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## Novel founder intronic variant in *SLC39A14* in two families causing Manganism and potential treatment strategies

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### Abstract

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Congenital disorders of manganese metabolism are rare occurrences in children, and medical management of these disorders is complex and challenging. Homozygous exonic mutations in the manganese transporter *SLC39A14* have recently been associated with a pediatric-onset neurodegenerative disorder characterized by brain manganese accumulation and clinical signs of manganese neurotoxicity, including parkinsonism-dystonia. We performed whole exome sequencing on DNA samples from two unrelated female children from the United Arab Emirates with progressive movement disorder and brain mineralization, identified a novel homozygous intronic mutation in *SLC39A14* in both children, and demonstrated that the mutation leads to aberrant splicing. Both children had consistently elevated serum manganese levels and were diagnosed with *SLC39A14*-associated manganism. Over a four-year period, we utilized a multidisciplinary management approach for Patient 1 combining decreased manganese dietary intake and chelation with symptomatic management of dystonia. Our treatment strategy appeared to slow disease progression, but did not lead to a cure or reversal of already established deficits. Clinicians should consider testing for noncoding mutations in the diagnosis of congenital disorders of manganese metabolism and utilizing multidisciplinary approaches in the management of these disorders.

### Keywords

Congenital manganism; *SLC39A14*; Manganese toxicity

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## 1. Introduction

Congenital disorders of manganese metabolism in children are rare occurrences. Manganese is an essential trace metal that is crucial for numerous metabolic pathways and normal cell function. However, in chronic overdose states, the metal can cause clinical toxicity by a variety of upstream subcellular-level mechanisms. Manganese toxicity is associated with a parkinsonism-dystonia syndrome termed manganism, the neuroanatomic substrate for which is manganese deposition in the globus pallidus and substantia nigra of the basal ganglia [1, 2]. The neuroimaging correlate for manganese deposition is progressive T1 shortening, in the absence of significant abnormalities on susceptibility weight sequencing [1, 3].

There are currently 3 known genetic transportopathies that impair manganese homeostasis (Table 1). *SLC39A8*, an intestinal manganese transporter, is associated with an autosomal recessive disorder of manganese uptake that presents with short limb dwarfism, neurodevelopmental impairment, epilepsy, deafness, and altered glycosylation related to intracellular manganese deficiency [4–6]. This disorder may be amenable to supplementation with manganese and galactose. *SLC30A10* is a transporter that serves to export hepatocellular manganese into bile. Biallelic mutations in this gene are associated with a recessive disorder that presents from childhood to early adulthood with progressive manganism, polycythemia, liver disease, and skin hyperpigmentation [7–12]. *SLC39A14*-related manganism is the most recent of these disorders to be reported in 2016 [13]. This disorder presents with childhood onset dystonia and parkinsonism, in the absence of systemic involvement, such as the polycythemia or liver disease seen in *SLC30A10*-related manganism. Biallelic exonic mutations in eleven patients from seven families have been

reported to date [8, 13–15]. Here, we report 2 additional unrelated patients from the United Arab Emirates (UAE) presenting with this disorder in association with a novel homozygous intronic variant that causes aberrant splicing. We describe the natural history of the disorder, and additionally detail our treatment strategy over a four-year period.

## 2. Materials and Methods

### 2.1 Patients and Sequencing

**Patient 1**—The proband and parents were enrolled in a research study at the Manton Center for Orphan Disease Research at Boston Children’s Hospital, approved by the Boston Children’s Hospital Institutional Review Board. Whole exome sequencing (WES) was performed using methods that have been previously described [16]. FASTQ files were filtered, aligned, and variants were filtered and annotated by Codified Genomics (proprietary algorithm, Houston, TX). Candidate variants were confirmed by dideoxy (Sanger) sequencing using standard methods. Likely pathogenic variants were selected to include nonsynonymous, splice site, and indel variants with an allele frequency <1% in the National Heart, Lung, and Blood Institute exome variant server database (<http://evs.gs.washington.edu/EVS/>) or 1000 genomes project (<http://www.1000genomes.org>) and were evaluated in the Exome Aggregation Consortium database (<http://www.exac.broadinstitute.org>). The pathogenicity of the variants was evaluated in silico using Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>), Sorting Intolerant From Tolerant (<http://sift.bii.a-star.edu.sg/>), and MutationTaster (<http://www.mutationtaster.org/>).

**Patient 2**—Trio WES of the proband and parents, and analysis to identify pathogenic variants was performed by GeneDx (Gaithersburg, MD). The Neurodegenerative Brain Iron Accumulation (NBIA) sequencing panel was performed by the Knight Diagnostic Laboratory at Oregon Health Sciences University (OHSU).

### 2.2 Real-time Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) and Sanger Sequencing

RNA was extracted from Patient 1 and control fibroblast cell lines and cDNA was synthesized using the Superscript IV kit (ThermoFisher, Waltham, MA) according to the manufacturer’s protocol. Complementary DNA (cDNA) was amplified using polymerase chain reaction (PCR) with three sets of primers and sent for Sanger sequencing. Set 1 was proximal to the mutation (forward primer in exon 3: GAGAATGAGCAGACGGAGGA, reverse primer in exon 4: CAATGGACAGGGCGATGAAG). Set 2 surrounded the mutation (forward primer in exon 5: TCCAAGTCTGCAGTGGTGTT, reverse primer in exon 6: GGAAGCGACTCAGAGGCATA). Set 3 also surrounded the mutation (forward primer in exon 5: ATTATGTCTCCAAGTCTGCAGT, reverse primer in exon 7: AGAGTAGCGGACACCTTTCA). qRT-PCR was performed using the Fast SYBR Green Master Mix (ThermoFisher, Waltham, MA) with the three sets of primers according to the manufacturer’s protocol. Delta cycle threshold (dCT) values were calculated by normalizing the cycle threshold (CT) value of each sample to the loading control (GAPDH). Double delta cycle threshold (ddCT) values were calculated by normalizing the dCT value of each patient sample to the corresponding control sample. Relative expression levels were then

calculated for each primer set and compared using a one-way Analysis of Variance and post-hoc Tukey honest significant difference Test.

### 2.3 Trace element analysis

Fibroblasts from controls and Patient 1 were grown to confluence in 10 cm<sup>2</sup> tissue culture dishes in Dulbecco's Modified Eagle Medium (ThermoFisher, Waltham, MA) with penicillin/streptomycin and 10% Fetal Bovine Serum (Sigma-Aldrich, St. Louis, MO) and analyzed for intracellular metals by inductively coupled plasma mass spectrometry (Trace Metals Laboratory, Harvard TH Chan School of Public Health, Boston, MA) as described previously [17]. The internal standard was 50 ppb indium. Briefly, cell samples were digested with 2 mL/g total wet weight nitric acid for 24 hours at room temperature. Ultrapure water was used for final sample dilution.

## 3. Results

### 3.1 Patient 1

Patient 1 is a 9-year old female from the UAE with progressive generalized dystonia and brain mineralization. She was the product of an uncomplicated term pregnancy. Her initial motor development was delayed: she sat at 8 months, and never stood. She said her first words around 1 year of age. At 2 years of age, she developed progressive generalized dystonia. She also displayed failure to thrive and required a G-tube for supplemental nutrition. Her parents are first cousins, and she has 3 healthy brothers, ages 3, 13, and 14 years (Figure 1A). There are no additional affected family members. There were no significant environmental exposures to heavy metals.

We first evaluated her at the age of 5 years. At that time, she could speak about 40-50 words, but no phrases or sentences. Her speech was dysarthric. She could W-sit and crawl but could not walk. She had a generalized dystonia and a dystonic smile. Ophthalmology evaluation was normal.

Her initial brain magnetic resonance imaging (MRI) was significant for T1 hyperintensity of bilateral globi pallidi and substantia nigra (Figure 2A–F). There was no abnormal T2 hyperintensity. Susceptibility weighted imaging disclosed no evidence of abnormal susceptibility artifact. Computed tomography scan of the brain did not demonstrate any calcifications.

Initial laboratory parameters demonstrated an elevated serum manganese (Mn) level of 64.2 µg/L (normal range 4-16.5 µg/L). Liver function tests, serum copper, ceruloplasmin, calcium, parathyroid hormone, ferritin, and urine oligosaccharides were normal. The complete blood count (CBC) demonstrated neutropenia. Serum iron was elevated at 207 mg/dL. Hair analysis corroborated elevated manganese storage, with a hair Mn concentration of 516 ppb. By comparison, the mother's hair Mn was 147 ppb and her brother's hair Mn concentration was 214 ppb. Serum Mn levels in her younger brother were initially elevated at 44.1 µg/L, but subsequently normalized. Her mother's serum Mn level was mildly elevated at 28.7 µg/L. Her father's serum Mn level was normal. Manganese excretion in urine was undetectable. Blood and urine levels of additional divalent heavy

metals (arsenic, mercury, lead, copper, and zinc) were normal. Liver ultrasound was normal, with no evidence of portosystemic shunting or arteriovenous malformation.

Analysis of cultured skin fibroblasts for heavy metal content in Patient 1 compared to 2 healthy controls demonstrated similar levels of manganese and iron, but significantly increased levels of zinc ( $p < 0.05$ , Student's t-test) (Table 2). Sequencing and deletion/duplication analysis of the *SLC30A10* gene was negative [7, 8, 10, 12]. Whole exome sequencing demonstrated a homozygous intronic variant in the *SLC39A14* gene, c. 751-9C>G (NM\_001128431, [Hg19] chr8: 22273273 C>G) (Figure 1B), which was not found in the gnomAD database. Both parents and two of the three unaffected siblings were heterozygous carriers, and the third unaffected sibling was homozygous for the wild type allele. Gel electrophoresis suggested both normal and abnormal splicing in Patient 1 fibroblast cells, and sequencing of cDNA demonstrated inclusion of the entire intronic sequence between exons 5 and 6. qRT-PCR analysis on fibroblast cells confirmed aberrant splicing, demonstrating significantly decreased *SLC39A14* transcript levels around the mutation compared to proximal to the mutation (Figure 1C–E).

Patient 1 has been followed intermittently for 4 years at our institution, although she and her family returned to the UAE for long periods between hospitalizations. During this time, her blood manganese levels have ranged between 49.3–79.6  $\mu\text{g/L}$  (Figure 3). Beginning at the age of 5 years, we treated her with repeated 5-day cycles of chelation with parenteral edetate calcium disodium ( $\text{CaNa}_2\text{EDTA}$ ) (40 mg/kg/day) over a 4-year period. The  $\text{CaNa}_2\text{EDTA}$  was well tolerated, except for a couple of occasions when it had to be held because of worsening neutropenia. The  $\text{CaNa}_2\text{EDTA}$  effected a urine Mn diuresis from negligible concentrations pretreatment to  $>25 \mu\text{g/L}$ ; however, serum Mn was not significantly altered. We attempted to coordinate monthly courses of chelation, but because of practical limitations with an international patient, she would often go several months or more between courses. Each course of chelation was associated with a small window of subjective improvement in dystonia and motor functioning, typically lasting weeks.

Two oral chelants, dimercaptosuccinic acid (DMSA, 200 mgs PO BID for 28 days) and d-penicillamine (125 mg PO BID for 30 days), were trialed and proved ineffectual in increasing urinary Mn excretion or lowering blood Mn levels. The two oral chelants were given in separate trials, with a substantial wash-out period in-between their use. We attempted a treatment trial with para-amino salicylic acid (PAS), a drug historically used for tuberculosis with some evidence of benefit in occupational studies of Mn toxicity [18, 19] and animal studies [20, 21]. The patient was not able to swallow the medication, and it could not be administered through her G-tube or effectively dissolved because of its enteric coating.

In addition, since the age of 7 years, the patient has been maintained on a reduced manganese diet using modular formulas. At the age of 8 years, we introduced days with a “Mn free diet”, or days of the week when she receives negligible Mn from oral food. To achieve this, she is entirely fed via manganese-depleted formula for these days, and is supplemented with a manganese free multivitamin. Her macronutrient and micronutrient profile on this diet is summarized in Supplementary Tables 1–3. We began with 2 days a

week of “Mn free diet”, and then ultimately increased to 3 days a week. We considered supplementation with iron to decrease gastrointestinal manganese absorption, but opted not to because of baseline high-normal serum iron levels.

There were 2 longer periods of time when the patient went off diet and parenteral chelation therapy while temporarily returning home to the UAE due to logistical hurdles in securing the customized Mn depleted formula. There was a 6-month period between ages 6-7 years, and another 6-month period between ages 7-8 years. Both of these treatment hiatuses were associated with a marked clinical deterioration from which she did not entirely recover following subsequent chelation treatments.

She has also been managed symptomatically for her dystonia with oral trihexyphenidyl and botulinum toxin injections. In addition, she underwent tendon lengthening surgery. She was previously trialed on baclofen, clonazepam, and L-dopa/carbidopa with little clinical benefit. She is being considered for deep brain stimulation.

Despite these interventions, her serum manganese levels have remained essentially unchanged (Figure 3) and she has demonstrated a gradual disease progression. She currently has more difficulty W-sitting and crawling. Her dysarthria has progressed. She reaches with a significant action tremor. Her intelligence has scored within the average range on formal neuropsychological evaluations, within the limits imposed by her motor impairment. She receives some of her nutrition through a G-tube because of feeding difficulties.

Serial brain MRIs, the most recent of which was at the age of 7 years, have demonstrated stable mineralization of brain parenchyma evidenced on her most recent imaging as T1 hyperintensity of basal ganglia, hypothalamus, anterior commissure, amygdala, cerebral peduncles (including the substantia nigra), dorsal pons, superior cerebellar peduncles, dentate nuclei, cerebellar folia, and pyramids. Abdominal MRI did not demonstrate hepatic manganese accumulation, and liver function tests in blood have remained normal.

### 3.2 Patient 2

Patient 2 presented at 2 years old for evaluation of a progressive movement disorder and loss of ambulation skills. She was born full term via non-spontaneous vaginal delivery, weighing 3.1 kg. There were no post-birth complications. Her mother received prenatal care. There were no maternal or fetal infections noted. The pregnancy was complicated by gestational diabetes, which was well controlled throughout the pregnancy.

Her early development was normal. At the age of 18 months, she first started having trouble walking. The parents first noticed that she started to limp and then started falling frequently. This progressed to her walking on the tips of her toes and then over a few months, she became unable to walk at all. A few weeks later, she was no longer able to sit independently.

Along with this regression in motor milestones, her parents also noticed increased stiffness in all of her extremities and odd positioning of her upper extremities and fingers. Symptoms have continued to progress in a caudal to rostral direction—she subsequently began to develop problems with chewing food and sticking out her tongue. She also lost the ability to say a few words, such as “dada” and “mama”, which she was able to do before the



regression started. She is still able to swallow but the difficulty in chewing has resulted in poor weight gain. There is no history of seizures.

There is an older sister (10 years old) and younger brother (6 months old) who are unaffected. The parents are healthy and deny consanguinity. Two maternal uncles died at 18 and 19 years of age of an unspecified liver condition. There are no other early deaths in the family. There is a paternal niece with dystonia diagnosed by another provider at the same hospital, but no genetic testing was pursued.

Patient 2 was seen by specialists in her home country (UAE) and baseline investigation included a gene panel for pantothenate kinase-associated neurodegeneration (PKAN), mitochondrial DNA sequencing, full gene sequencing of *SLC30A10*, and whole exome sequencing, which were all negative. Her MRI showed abnormal T1 shortening within the globus pallidus, brainstem, cerebellum, and, to a lesser extent, within the surrounding cerebral white matter (Figure 2G–I).

Additional testing included normal electroencephalography and normal biochemical labs, including plasma amino acids, urine organic acids, serum lactate, plasma carnitine, and plasma acylcarnitines. Initial testing demonstrated an elevated serum manganese level of 78 µg/L. Liver function tests, serum copper, ceruloplasmin, calcium and ferritin were normal. Zinc level was elevated at 123 mcg/dL (normal range: 29–115 mcg/dL). CBC and serum iron were normal. The NBIA sequencing panel completed by the Knight Diagnostic Laboratory at OHSU identified the same homozygous variant in intron 5 of *SLC39A14*, c.751-9C>G.

Feeding and speech evaluations performed in the United States demonstrated decreased oral motor skills, using suckling, munching, and suction to eat. She did not exhibit tongue lateralization and mastication to manipulate food. A soft pureed diet was recommended as well as a swallow study. Patient 2 returned to the UAE and received chelation therapy for a year but parents decided not to continue with it.

## 4. Discussion

### 4.1 SLC39A14-associated manganism

Biallelic exonic mutations in the manganese transporter SLC39A14 have recently been implicated in a pediatric-onset neurodegenerative disorder associated with brain manganese accumulation and clinical signs of manganese neurotoxicity, including parkinsonism-dystonia. Previously, 11 patients from 7 families had been reported with exonic missense, nonsense, and frameshift mutations[13–15]. In this article, we report 2 additional unrelated patients with a novel homozygous intronic variant that leads to aberrant splicing. Inclusion of the intron between exons 5 and 6, confirmed by cDNA sequencing, results in a stop codon at the beginning of the intron and suggests there is thus less functional protein. All 13 patients presented in infancy or early childhood with rapidly progressive dystonia, loss of developmental milestones, and/or bulbar dysfunction, and all had elevated levels of Mn in blood or serum.

The function of the SLC39A14 transporter has not been fully elucidated; however, it is presumed to function in hepatic Mn uptake for biliary excretion through SLC30A10 [8]. Aberrant SLC39A14 function likely results in insufficient clearance of manganese from blood, and subsequent accumulation in brain. It is not known what additional transport roles it may have in other organs or cellular compartments. The SLC39A14 protein localizes to the plasma membrane, specifically to the basolateral domain in polarized hepatocytes where it likely functions in hepatic Mn uptake from blood, and also localizes intracellularly where it may play a role in intracellular trafficking [8, 22, 23]. In addition to manganese toxicity in brain, it is not yet clear if intracellular manganese deficiency in other cellular compartments may contribute to the pathogenesis of this disorder. Arguing against this, our patient did not have any abnormalities on transferrin mass spectrometry to suggest dysfunction of the manganese dependent enzyme  $\beta$ -1,4-galactosyltransferase that is seen in *SLC39A8* gene related disorder.

#### 4.2 Effectiveness of Mn Chelation with CaNaEDTA

There have been case reports that SLC30A10 can be effectively treated with IV chelation with calcium disodium edetate and iron supplementation, the latter to reduce intestinal manganese absorption through competitive inhibition across shared transporters. The previous report on this disorder suggested biochemical, clinical, and neuroimaging improvement with monthly chelation with intravenous calcium disodium edetate [10, 18, 24]. In terms of *SLC39A14*-associated manganese, the previous reports suggest variable clinical response with chelation with CaNaEDTA. In three patients, chelation led to a reduction in Mn levels; one patient started on chelation at 17 years old continued to deteriorate, a second patient had reported worsening of cervical dystonia and discontinued treatment, and a third patient had some clinical improvement in terms of dystonia and motor abilities [13–15]. A fourth patient started on IV chelation at five years old had major clinical improvement and was able to walk again [13]. Patient 1 in this article was also treated with IV chelation over a four year period, and although urine diuresis of Mn was observed, there was no change in serum Mn levels and her disease continued to progress. Thus, further investigation is needed to understand the variable clinical response, which may be influenced by the age at which treatment is started, disease severity, the specific mutation, or other factors. One limitation of parenteral CaNa<sub>2</sub> EDTA is its inability to cross the blood brain barrier, and so its ability to remove brain manganese may be quite limited. Another practical limitation was that parenteral chelation in our patient required hospitalization, which made consistent monthly courses difficult to arrange. It is possible that this treatment may have been more effective if the frequency of administration was increased.

#### 4.3 Other Chelants for Mn

PAS, an anti-tuberculous drug that has some chelating properties for manganese, has potential for treatment of this disorder since it can cross the blood brain barrier [18–21]. Further, it is more practical to administer since it is an oral medication and can be taken continuously. Unfortunately, our patient was not able to tolerate PAS because she could not swallow capsules and they could not be administered through her G-tube or dissolved given their enteric coating.



#### 4.4 Clinical Outcomes

For Patient 1, despite significant dietary restriction of manganese and multiple courses of parenteral chelation, we were not able to lower serum manganese levels to an appreciable degree or effect any significant improvement in disease course. Further, brain imaging did not demonstrate any improvement in established mineralization. However, it is likely that this treatment has slowed the progression of her disease as evidenced by her dramatic clinical deterioration when stopping her therapies for 6 month intervals. We have lost contact with Patient 2 as she returned to the UAE and we have not been able to sustain communication with her family.

#### 5. Conclusions

Chronic hypermanganesemia due to SLC39A14 transporter defect is a rare and disabling pediatric neuro-metabolic disorder. We report here two additional cases of *SLC39A14* gene-related manganese in association with a common homozygous splice region variant. This is the first case series to describe a multidisciplinary approach to pediatric patient management, combining decreased manganese dietary intake and chelation with symptomatic dystonia treatment.

Our study had limitations, notably that logistical hurdles prevented us from optimally managing Patient 1 as she travelled back and forth between the United States and the UAE, and prevented us from starting any treatment for Patient 2 as she returned to the UAE and we lost contact with her family. In particular, treatment for Patient 1 may have been more effective if these logistical hurdles were not present. Given the rarity of this disorder and thus the small sample size, future studies of patients with this condition and treatment outcomes over long term periods will be critical to understand the most effective management approaches.

It appears based on our results that while parenteral chelation therapy with CaNa<sub>2</sub>EDTA and dietary manganese restriction may slow disease progression, it is not curative and does not appear to significantly reverse established deficits. This may in part relate to the inability of CaNa<sub>2</sub>EDTA to cross the blood brain barrier. It is possible that treatment would have been more effective if instituted at a younger age, and administered with a greater frequency and perhaps for a longer duration.

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## Abbreviations

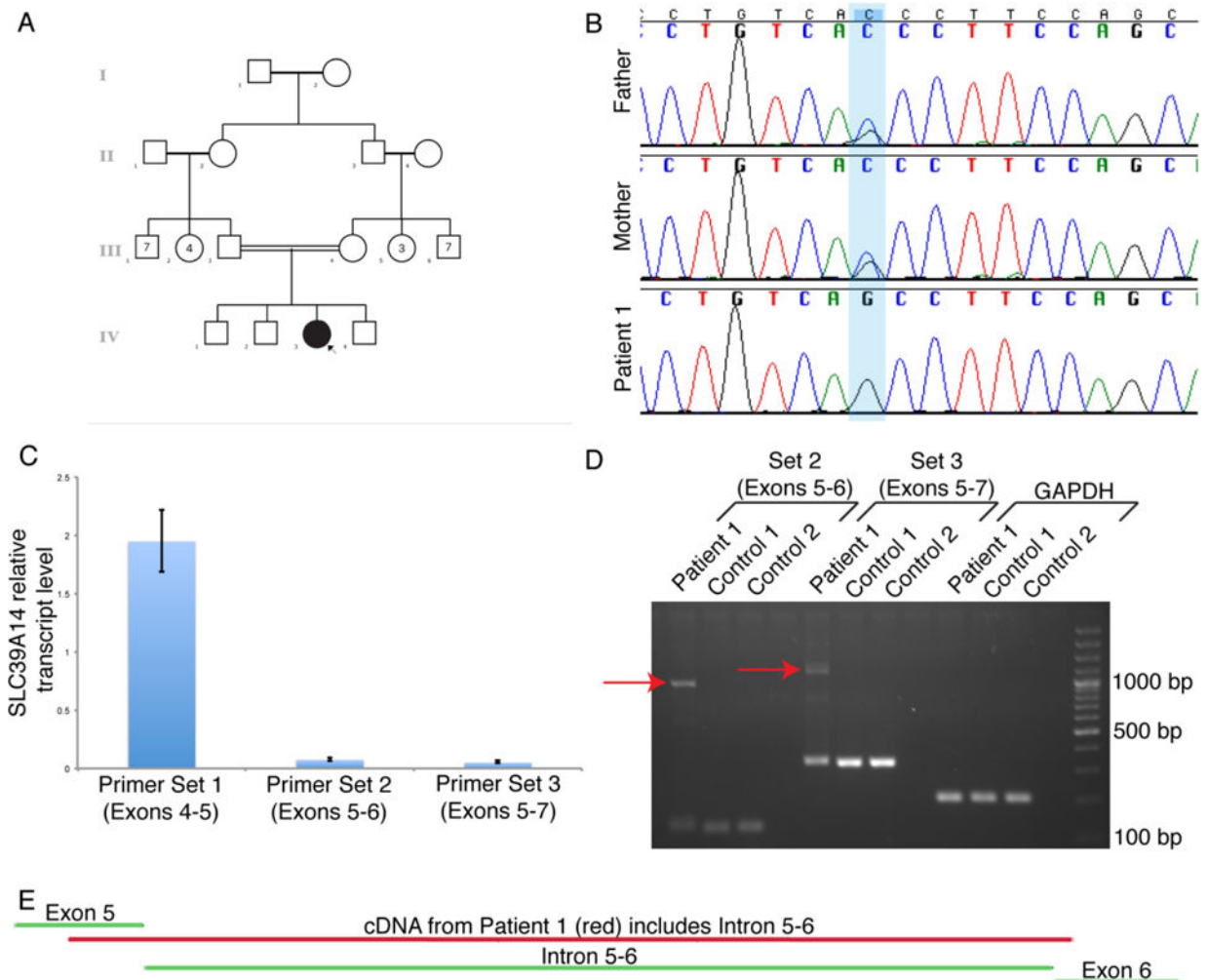
<b>BID</b>	twice daily, CaNa <sub>2</sub> EDTA, edetate calcium disodium
<b>CBC</b>	complete blood count
<b>cDNA</b>	complementary DNA
<b>dCT</b>	delta cycle threshold
<b>ddCT</b>	double delta cycle threshold
<b>DMSA</b>	dimercaptosuccinic acid
<b>Mn</b>	manganese
<b>MRI</b>	magnetic resonance imaging
<b>NIDA</b>	Neurodegenerative Brain Iron Accumulation
<b>PAS</b>	para-amino salicylic acid
<b>OHSU</b>	Oregon Health Sciences University
<b>PCR</b>	polymerase chain reaction
<b>PKAN</b>	pantothenate kinase-associated neurodegeneration
<b>PO</b>	by mouth, qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction
<b>UAE</b>	United Arab Emirates
<b>WES</b>	whole exome sequencing

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**Figure 1.**

Identification of a homozygous intronic variant in *SLC39A14* that leads to aberrant splicing (2-column figure)

A) Pedigree of Patient 1's family. The square represents a male individual, the circle represents a female individual, the filled in circle designates Patient 1, and the double horizontal line between Patient 1's parents indicates consanguinity.

B) Sanger sequencing chromatograms of genomic DNA demonstrate the homozygous intronic variant in *SLC39A14* in Patient 1 and that both of her parents are heterozygous carriers of the variant.

C) qRT-PCR using RNA extracted from patient fibroblasts demonstrates significantly decreased *SLC39A14* transcript levels surrounding the mutation (Sets 2 and 3) compared to proximal to the mutation (Set 1) (one-way ANOVA  $p < 0.01$ ; Tukey HSD  $p < 0.01$  for Set 1 versus Set 2,  $p < 0.01$  for Set 1 versus Set 3,  $p > 0.05$  for Set 2 versus Set 3).

D) Gel electrophoresis demonstrates that cDNA from Patient 1 fibroblasts contains an additional, larger band representing the included intronic sequence compared to cDNA from control fibroblasts. The red arrows indicate the abnormal splicing products that contain the entire intron between exons 5 and 6.

E) Sanger sequencing of cDNA from Patient 1 fibroblasts confirms inclusion of the intronic sequence between exons 5 and 6 (image modified from Sequencher).

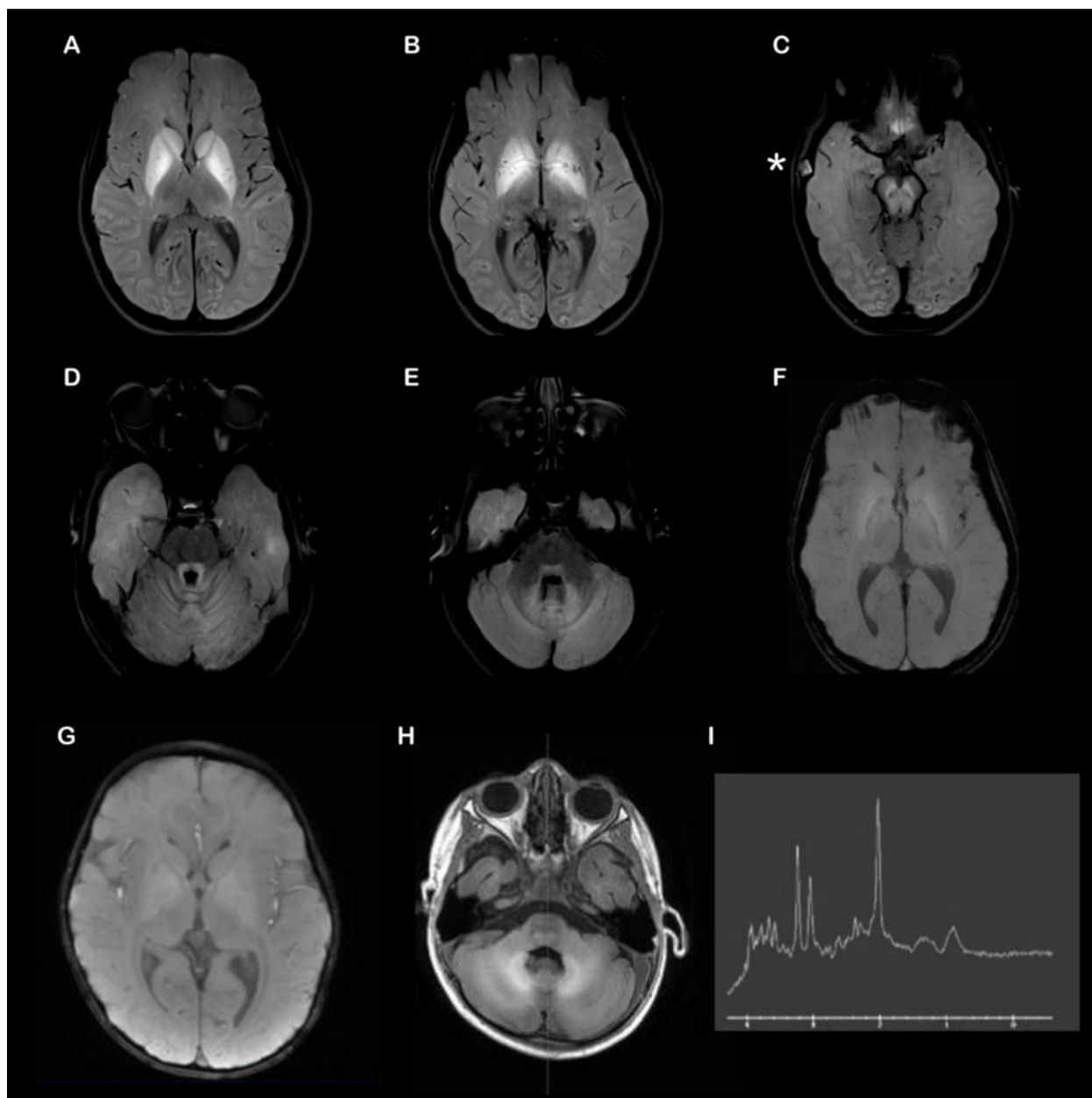
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**Figure 2.**

Brain magnetic resonance imaging (MRI) of Patients 1 and 2 (2-column figure) Axial T1 fat suppressed images of the brain from Patient 1 (A–F) demonstrate T1 shortening in the basal ganglia (A,B), subthalamic nuclei (B), midbrain including the substantia nigra (C), pontine tegmentum including the central tegmental tracts (D), superior/middle cerebellar peduncles (D,E), cerebellar folia (D,E), and the dentate nuclei (E). There are also questionable areas of T1 shortening in the perisylvian cortex (A) and occipital cortex (B,C). No pathologic susceptibility artifact was encountered in the areas of T1 shortening (representative slice in F). There was an incidental right temporal bone dermoid cyst (\* in C). MRI of Patient 2 (G–H) shows abnormal T1 shortening within the globus pallidus, brainstem, cerebellum, and, to a lesser extent, within the surrounding cerebral white matter (G) and stable diffuse volume

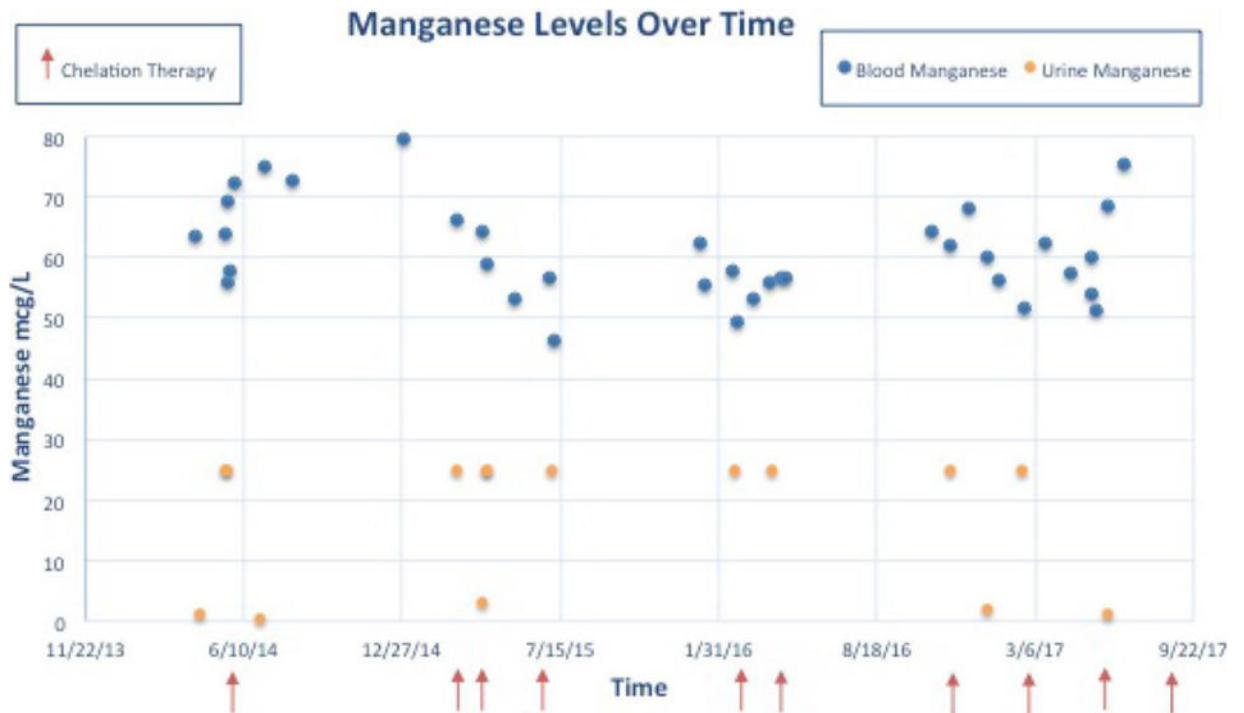
loss (H). The signal changes can be seen in the setting of hypermagnesemia or other metabolic disorders. MR spectroscopy (I) demonstrates normal metabolic peaks within the L basal ganglia. Technically limited left parietal white matter voxel demonstrates an abnormal decreased creatine peak but preserved choline and NAA peaks, which is of uncertain clinical significance.

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**Figure 3.** Treatment outcome over a four-year period for Patient 1 (2-column figure) Blue dots indicate blood manganese levels, orange dots indicate urine manganese levels, and red arrows indicate when chelation therapy occurred.

**Table 1**

Genetic transportopathies that impair manganese homeostasis

	<i>SLC39A14</i> gene disorder	<i>SLC30A10</i> gene disorder	<i>SLC39A8</i> gene disorder
Inheritance	Autosomal recessive	Autosomal recessive	Autosomal recessive
Serum manganese levels	Elevated	Elevated	Low
Cognition	Relatively spared	Relatively spared	Developmental delay, intellectual disability
Extrapyramidal symptoms	Parkinsonism, dystonia	Parkinsonism, dystonia	Dystonia
Seizures	None	None	Yes
Systemic symptoms	None	Liver disease, polycythemia	Skeletal dysplasia, deafness, liver disease
Treatment	Chelation	Chelation, iron supplementation	Oral galactose

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**Table 2**

Heavy metal content in cultured skin fibroblasts.

	<b>Control Fibroblasts #1</b>	<b>Control Fibroblasts #2</b>	<b>Patient Fibroblasts</b>
<i>Manganese</i> (ng/mg)	510.3 ± 0.40	509.8 ± 0.65	519.5 ± 1.20
<i>Iron</i> (µg/mg)	2.48 ± 0.27	1.85 ± 0.03	2.42 ± 0.28
<i>Zinc</i> (ug/mg)	1.84 ± 0.05	1.58 ± 0.04	5.09 ± 0.18*

Fibroblasts were collected from 10 cm<sup>2</sup> dish and intracellular metal levels were measured by ICP-MS. Data are means ± SD from 10 measurements.

\* P < 0.05 between control and patient fibroblasts; Student's *t*-test.

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