J BIOCHEM MOLECULAR TOXICOLOGY
Volume 22, Number 5, 2008

Manganese Accumulation in the Mouse Ear Following Systemic Exposure

Ci Ma,[1] Scott N. Schneider,[1] Marian Miller,[1] Daniel W. Nebert,[1] Caroline Lind,[1] Sandy M. Roda,[1] Scott E. Afton,[2] Joseph A. Caruso,[2] and Mary Beth Genter[1]

[1]Department of Environmental Health, University of Cincinnati, Cincinnati, OH 45267-0056, USA; E-mail: *Marybeth.genter@uc.edu*

[2]Department of Chemistry, University of Cincinnati, Cincinnati, OH 45267-0056, USA

Received 11 April 2008; revised 1 May 2008; accepted 7 May 2008

ABSTRACT: There is evidence in human populations that exposure to manganese (Mn), or Mn in combination with excessive noise exposure, results in hearing loss. Quantitative reverse-transcriptase polymerase chain reaction revealed expression of the metal transporters DMT1, ZIP8, and ZIP14 in control mouse ears. ZIP8 is known to have a high affinity ($K_m = 2.2$ μM) for Mn transport, and ZIP8 protein was localized to the blood vessels of the ear by immunohistochemistry. We treated mice (strains C57BL/6J and DBA/2J) with Mn (100 mg/kg $MnCl_2$, by subcutaneous injection, on three alternating days), and Mn was significantly elevated in the ears of the treated mice. Mn concentrations remained elevated over controls for at least 2 weeks after treatment. These studies demonstrate that metal transporters are present in the mouse ear and that Mn can accumulate in the ear following systemic exposure. Future studies should focus on whether Mn exposure is associated with hearing deficits. © 2008 Wiley Periodicals, Inc. J Biochem Mol Toxicol 22:305–310, 2008; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10:1002/jbt.20241

KEYWORDS: **Manganese; Mouse; Ear; ZIP8; *Slc39a8*; *Slc39a14*; *Dmt1***

INTRODUCTION

Manganese (Mn) is an essential element that can be toxic both as a result of dietary deficiency and as a consequence of excessive exposure via drinking water or occupational exposure [1,2]. There are reports of hearing loss in welders exposed to Mn, as well as in individuals with concomitant exposures to noise and Mn [3–8]; in addition, a recent publication demonstrated dose-related decreases in the acoustic startle response in Mn-treated rats [9].

Dietary Mn deficiency in animals results in a wide variety of structural and physiological defects, including growth retardation, skeletal and cartilage malformations, impaired reproductive function, congenital ataxia due to abnormal inner ear development, optic nerve abnormalities, impaired insulin metabolism, abnormal glucose tolerance, and an impaired oxidant defense system [1]. Excessive exposure to Mn has been recognized for decades to be associated with a Parkinsonian-like state in humans, with signs including abnormal gait, altered balance, exaggerated reflexes in the lower limbs, tremors in the upper limbs, and masked facies [10,11]. Whereas consumption of drinking water containing high levels of Mn has been suggested as a cause of neurological deficits and Parkinsonian symptoms [2], occupational exposures are by far of greater concern. Because of the essential nature of Mn in the development of various sensory systems, including the ear and the eye [12,13], we hypothesized that one or more metal transporters may be responsible for the transport of Mn into these tissues.

Slc39a8 has recently been identified as the gene that confers susceptibility to cadmium (Cd)-induced testicular necrosis and acute renal failure in mice [14]. The mouse *Slc39a8* gene encodes the metal transporter ZIP8, which shows 96% amino acid identity to the human *SLC39A8* gene product—confirming a high degree of evolutionary conservation of this gene during the past 65 million years. Inbred mice that are susceptible to Cd-induced testicular necrosis, such as the DBA/2J and 129/SvJ strains, express ZIP8 at high levels in testicular endothelial cells; on the contrary, mice such

Ci Ma and Scott N. Schneider contributed equally to the studies described in this manuscript.

Correspondence to: Mary Beth Genter.

Contract Grant Sponsor: National Institutes of Health.

Contract Grant Number: T42/OH008432-01 (to Scott N. Schneider and Mary Beth Genter), ES010416 (Daniel W. Nebert), and ES06096 (Daniel W. Nebert and Mary Beth Genter).

© 2008 Wiley Periodicals, Inc.

as the C57BL/6J and A/J strains that are resistant to Cd-induced testicular necrosis express negligible amounts of ZIP8 in testicular endothelial cells [15]. Competition studies revealed that Mn is the most potent inhibitor of Cd transport by ZIP8, and the K_m for Mn transport (2.2 μM) is very low and close to that for Cd (0.62 μM). Therefore, ZIP8 is also a Mn transporter, and we believe that Mn is likely to be the normal physiological substrate for ZIP8. Further analysis suggests that ZIP8 is a Mn^{2+}/HCO_3^- symporter [16], transporting two anions for each cation and therefore is electroneutral [17].

While it appears that the physiological substrate for ZIP8 is Mn, ZIP8 is also clearly a "rogue" transporter of Cd [15,16]. ZIP8 is localized to the apical surfaces of all polarized endothelial and epithelial cells in which it is expressed [16]. ZIP8 transcript levels between susceptible and resistant mouse strains do not differ either by quantitative PCR or by Northern blot analysis in the testis or in any other tissues examined—rather, it is the specific cellular localization in the testicular vascular endothelial cells in the DBA/2J mice that confers the genetically distinct susceptibility to Cd-induced testicular necrosis [14].

METHODS

Animals and Treatment

Female mice [inbred strains C57BL/6J (B6) and DBA/2J (D2)] were purchased from Jackson Labs (Bar Harbor, ME) at approximately 7 weeks of age. Tissues were isolated from four mice of each strain for quantitative polymerase chain reaction (PCR) following carbon dioxide asphyxiation. Tissues (ears, ethmoid turbinates, and kidneys) were frozen at −80°C until processing. For Mn dosing, B6 and D2 mice ($n = 4$ per treatment) received $MnCl_2$ or distilled water by subcutaneous injection (in the interscapular area), as previously described [18]. In brief, mice were treated with 100 mg/kg $MnCl_2$ dissolved in water with a final volume of 100 μL/20 g b.w. Mice received three doses on alternating days and were sacrificed 24 h after the last dose. All mice were euthanized with an overdose of carbon dioxide, and ear tissues were dissected, frozen on dry ice, and stored at −80°C until analysis for Mn content.

Metal Transporter Gene Expression

Tissues (bony otic capsule [dissected free of surrounding tissue], olfactory epithelium, and kidney) were homogenized in TriReagent (Molecular Research Center, Cincinnati, OH) at 1 mL/50 mg of tissue using a polytron-type homogenizer, and RNA was isolated per the manufacturer's protocol. RNA concentration was analyzed using a Nanodrop 1200 detection system, and RNA integrity was analyzed using an Agilent bioanalyzer. cDNA was synthesized using the reverse-iT (AbGene) kit using oligo-dT primers and 1 μg of total RNA. Real-time quantitative PCR (Q-PCR) was performed using a DNA engine Opticon-2 real-time PCR detection system (MJ Research, Waltham, MA). Samples were prepared as 20-μL volume reactions with 1 μL of cDNA added to 7 μL of water, 1 μL of each primer (prepared as 10 μM solutions), and 10 μL of iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA) for each sample. The primer sets used were

ZIP8
5′-CTCGCCTTCAGTGAGGATGT-3′
5′-GCTTTGCGTTGTGCTTTCTT-3′
ZIP14
5′–TCTGGAGACCTCTTTGCGG–3′
5′–ACAGGGCGATGAAGTAAGTG–3′

DMT1
5′-TCAGAGCTCCACCATGACTG-3′
5′-TGTGAACGTGAGGATGGGTA-3′
β-actin
5′-CATCCGTAAAGACCTCTATGCC-3′
5′-ACGCAGCTCAGTAACAGTCC-3′

Immunohistochemistry

Immunohistochemistry was performed using an anti-ZIP8 antibody [14] and the Renaissance TSA biotin system kit (Perkin Elmer, Waltham, MA). Paraffin-embedded tissue sections were deparaffinized, rehydrated through a graded series of ethanol dilutions, and blocked with the TSA biotin blocking reagent, prepared in 10 mM phosphate-buffered saline containing 0.1% Tween 20 (PBST) for 20 min at room temperature in a humidified chamber. Sections were then incubated with anti-ZIP8 antibody (1/500) for 2 h at room temperature or at 4°C overnight. Following washes in PBST, sections were sequentially incubated in horseradish peroxidase (HRP)-conjugated antirabbit secondary antibody (1:100; Dako, Carpinteria, CA), the biotin tyramide reagent (1:50; supplied with the kit), and finally with streptavidin-HRP reagent (1:100, supplied with the kit). Slides were washed in PBST, followed by three changes of water, and allowed to dry nearly completely. Antibody binding was visualized using 3-amino-9-ethyl carbazole (AEC) as follows: thirty-five microliters of AEC stock solution (0.4 g dissolved in 100 mL dimethylformamide) was combined with 500 μL of sodium acetate (0.1 M, pH 5.2) and filtered (Amicon Ultrafree-MC centrifugal filter). After

addition of 1 μL of fresh 30% hydrogen peroxide, the chromagen solution was incubated on tissue sections for 20–30 min, allowing development of the reddish-brown reaction product. Slides were rinsed in water, and mounted in an aqueous mounting medium (Fluoromount). Photomicrographs were acquired using a Nikon E400 light microscope with a Nikon D80 camera body.

Manganese Concentration in Ears

Mn Trial Experiment

To establish that Mn accumulates in the ear following systemic exposure, ears [otic capsules, isolated as described earlier] from mice sacrificed 24 h after three Mn treatments were analyzed by graphite furnace atomic absorption spectroscopy. Briefly, ears (both ears from each mouse comprised a single sample) were dried to a constant weight in a dessicant box at room temperature. Samples were then submerged in approximately 2 mL of trace metal grade nitric acid and digested at 125°C (approximately 30 min). Analysis was performed using a graphite furnace 5100 zl (Perkin Elmer), and results were interpreted with the AA Winlab version 3.2 software. Instrument parameters were as follows: Wavelength: 279.5 nm; read time: 5.0 s; slit width: 0.2; read delay: 0.0; BOC time: 2 s; signal type: Zeeman AA; signal measurement: peak area. A reagent blank and two spiked reagent samples were prepared for each set of samples. Mn concentrations were interpolated from a standard curve. All results were reported in μg/g tissue. Recoveries were calculated as follows: result/sample + spike × 100. All recoveries and internal quality control samples were within ±10% of the actual value.

Persistence of Mn in the Ear

An additional set of mice was dosed with Mn as described earlier, and sacrificed 14 days later to ascertain whether Mn persists in the ear. Because the core facility that analyzed our initial samples (earlier) closed, we sought additional collaborators to analyze the second set of samples. This analysis was performed by inductively coupled plasma mass spectrometry (ICP-MS). All solutions were prepared in double-distilled (18 MΩ cm) deionized water (Sybron Barnstead, Boston, MA). Metal-free polypropylene tubes for sample preparation and storage were purchased from CPI International (Santa Rosa, CA). The Mn elemental standard used for quantification was acquired from SpexCertiPrep (Metuchen, NJ). Calibration standards of 1.0–500 μg/L were prepared through dilution from a stock solution with 5% v/v HNO_3 (Pharmaco, Hartford, CT).

Briefly, the dried ear tissues (16–40 mg) were placed in septum-sealed glass tubes, treated with 1.0 mL of 25% (v/v) HNO_3 and subjected to first stage of microwave digestion [CEM intelligent explorer/discover system (CEM Corporation, Mathews, NC)]. The microwave was equipped with a 24-vial autosampler, a self-contained microwave chamber, and allowed for simultaneous monitoring of wattage, temperature, and pressure. Digestion parameters were as follows: power, 125 W; ramp, 2:00 min; hold, 2:00 min; temperature, 110°C. Subsequently, 0.1 mL of 30% H_2O_2 was added and the samples were processed through the second stage: power, 125 W; ramp, 2:00 min; hold, 2:00 min; temperature, 165°C. Following the microwave digestion sequence, the resulting solutions were diluted with double-distilled water to 5 mL and analyzed in continuous flow mode by ICP-MS (Agilent 7500ce, Agilent Technologies, Tokyo, Japan). The instrument was equipped with a glass expansion microconcentric nebulizer, a Scott double-channel spray chamber (2°C), a shielded torch with a sampling depth of 7 mm, nickel sampling and skimmer cones, an octopole ion guide enclosed in a collision/reaction cell with hydrogen (Matheson Gas Products, Parisppany, NJ) pressurization (purity of 99.999%), a quadrupole mass analyzer with a dwell time of 100 ms per isotope, and an electron multiplier for detection. Instrumental parameters were as follows: forward power, 1500 W; plasma gas flow rate, 15.0 L min^{-1}; auxiliary gas flow rate, 1.0 L min^{-1}; carrier gas flow rate, 0.99 L min^{-1}; makeup gas flow rate, 0.14 L min^{-1}; monitored isotope, ^{55}Mn.

Data Analysis

Mn concentrations were compared between treated and control mice of each strain by Student's *t*-test.

RESULTS

Gene Expression

Slc39a14 is evolutionarily the closest neighbor to *Slc39a8*, and the encoded proteins, ZIP14 and ZIP8, have been shown to transport a number of divalent metal ions, including iron, cadmium, and Mn. *Slc11a2* encodes DMT1, a proton-coupled transporter of several metal divalent cations. Therefore, we performed quantitative RT-PCR to compare the expression of ZIP8, ZIP14, and DMT1 in the ear to that in the olfactory epithelium (where DMT1 has previously been described) and in kidney (previously been shown to express ZIP8) [16,19]. As shown in Table 1, C_t values for ZIP8 and DMT1 were comparable in D2 and B6 mouse ears, whereas ZIP14 expression is considerably lower (as

TABLE 1. Metal Transporter mRNA Levels in Ear, Olfactory Epithelium (OE), and Kidney of D2 and B6 Mice

Strain and Tissue	C_t *ZIP8*	C_t *ZIP14*	C_t *DMT1*	C_t β-*Actin*
D2 ear	21.9 ± 0.9	25.1 ± 0.2	19.9 ± 0.4	14.7 ± 0.2
B6 ear	21.7 ± 0.6	25.0 ± 0.1	20.0 ± 0.5	15.2 ± 0.4
D2 OE	19.7 ± 0.4	23.2 ± 0.2	17.1 ± 0.7	12.2 ± 0.3
B6 OE	19.4 ± 0.9	22.9 ± 0.2	17.0 ± 0.4	14.1 ± 0.1
D2 kidney	18.1	18.4	17.4	13.7
B6 kidney	15.2	18.8	17.4	13.7

$N = 4$ for ear and olfactory epithelium; $N = 1$ for kidney samples.
C_t values are expressed as means ± SEM.

indicated by a higher C_t value, indicating that the low transcript level necessitated a higher number of PCR cycles to attain the threshold level).

Immunohistochemistry

ZIP8 immunohistochemistry revealed immunoreactivity in blood vessel walls throughout the ear (Figure 1). While the stria vascularis was negative for ZIP8 immunoreactivity, the underlying spiral ligament, as well as several larger blood vessels, showed strong immunoreactivity.

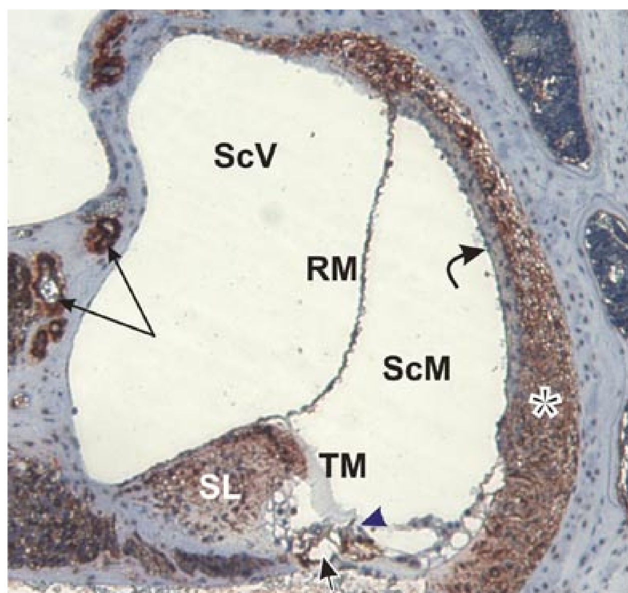

FIGURE 1. Frontal section taken through the ear of a D2 mouse, labeled as follows: single arrowhead: outer hair cells; curved arrow: stria vascularis; short black arrow: tunnel of Corti; ScM: scala media (cochlear duct); ScV: scala vestibuli; RM: Reissner's membrane. Note that the stria vascularis itself is negative for ZIP8 immunoreactivity, but the underlying spiral ligament stains strongly (*). In addition, note positive immunoreactivity in the spiral limbus (SL) and the walls of large blood vessels (double arrow).

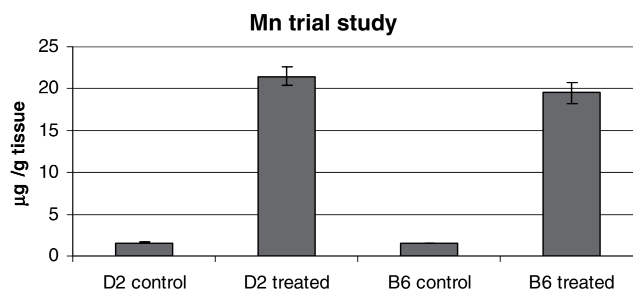

FIGURE 2. Trial study to determine whether Mn accumulates in the ears of Mn-treated mice. Mice were treated with 100 mg/kg $MnCl_2$ or saline vehicle on three alternating days and sacrificed 24 h after the last dose. Mn concentration was measured by atomic absorption spectrometry. In both C57BL/6J (B6) and DBA/2J mice (D2), the Mn concentration in the ears of treated mice was significantly higher than in vehicle-treated control mice (mean ± SEM, $p < 0.05$).

Mn Concentration in Ears

Mn concentration was clearly increased in the ears of the mice receiving subcutaneous $MnCl_2$ injections and sacrificed 24 h after the third dose, compared to the respective control group (Figure 2); there was no difference between the two mouse inbred strains with regard to Mn accumulation. Ears from mice held for 2 weeks after dosing still contained significantly more Mn than untreated controls (Figure 3); in fact, the concentrations decreased by only approximately 50% in the 2 weeks after the last dose. Mn concentrations in the ears of untreated control mice did not differ when analyzed by atomic absorption spectrometry or by ICP-MS.

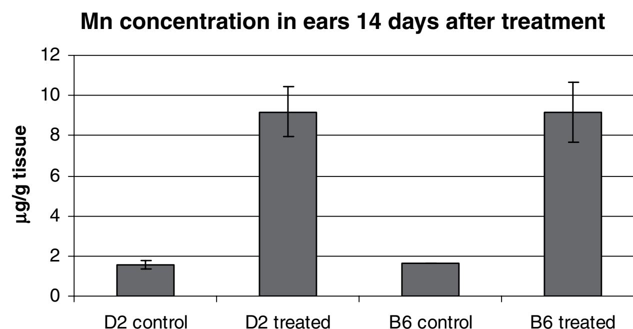

FIGURE 3. Measurement of Mn concentration in the ears of Mn-treated mice 14 days after the end of treatment. Mice were treated with 100 mg/kg $MnCl_2$ or saline vehicle on three alternating days and sacrificed 14 days after the last dose. Mn concentration was measured by ICP-MS. In both C57BL/6J (B6) and DBA/2J (D2) mice, the Mn concentration in the ears of treated mice was significantly higher than in vehicle-treated control mice (mean ± SEM, $p < 0.05$).

DISCUSSION

Previous studies suggest that Mn exposure, alone, or in combination with excessive noise exposure, is associated with hearing deficits [3–8]. Oxidative stress likely contributes to noise- and possibly Mn-induced hearing loss, based on several observations. First, antioxidant supplementation (vitamin C) was shown to protect against noise-induced hearing loss in guinea pigs, suggesting that free radical generation was responsible, at least in part, for noise-induced hearing loss [20]. Mn has been shown to cause oxidative stress when administered (as the gasoline supplement methylcyclopentadienyl manganese tricarbonyl (MMT)) to cells in vitro [21]; furthermore, Mn has been demonstrated to catalyze the oxidation of dopamine, which could cause oxidative damage and cell death in Mn-sensitive regions of the central nervous system [22].

The mouse strains used for the present studies were selected because of previous data from our labs that showed that B6 mice were resistant to cadmium-induced testicular necrosis due to the lack of expression (and thus the lack of Cd uptake) of ZIP8 of the testis of B6 mice; conversely, D2 mice, which are susceptible to Cd-induced testicular necrosis, express ZIP8 in testicular endothelial cells. We hypothesized that the same distinction would hold true between these mouse strains with regard to expression of ZIP8 in the blood vessels of the inner ear; this turned out not to be the case. Instead, q-PCR suggested equivalent ZIP8 expression between B6 and D2 mice, and immunohistochemistry indicated identical localization of ZIP8 protein between the two strains. We were unable to perform hearing tests on the mice in the current study because both B6 and D2 mice lose hearing at relatively young ages, and are thus inappropriate for testing for hearing loss in response to toxicant exposures [23]. Certainly, a logical extension of the current studies would be to use an appropriate mouse strain and compare hearing in control versus Mn-treated mice.

Our results demonstrate the presence of metal transporters in the mouse ear, and also the accumulation of Mn in the ear following systemic Mn administration. Previous studies in which mice were dosed systemically with Cd, another metal that is transported by ZIP8, did not have increased Cd concentration in all tissues. Therefore, tissues such as liver and kidney, which express ZIP8, did accumulate Cd, whereas brain, in which ZIP8 protein and gene expression are undetectable, did not accumulate Cd [14]. Given that ZIP8 is also a high-affinity Mn transporter [16], we believe that the same observation would hold true, but this experiment was not performed. We have also shown that Mn can persist in the ear for at least 2 weeks after cessation of dosing. The observation that Mn persists in the ear after cessation of exposure further suggests that noise exposures in Mn-exposed individuals may not have to occur concurrently in order for oxidative injury potentiation to occur. Thus, it is plausible that Mn can contribute to hearing loss, either alone or in concert with excess noise exposure.

ACKNOWLEDGMENTS

We would like to thank Jaye Ward, Cincinnati Children's Hospital for teaching us to dissect ears, and Michelle Stegemoller and Kathy LaDow for assistance in treating mice.

REFERENCES

1. Keen CL, Ensunsa JL, Watson MH, Baly DL, Donovan SM, Monaco MH, Clegg MS. Nutritional aspects of manganese from experimental studies. Neurotoxicology 1999;20:213–223.
2. Wasserman GA, Liu X, Parvez F, Ahsan H, Levy D, Factor-Litvak P, Kline J, van Geen A, Slavkovich V, LoIacono NJ, Cheng Z, Zheng Y, Graziano JH. Water manganese exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Perspect 2006;114:124–129.
3. Nikolov Z. Hearing reduction caused by manganese and noise. JFORL J Fr Otorhinolaryngol Audiophonol Chir Maxillofac 1974;23:231–234.
4. Khalkova Zh, Kostadinova G. Auditory-vestibular changes in workers in ferrous metallurgy manufacture. Probl Khig 1986;11:134–138 (in Bulgarian).
5. Korczynski RE. Occupational health concerns in the welding industry. Appl Occup Environ Hyg 2000;15:936–945.
6. Josephs KA, Ahlskog JE, Klos KJ, Kumar N, Fealey RD, Trenerry MR, Cowl CT. Neurologic manifestations in welders with pallidal MRI T1 hyperintensity. Neurology 2005;64:2033–2039.
7. Park RM, Bowler RM, Eggerth DE, Diamond E, Spencer KJ, Smith D, Gwiazda R. Issues in neurological risk assessment for occupational exposures: the Bay Bridge welders. Neurotoxicology 2006;27:373–384.
8. da Silva CJ, da Rocha AJ, Jeronymo S, Mendes MF, Milani FT, Maia AC Jr, Braga FT, Sens YA, Miorin, LA. A preliminary study revealing a new association in patients undergoing maintenance hemodialysis: Manganism symptoms and T1 hyperintense changes in the basal ganglia. Am J Neuroradiol 2007;28:1474–1479.
9. Vezér T, Kurunczi A, Náray M, Papp A, Nagymajtényi L. Behavioral effects of subchronic inorganic manganese exposure in rats. Am J Ind Med 2007;50:841–852.
10. Olanow CW, Good PF, Shinotoh H, Hewitt KA, Vingerhoets F, Snow BJ, Beal MF, Calne DB, Perl DP. Manganese intoxication in the rhesus monkey: A clinical, imaging, pathologic, and biochemical study. Neurology 1996;46:492–498.
11. Seth PK, Chandra SV. Neurotoxic effects of manganese. In: Bondy, SA, editor. Neurotoxicology of metals. Boca Raton, FL: CRC; 1989.

12. Erway LC, Purichia NA, Netzler ER, D'Amore MA, Esses D, Levine M. Genes, manganese, and zinc in formation of otoconia: labeling, recovery, and maternal effects. Scan Electron Microsc 1986;(Pt 4):1681–1694.
13. Gong H, Amemiya T. Ultrastructure of retina of manganese-deficient rats. Invest Ophthalmol Vis Sci 1996;37:1967–1974.
14. Wang B, Schneider S, Dragin N, Girijashanker K, Dalton TP, He L, Miller ML, Stringer KF, Soleimani M, Richardson DD, Nebert DW. Enhanced cadmium-induced testicular necrosis and renal proximal tubular damage caused by gene-dose increase in a *Slc39a8*-transgenic mouse line. Am J Physiol Cell Physiol 2007;292:C1523–C1535.
15. Dalton TP, He L, Wang B, Miller ML, Jin L, Stringer KF, Chang X, Baxter CS, Nebert DW. Identification of mouse SLC39A8 as the transporter responsible for cadmium-induced toxicity in the testis. Proc Natl Acad Sci USA 2005;102:3401–3406.
16. He L, Girijashanker K, Dalton TP, Reed J, Li H, Soleimani M, Nebert D. ZIP8, member of the solute-carrier-39 (SLC39) metal-transporter family: characterization of transporter properties. Mol Pharmacol 2006;70:171–180.
17. Liu Z, Li H, Soleimani M, Girijashanker K, Reed JM, He L, Dalton TP, Nebert DW. Cd^{2+} versus Zn^{2+} uptake by the ZIP8 HCO3-dependent symporter: kinetics, electrogenicity and trafficking. Biochem Biophys Res Commun 2008;365:814–820.
18. Dodd CA, Ward DL, Klein BG. Basal ganglia accumulation and motor assessment following manganese chloride exposure in the C57BL/6 mouse. Int J Toxicol 2005:24:389–397.
19. Thompson K, Molina RM, Donaghey T, Schwob JE, Brain JD, Wessling-Resnick M. Olfactory uptake of manganese requires DMT1 and is enhanced by anemia. FASEB J 2007;21:223–230.
20. McFadden SL, Woo JM, Michalak N, Ding D. Dietary vitamin C supplementation reduces noise-induced hearing loss in guinea pigs. Hear Res 2005;202:200–208.
21. Kitazawa M, Wagner JR, Kirby ML, Anantharam V, Kanthasamy AG. Oxidative stress and mitochondrial-mediated apoptosis in dopaminergic cells exposed to methylcyclopentadienyl manganese tricarbonyl. J Pharmacol Exp Ther 2002;302:26–35.
22. Graham DG. Catecholamine toxicity: a proposal for the molecular pathogenesis of manganese neurotoxicity and Parkinson's disease. NeuroToxicology 1984;5:83–96.
23. Davis RR, Newlander JK, Ling X, Cortopassi GA, Krieg EF, Erway LC. Genetic basis for susceptibility to noise-induced hearing loss in mice. Hear Res 2001;155:82–90.