Mn was efficiently absorbed from the apical side into S3 cells,[110] where ZIP8 is highly expressed, suggesting that some of the glomerulus-filtered Mn may be reabsorbed by ZIP8 at the S3 segment of proximal tubules in the kidney, providing another route of Mn recovery in the body. In support of this hypothesis, a clinical study showed that high amounts of Mn were excreted in the urine of ZIP8-mutated patients receiving high doses of Mn for therapy.[111] Further studies are required to elucidate the contribution of ZIP8 to the reabsorption of Mn in proximal tubule systems.

Genome-wide association studies have revealed that the SNP, rs13107325, in *SLC39A8/ZIP8* is associated with multiple clinical phenotypes. The related diseases and conditions include schizophrenia,[112–114] hypotension,[115,116] obesity,[117,118] decreased HDL cholesterol levels,[119,120] acute coronary syndrome (ACS),[121] and Crohn's disease.[122] The SNP, rs13107325, which has a frequency of more than 5% in Europe, causes a missense mutation, A391T, at a location close to TM7. However, the effects of the A391T mutation on the Mn transporting ability of ZIP8 and its association with a broad range of diseases remain elusive. Human carriers of rs13107325 were shown to have lower plasma Mn concentrations than non-carriers, but the extent of this decrease in concentration was only about 10%–20%.[76,123] Ectopic expression of A391T mutant ZIP8 in cultured cells resulted in minimal changes in Mn uptake in HEK293 cells,[123] whereas it caused a decrease in Cd uptake in HEK293 cells,[124] and in Zn uptake in CHO cells.[125] Recently, two groups have generated knock-in mice with the A391T mutant ZIP8.[74,75] In both these studies, the A391T mutation of ZIP8 resulted in lowered Mn levels in the intestine, leading to impaired intestinal barrier function and symptoms similar to those of Crohn's disease. These results suggest that the alteration in Mn transport by the A391T mutation of ZIP8 is involved at least in Crohn's disease. Further studies are required to elucidate the relationship between ZIP8 SNP mutations and the development of a wide range of diseases.

Congenital disorders related to ZNT10, ZIP14, and ZIP8 in humans have led to an understanding of the tissue-specific roles of these Mn transporters, including Mn excretion by ZNT10 and ZIP14,

or Mn reabsorption by ZIP8. Further mechanistic studies on the physiological and pathological functions of ZIP8, ZIP14, and ZNT10 will provide a complete understanding of the regulatory mechanisms of systemic and cellular Mn homeostasis.

1.4. Perspectives

Accumulating evidence has helped clarify the importance of Zn transporters in cellular and systemic Mn metabolism through the mobilization of Mn across the cellular membrane. Simultaneously, new questions have arisen about their functions. Can these transporters flexibly discriminate between Zn and Mn? Can their Mn transport activity be flexibly regulated? Considering the properties of ZNT and ZIP transporters, it would be interesting to consider the possibility that heterodimerization may occur between ZNT10 and other ZNT proteins, or between ZIP8 or ZIP14 and other ZIP proteins to control their Mn/Zn transport activity. ZNT10 has been shown to heterodimerize with ZNT3,[126] although this heterodimerization needs to be investigated from the perspective of Mn pathophysiology. Answers to these questions need to be obtained for a understanding of Mn homeostasis.

The impairment of Mn homeostasis results in various diseases. Genetic variation associated with Mn homeostasis likely leads to a variety of symptoms and risk of diseases. Dietary Mn deficiency is unlikely to occur in a normal individual; thus, an excess of Mn, which may be associated with both rare and common neurodegenerative disorders,[127] need to be intensively examined in the future. In patients with parkinsonism caused by mutations in *ZNT10* and *ZIP14*, chelation therapy with disodium calcium edetate is reported to show significant improvement,[18,20,95] along with supplementation of iron, which is a competitive inhibitor of intestinal Mn uptake. Thus, compounds that reduce Mn toxicity may lead to the discovery and development of drugs for a number of human diseases associated with altered Mn homeostasis. The recent emergence of several small molecule inhibitors of ZIP transporters[128,129] show the high possibility for novel pharmacological applications by directly targeting ZNT10, ZIP8, and ZIP14 in human diseases associated with these transporters. However, these inhibitors have not yet been used in animal studies or in human patients. Considering ZNT10, ZIP8, and ZIP14 are expressed on the cell surface, an antibody–drug conjugate may be useful. In this regard, an antibody–drug conjugate consisting of an anti-ZIP6 humanized monoclonal antibody and a microtubule-disrupting agent provides useful information because it showed the efficacy in both *in vitro* and *in vivo* antitumor activity.[130] It is needless to say that molecular mechanisms underlying the association of ZNT10, ZIP8, and ZIP14 with Zn and Mn metabolism should be clarified for potential pharmacological.

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Declaration of competing interest

Both authors declare no competing financial and non-financial interests.

References

1. Aschner M, Vrana KE, Zheng W. Manganese uptake and distribution in the central nervous system (CNS). *Neurotoxicology*. 1999;20(2–3):173–180.
2. Post JE. Manganese oxide minerals: crystal structures and economic and environmental significance. *Proc Natl Acad Sci U S A*. 1999;96(7):3447–3454.
3. Bowman AB, Erikson KM, Aschner M. In: Huang S, ed. *Manganese - the two faces of essentiality and neurotoxicity. Metals and neurodegeneration*. Kerala, India: Research Signpost; 2010.
4. Christianson DW. Structural chemistry and biology of manganese metalloenzymes. *Prog Biophys Mol Biol*. 1997;67(2–3):217–252.
5. Couper J. On the effects of black oxide of manganese when inhaled into the lungs. *Br Ann Med Pharmacol*. 1837;1(1):41–42.
6. Nelson K, Golnick J, Korn T, Angle C. Manganese encephalopathy: utility of early magnetic resonance imaging. *Br J Ind Med*. 1993;50(6):510–513.
7. Kawata F. [Studies on monoamine metabolism in the rat brain with overdosage of manganese]. *Nihon Hoigaku Zasshi*. 1990;44(2):137–146.
8. Racette BA. Manganism in the 21st century: the Hanninen lecture. *Neurotoxicology*. 2014;45:201–207.
9. Schroeder HA, Balassa JJ, Tipton IH. Essential trace metals in man: manganese. A study in homeostasis. *J Chron Dis*. 1966;19(5):545–571.
10. Greenberg DM, Campbell WW. Studies in mineral metabolism with the aid of induced radioactive isotopes: IV-manganese. *Proc Natl Acad Sci U S A*. 1940;26(7):448–452.
11. Pollack S, George JN, Reba RC, Kaufman RM, Crosby WH. The absorption of nonferrous metals in iron deficiency. *J Clin Invest*. 1965;44:1470–1473.
12. Gunshin H, Mackenzie B, Berger UV, et al. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature*. 1997;388(6641):482–488.
13. Fujishiro H, Doi M, Enomoto S, Himeno S. High sensitivity of RBL-2H3 cells to cadmium and manganese: an implication of the role of ZIP8. *Metall*. 2011;3(7):710–718.
14. Fujishiro H, Yano Y, Takada Y, Tanihara M, Himeno S. Roles of ZIP8, ZIP14, and DMT1 in transport of cadmium and manganese in mouse kidney proximal tubule cells. *Metall*. 2012;4(7):700–708.
15. Fujishiro H, Yoshida M, Nakano Y, Himeno S. Interleukin-6 enhances manganese accumulation in SH-SY5Y cells: implications of the up-regulation of ZIP14 and the down-regulation of ZnT10. *Metall*. 2014;6(4):944–949.
16. Dalton TP, He L, Wang B, et al. Identification of mouse SLC39A8 as the transporter responsible for cadmium-induced toxicity in the testis. *Proc Natl Acad Sci U S A*. 2005;102(9):3401–3406.
17. He L, Girijashanker K, Dalton TP, et al. ZIP8, member of the solute-carrier-39 (SLC39) metal-transporter family: characterization of transporter properties. *Mol Pharmacol*. 2006;70(1):171–180.
18. Quadri M, Federico A, Zhao T, et al. Mutations in SLC30A10 cause parkinsonism and dystonia with hypermanganesemia, polycythemia, and chronic liver disease. *Am J Hum Genet*. 2012;90(3):467–477.
19. DeWitt MR, Chen P, Aschner M. Manganese efflux in Parkinsonism: insights from newly characterized SLC30A10 mutations. *Biochem Biophys Res Commun*. 2013;432(1):1–4.
20. Tuschl K, Clayton PT, Gospe Jr SM, et al. Syndrome of hepatic cirrhosis, dystonia, polycythemia, and hypermanganesemia caused by mutations in SLC30A10, a manganese transporter in man. *Am J Hum Genet*. 2012;90(3):457–466.
21. Nishito Y, Tsuji N, Fujishiro H, et al. Direct comparison of manganese detoxification/efflux proteins and molecular characterization of ZnT10 as a manganese transporter. *J Biol Chem*. 2016;291(28):14773–14787.
22. Mukhopadhyay S. Familial manganese-induced neurotoxicity due to mutations in SLC30A10 or SLC39A14. *Neurotoxicology*. 2018;64:278–283.
23. Winslow JWW, Limesand KH, Zhao N. The functions of ZIP8, ZIP14, and ZnT10 in the regulation of systemic manganese homeostasis. *Int J Mol Sci*. 2020;21(9).
24. Anagianni S, Tuschl K. Genetic disorders of manganese metabolism. *Curr Neurol Neurosci Rep*. 2019;19(6):33.
25. Balachandran RC, Mukhopadhyay S, McBride D, et al. Brain manganese and the balance between essential roles and neurotoxicity. *J Biol Chem*. 2020;295(19):6312–6329.
26. Lu M, Fu D. Structure of the zinc transporter YiiP. *Science*. 2007;317(5845):1746–1748.
27. Lu M, Chai J, Fu D. Structural basis for autoregulation of the zinc transporter YiiP. *Nat Struct Mol Biol*. 2009;16(10):1063–1067.
28. Coudray N, Valvo S, Hu M, et al. Inward-facing conformation of the zinc transporter YiiP revealed by cryoelectron microscopy. *Proc Natl Acad Sci U S A*. 2013;110(6):2140–2145.
29. Gupta S, Chai J, Cheng J, D'Mello R, Chance MR, Fu D. Visualizing the kinetic power stroke that drives proton-coupled zinc(II) transport. *Nature*. 2014;512(7512):101–104.
30. Lopez-Redondo ML, Coudray N, Zhang Z, Alexopoulos J, Stokes DL. Structural basis for the alternating access mechanism of the cation diffusion facilitator YiiP. *Proc Natl Acad Sci U S A*. 2018;115(12):3042–3047.
31. Xue J, Xie T, Zeng W, Jiang Y, Bai X-C. Cryo-EM structures of human ZnT8 in both outward- and inward-facing conformations. *eLife*. 2020;9.

32. Fukue K, Itsumura N, Tsuji N, et al. Evaluation of the roles of the cytosolic N-terminus and His-rich loop of ZNT proteins using ZNT2 and ZNT3 chimeric mutants. *Sci Rep*. 2018;8(1):14084.
33. Kambe T, Taylor KM, Fu D. Zinc transporters and their functional integration in mammalian cells. *J Biol Chem*. 2021;296:100320.
34. Suzuki E, Ogawa N, Takeda TA, et al. Detailed analyses of the crucial functions of Zn transporter proteins in alkaline phosphatase activation. *J Biol Chem*. 2020;295(17):5669–5684.
35. Ohana E, Hoch E, Keasar C, et al. Identification of the Zn2+ binding site and mode of operation of a mammalian Zn2+ transporter. *J Biol Chem*. 2009;284(26):17677–17686.
36. Shusterman E, Beharier O, Shiri L, et al. ZnT-1 extrudes zinc from mammalian cells functioning as a Zn(2+)/H(+) exchanger. *Metall*. 2014;6(9):1656–1663.
37. Wei Y, Fu D. Selective metal binding to a membrane-embedded aspartate in the Escherichia coli metal transporter YiiP (FieF). *J Biol Chem*. 2005;280(40):33716–33724.
38. Fujimoto S, Itsumura N, Tsuji T, et al. Cooperative functions of ZnT1, metallothionein and ZnT4 in the cytoplasm are required for full activation of TNAP in the early secretory pathway. *PLoS One*. 2013;8(10), e77445.
39. Tsuji T, Kurokawa Y, Chiche J, et al. Dissecting the process of activation of cancer-promoting zinc-requiring ectoenzymes by zinc metalation mediated by ZNT transporters. *J Biol Chem*. 2017;292(6):2159–2173.
40. Gustin JL, Zanis MJ, Salt DE. Structure and evolution of the plant cation diffusion facilitator family of ion transporters. *BMC Evol Biol*. 2011;11:76.
41. Pedas P, Schiller Stokholm M, Hegelund JN, Ladegard AH, Schjoerring JK, Husted S. Golgi localized barley MTP8 proteins facilitate Mn transport. *PLoS One*. 2014;9(12), e113759.
42. Ueno D, Sasaki A, Yamaji N, et al. A polarly localized transporter for efficient manganese uptake in rice. *Native Plants*. 2015;1:15170.
43. Tsunemitsu Y, Genga M, Okada T, et al. A member of cation diffusion facilitator family, MTP11, is required for manganese tolerance and high fertility in rice. *Planta*. 2018;248(1):231–241.
44. Leyva-Illades D, Chen P, Zogzas CE, et al. SLC30A10 is a cell surface-localized manganese efflux transporter, and parkinsonism-causing mutations block its intracellular trafficking and efflux activity. *J Neurosci*. 2014;34(42):14079–14095.
45. Kambe T, Suzuki E, Komori T. In: Fukada T, Kambe T, eds. *Zinc transporters-A review and a new view from biochemistry. Zinc Signaling*. 2nd ed. Springer-Nature; 2019:23–56.
46. Kambe T, Tsuji T, Fukue K. In: Fukada T, Kambe T, eds. *Zinc transport proteins and zinc signaling. Zinc signals in cellular functions and disorders*. Tokyo: Springer; 2014:27–53.
47. Palmiter RD, Findley SD. Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc. *EMBO J*. 1995;14(4):639–649.
48. Nishito Y, Kambe T. Zinc transporter 1 (ZNT1) expression on the cell surface is elaborately controlled by cellular zinc levels. *J Biol Chem*. 2019;294:15686–15697.
49. Levy M, Elkoshi N, Barber-Zucker S, et al. Zinc transporter 10 (ZnT10)-dependent extrusion of cellular Mn2+ is driven by an active Ca2+-coupled exchange. *J Biol Chem*. 2019;294(15):5879–5889.
50. Udayalaxmi S, Gangula MR, Ravikiran K, Ettaiah P. Investigation of manganese metal coordination in proteins: a comprehensive PDB analysis and quantum mechanical study. *Struct Chem*. 2020;31(3):1057–1064.
51. Martin JE, Giedroc DP. Functional determinants of metal ion transport and selectivity in paralogous cation diffusion facilitator transporters CzcD and MntE in Streptococcus pneumoniae. *J Bacteriol*. 2016;198(7):1066–1076.
52. Zogzas CE, Aschner M, Mukhopadhyay S. Structural elements in the transmembrane and cytoplasmic domains of the metal transporter SLC30A10 are required for its manganese efflux activity. *J Biol Chem*. 2016;291(31):15940–15957.
53. Hu J. Toward unzipping the ZIP metal transporters: structure, evolution, and implications on drug discovery against cancer. *FEBS J*. 2020;288(20):5805–5825.
54. Aydemir TB, Cousins RJ. The multiple faces of the metal transporter ZIP14 (SLC39A14). *J Nutr*. 2018;148(2):174–184.
55. Taylor KM, Nicholson RI. The LZT proteins; the LIV-1 subfamily of zinc transporters. *Biochim Biophys Acta*. 2003;1611(1–2):16–30.
56. Antala S, Ovchinnikov S, Kamisetty H, Baker D, Dempski RE. Computation and functional studies provide a model for the structure of the zinc transporter hZIP4. *J Biol Chem*. 2015;290(29):17796–17805.
57. Zhang T, Liu J, Fellner M, Zhang C, Sui D, Hu J. Crystal structures of a ZIP zinc transporter reveal a binuclear metal center in the transport pathway. *Sci Adv*. 2017;3(8), e1700344.
58. Zhang T, Sui D, Zhang C, Cole L, Hu J. Asymmetric functions of a binuclear metal center within the transport pathway of a human zinc transporter ZIP4. *Faseb J : official publication of the Federation of American Societies for Experimental Biology*. 2020;34(1):237–247.
59. Eide DJ. Zinc transporters and the cellular trafficking of zinc. *Biochim Biophys Acta*. 2006;1763(7):711–722.
60. Gupta S, Merriman C, Petzold CJ, Ralston CY, Fu D. Water molecules mediate zinc mobility in the bacterial zinc diffusion channel ZIPB. *J Biol Chem*. 2019;294(36):13327–13335.
61. Bin BH, Fukada T, Hosaka T, et al. Biochemical characterization of human ZIP13 protein: a homo-dimerized zinc transporter involved in the spondylocheiro dysplastic Ehlers-Danlos syndrome. *J Biol Chem*. 2011;286(46):40255–40265.
62. Lin W, Chai J, Love J, Fu D. Selective electrodiffusion of zinc ions in a Zrt-, Irt-like protein, ZIPB. *J Biol Chem*. 2010;285(50):39013–39020.
63. Taylor KM, Muraina IA, Brethour D, et al. Zinc transporter ZIP10 forms a heteromer with ZIP6 which regulates embryonic development and cell migration. *Biochem J*. 2016;473(16):2531–2544.
64. Gaither LA, Eide DJ. Functional expression of the human hZIP2 zinc transporter. *J Biol Chem*. 2000;275(8):5560–5564.
65. Girijashanker K, He L, Soleimani M, et al. Slc39a14 gene encodes ZIP14, a metal/bicarbonate symporter: similarities to the ZIP8 transporter. *Mol Pharmacol*. 2008;73(5):1413–1423.
66. Franz MC, Pujol-Gimenez J, Montalbetti N, et al. Reassessment of the transport mechanism of the human zinc transporter SLC39A2. *Biochemistry*. 2018;57(26):3976–3986.
67. Aydemir TB, Liuzzi JP, McClellan S, Cousins RJ. Zinc transporter ZIP8 (SLC39A8) and zinc influence IFN-gamma expression in activated human T cells. *J Leukoc Biol*. 2009;86(2):337–348.
68. Guthrie GJ, Aydemir TB, Troche C, Martin AB, Chang SM, Cousins RJ. Influence of ZIP14 (slc39A14) on intestinal zinc processing and barrier function. *Am J Physiol Gastrointest Liver Physiol*. 2015;308(3):G171–G178.
69. Jenkitkasemwong S, Wang CY, Mackenzie B, Knutson MD. Physiologic implications of metal-ion transport by ZIP14 and ZIP8. *Biometals*. 2012;25(4):643–655.
70. Nebert DW, Galvez-Peralta M, Hay EB, et al. ZIP14 and ZIP8 zinc/bicarbonate symporters in Xenopus oocytes: characterization of metal uptake and inhibition. *Metall*. 2012;4(11):1218–1225.
71. Wang C-Y, Jenkitkasemwong S, Duarte S, et al. ZIP8 is an iron and zinc transporter whose cell-surface expression is up-regulated by cellular iron loading. *J Biol Chem*. 2012;287(41):34032–34043.
72. Kambe T, Tsuji T, Hashimoto A, Itsumura N. The physiological, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism. *Physiol Rev*. 2015;95(3):749–784.
73. Pickrell JK, Berisa T, Liu JZ, Segurel L, Tung JY, Hinds DA. Detection and interpretation of shared genetic influences on 42 human traits. *Nat Genet*. 2016;48(7):709–717.
74. Nakata T, Creasey EA, Kadoki M, et al. A missense variant in SLC39A8 confers risk for Crohn's disease by disrupting manganese homeostasis and intestinal barrier integrity. *Proc Natl Acad Sci U S A*. 2020;117(46):28930–28938.
75. Sunuwar L, Frkatovic A, Sharapov S, et al. Pleiotropic ZIP8 A391T implicates abnormal manganese homeostasis in complex human disease. *JCI Insight*. 2020;5(20).
76. Mealer RG, Jenkins BG, Chen C-Y, et al. The schizophrenia risk locus in SLC39A8 alters brain metal transport and plasma glycosylation. *Sci Rep*. 2020;10(1):13162.
77. Matsuura W, Yamazaki T, Yamaguchi-Iwai Y, et al. SLC39A9 (ZIP9) regulates zinc homeostasis in the secretory pathway: characterization of the ZIP subfamily I protein in vertebrate cells. *Biosci Biotechnol Biochem*. 2009;73(5):1142–1148.
78. Lin SJ, Culotta VC. Suppression of oxidative damage by Saccharomyces cerevisiae ATX2, which encodes a manganese-trafficking protein that localizes to Golgi-like vesicles. *Mol Cell Biol*. 1996;16(11):6303–6312.
79. Yamaji T, Hanamatsu H, Sekizuka T, et al. A CRISPR screen using subtilase cytotoxin identifies SLC39A9 as a glycan-regulating factor. *iScience*. 2019;15:407–420.
80. Claro da Silva T, Hiller C, Gai Z, Kullak-Ublick GA. Vitamin D3 transactivates the zinc and manganese transporter SLC30A10 via the Vitamin D receptor. *J Steroid Biochem Mol Biol*. 2016;163:77–87.
81. Ahmad TR, Higuchi S, Bertaggia E, et al. Bile acid composition regulates the manganese transporter Slc30a10 in intestine. *J Biol Chem*. 2020;295(35):12545–12558.
82. Go S, Kurita H, Yokoo K, Inden M, Kambe T, Hozumi I. Protective function of SLC30A10 induced via PERK-ATF4 pathway against 1-methyl-4-phenylpyridinium. *Biochem Biophys Res Commun*. 2017;490(4):1307–1313.
83. Liuzzi JP, Lichten LA, Rivera S, et al. Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozincemia of the acute-phase response. *Proc Natl Acad Sci U S A*. 2005;102(19):6843–6848.
84. Beker Aydemir T, Chang SM, Guthrie GJ, et al. Zinc transporter ZIP14 functions in hepatic zinc, iron and glucose homeostasis during the innate immune response (endotoxemia). *PLoS One*. 2012;7(10), e48679.
85. Lichten LA, Liuzzi JP, Cousins RJ. Interleukin-1beta contributes via nitric oxide to the upregulation and functional activity of the zinc transporter Zip14 (Slc39a14) in murine hepatocytes. *Am J Physiol Gastrointest Liver Physiol*. 2009;296(4):G860–G867.
86. Homma K, Fujisawa T, Tsuburaya N, et al. SOD1 as a molecular switch for initiating the homeostatic ER stress response under zinc deficiency. *Mol Cell*. 2013;52(1):75–86.
87. Zhao N, Zhang AS, Worthen C, Knutson MD, Enns CA. An iron-regulated and glycosylation-dependent proteasomal degradation pathway for the plasma membrane metal transporter ZIP14. *Proc Natl Acad Sci U S A*. 2014;111(25):9175–9180.
88. Thompson KJ, Wessling-Resnick M. ZIP14 is degraded in response to manganese exposure. *Biometals : an international journal on the role of metal ions in biology, biochemistry, and medicine*. 2019;32(6):829–843.

89. Begum NA, Kobayashi M, Moriwaki Y, Matsumoto M, Toyoshima K, Seya T. Mycobacterium bovis BCG cell wall and lipopolysaccharide induce a novel gene, BIGM103, encoding a 7-TM protein: identification of a new protein family having Zn-transporter and Zn-metalloprotease signatures. *Genomics*. 2002;80(6):630–645.
90. Liu MJ, Bao S, Galvez-Peralta M, et al. ZIP8 regulates host defense through zinc-mediated inhibition of NF-kappaB. *Cell Rep*. 2013;3(2):386–400.
91. Kim JH, Jeon J, Shin M, et al. Regulation of the catabolic cascade in osteoarthritis by the zinc-ZIP8-MTF1 axis. *Cell*. 2014;156(4):730–743.
92. Melia JMP, Lin R, Xavier RJ, et al. Induction of the metal transporter ZIP8 by interferon gamma in intestinal epithelial cells: potential role of metal dyshomeostasis in Crohn's disease. *Biochem Biophys Res Commun*. 2019;515(2):325–331.
93. Aiba I, Hossain A, Kuo MT. Elevated GSH level increases cadmium resistance through down-regulation of Sp1-dependent expression of the cadmium transporter ZIP8. *Mol Pharmacol*. 2008;74(3):823–833.
94. Song J, Kim D, Lee CH, Lee MS, Chun CH, Jin EJ. MicroRNA-488 regulates zinc transporter SLC39A8/ZIP8 during pathogenesis of osteoarthritis. *J Biomed Sci*. 2013;20:31.
95. Tuschl K, Meyer E, Valdivia LE, et al. Mutations in SLC39A14 disrupt manganese homeostasis and cause childhood-onset parkinsonism-dystonia. *Nat Commun*. 2016;7:11601.
96. Boycott KM, Beaulieu CL, Kernohan KD, et al. Autosomal-recessive intellectual disability with cerebellar atrophy syndrome caused by mutation of the manganese and zinc transporter gene SLC39A8. *Am J Hum Genet*. 2015;97(6):886–893.
97. Park JH, Hogrebe M, Gruneberg M, et al. SLC39A8 deficiency: a disorder of manganese transport and glycosylation. *Am J Hum Genet*. 2015;97(6):894–903.
98. Klaassen CD. Biliary excretion of manganese in rats, rabbits, and dogs. *Toxicol Appl Pharmacol*. 1974;29(3):458–468.
99. Taylor CA, Hutchens S, Liu C, et al. SLC30A10 transporter in the digestive system regulates brain manganese under basal conditions while brain SLC30A10 protects against neurotoxicity. *J Biol Chem*. 2019;294(6):1860–1876.
100. Mercadante CJ, Prajapati M, Conboy HL, et al. Manganese transporter Slc30a10 controls physiological manganese excretion and toxicity. *J Clin Invest*. 2019;129(12):5442–5461.
101. Papavasiliou PS, Miller ST, Cotzias GC. Role of liver in regulating distribution and excretion of manganese. *Am J Physiol*. 1966;211(1):211–216.
102. Jenkitkasemwong S, Akinyode A, Paulus E, et al. SLC39A14 deficiency alters manganese homeostasis and excretion resulting in brain manganese accumulation and motor deficits in mice. *Proc Natl Acad Sci U S A*. 2018;115(8):E1769–E1778.
103. Xin Y, Gao H, Wang J, et al. Manganese transporter Slc39a14 deficiency revealed its key role in maintaining manganese homeostasis in mice. *Cell Discov*. 2017;3:17025.
104. Aydemir TB, Thorn TL, Ruggiero CH, Pompilus M, Febo M, Cousins RJ. Intestine-specific deletion of metal transporter Zip14 (Slc39a14) causes brain manganese overload and locomotor defects of manganism. *Am J Physiol Gastrointest Liver Physiol*. 2020;318(4):G673–G681.
105. Fujishiro H, Himeno S. New insights into the roles of ZIP8, a cadmium and manganese transporter, and its relation to human diseases. *Biol Pharm Bull*. 2019;42(7):1076–1082.
106. Choi EK, Nguyen TT, Gupta N, Iwase S, Seo YA. Functional analysis of SLC39A8 mutations and their implications for manganese deficiency and mitochondrial disorders. *Sci Rep*. 2018;8(1):3163.
107. Maverakis E, Fung MA, Lynch PJ, et al. Acrodermatitis enteropathica and an overview of zinc metabolism. *J Am Acad Dermatol*. 2007;56(1):116–124.
108. DiDonato M, Sarkar B. Copper transport and its alterations in Menkes and Wilson diseases. *Biochim Biophys Acta*. 1997;1360(1):3–16.
109. Lin W, Vann DR, Doulias PT, et al. Hepatic metal ion transporter ZIP8 regulates manganese homeostasis and manganese-dependent enzyme activity. *J Clin Invest*. 2017;127(6):2407–2417.
110. Fujishiro H, Hamao S, Isawa M, Himeno S. Segment-specific and direction-dependent transport of cadmium and manganese in immortalized S1, S2, and S3 cells derived from mouse kidney proximal tubules. *J Toxicol Sci*. 2019;44(9):611–619.
111. Park JH, Hogrebe M, Fobker M, et al. SLC39A8 deficiency: biochemical correction and major clinical improvement by manganese therapy. *Genet Med*. 2018;20(2):259–268.
112. Carrera N, Arrojo M, Sanjuan J, et al. Association study of nonsynonymous single nucleotide polymorphisms in schizophrenia. *Biol Psychiatr*. 2012;71(2):169–177.
113. Schizophrenia Working Group of the Psychiatric Genomics C. Biological insights from 108 schizophrenia-associated genetic loci. *Nature*. 2014;511(7510):421–427.
114. Hertzberg L, Katsel P, Roussos P, Haroutunian V, Domany E. Integration of gene expression and GWAS results supports involvement of calcium signaling in Schizophrenia. *Schizophr Res*. 2015;164(1–3):92–99.
115. Ehret GB, Munroe PB, Rice KM, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*. 2011;478(7367):103–109.
116. Tragante V, Barnes MR, Ganesh SK, et al. Gene-centric meta-analysis in 87,736 individuals of European ancestry identifies multiple blood-pressure-related loci. *Am J Hum Genet*. 2014;94(3):349–360.
117. Speliotes EK, Willer CJ, Berndt SI, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet*. 2010;42(11):937–948.
118. Berndt SI, Gustafsson S, Magi R, et al. Genome-wide meta-analysis identifies 11 new loci for anthropometric traits and provides insights into genetic architecture. *Nat Genet*. 2013;45(5):501–512.
119. Waterworth DM, Ricketts SL, Song K, et al. Genetic variants influencing circulating lipid levels and risk of coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2010;30(11):2264–2276.
120. Willer CJ, Schmidt EM, Sengupta S, et al. Discovery and refinement of loci associated with lipid levels. *Nat Genet*. 2013;45(11):1274–1283.
121. Johansson A, Eriksson N, Lindholm D, et al. Genome-wide association and Mendelian randomization study of NT-proBNP in patients with acute coronary syndrome. *Hum Mol Genet*. 2016;25(7):1447–1456.
122. Li D, Achkar JP, Haritunians T, et al. A pleiotropic missense variant in SLC39A8 is associated with Crohn's disease and human gut microbiome composition. *Gastroenterology*. 2016;151(4):724–732.
123. Haller G, McCall K, Jenkitkasemwong S, et al. A missense variant in SLC39A8 is associated with severe idiopathic scoliosis. *Nat Commun*. 2018;9(1):4171.
124. Zhang R, Witkowska K, Afonso Guerra-Assunção J, et al. A blood pressure-associated variant of the SLC39A8 gene influences cellular cadmium accumulation and toxicity. *Hum Mol Genet*. 2016;25(18):4117–4126.
125. Tseng WC, Reinhart V, Lanz TA, et al. Schizophrenia-associated SLC39A8 polymorphism is a loss-of-function allele altering glutamate receptor and innate immune signaling. *Transl Psychiatry*. 2021;11(1):136.
126. Zhao Y, Feresin RG, Falcon-Perez JM, Salazar G. Differential targeting of SLC30A10/ZnT10 heterodimers to endolysosomal compartments modulates EGF-induced MEK/ERK1/2 activity. *Traffic*. 2016;17(3):267–288.
127. Hozumi I, Hasegawa T, Honda A, et al. Patterns of levels of biological metals in CSF differ among neurodegenerative diseases. *J Neurol Sci*. 2011;303(1–2):95–99.
128. Nolin E, Gans S, Llamas L, et al. Discovery of a ZIP7 inhibitor from a Notch pathway screen. *Nat Chem Biol*. 2019;15(2):179–188.
129. Pujol-Gimenez J, Poirier M, Buhlmann S, et al. Inhibitors of human divalent metal transporters DMT1 (SLC11A2) and ZIP8 (SLC39A8) from a GDB-17 fragment library. *ChemMedChem*. 2021;16(21):3306–3314.
130. Sussman D, Smith LM, Anderson ME, et al. SGN-LIV1A: a novel antibody-drug conjugate targeting LIV-1 for the treatment of metastatic breast cancer. *Mol Cancer Therapeut*. 2014;13(12):2991–3000.
131. Fujishiro H, Himeno S. Roles of zinc transporters that control the essentiality and toxicity of manganese and cadmium. *Yakugaku Zasshi*. 2021;141:695–703.