Disease Heterogeneity in Na⁺/Citrate Cotransporter Deficiency

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Abstract We report a novel mutation found in two siblings, a male and a female aged 8 and 5 years, respectively. Both patients presented with developmental delay and intractable seizures consistent with previous reports of SLC13A5 transporter deficiency. Both had two mutations in the *SLC13A5* gene, c.655G>A (G219R) and the novel mutation c.245A>G (Y82C). However, the phenotypes were not identical as the female had focal cortical dysplasia that led to brain surgery. This is another example of the heterogeneity in disease expression even when the genotype is identical in the affected individuals.

Introduction

The human Na⁺/citrate cotransporter (Na⁺CT; symbol, *SLC13A5*) was first cloned and characterized by Inoue et al. (2002). It is a plasma membrane transporter with

preferential selectivity toward citrate as a substrate. Na⁺CT mediates the cellular entry of citrate, and, as an active transporter, it is probably responsible for maintaining a cytosolic concentration gradient for citrate in certain cells such as neurons and hepatocytes. Citrate plays a major role in several important biochemical pathways. Most importantly, it is an intermediate in the citric acid cycle. Playing a role in the maintenance of the cytosolic pool of citrate, the Na⁺CT may assist in the establishment of an adequate pool of citrate in the mitochondrial matrix. Citrate is also the major precursor for cytoplasmic acetyl CoA (generated by ATP citrate lyase) and thus serves as an obligatory starting material for the synthesis of fatty acids, cholesterol, and, in neurons, acetylcholine. As such, Na⁺CT is likely to be a very important protein in neuronal homeostasis and cholinergic signaling.

In support of this hypothesis, mutations in the *SLC13A5* gene were recently associated with encephalopathic epilepsy (Thevenon et al. 2014; Hardies et al. 2015). We report two siblings with this neuronal citrate transporter defect with seizures but with different phenotypes, i.e., the presence or absence of intractable seizures and focal brain lesions, which underscore the heterogeneity in SLC13A5 disease expression.

Methods

Whole exome sequencing (WES) was performed in proband-parent quad analysis at GeneDX (Gaithersburg, MD, USA). Informed consent was obtained for each individual. Genomic DNA was extracted from whole blood from the two affected children and their unaffected parents, and the resultant DNA sequences were mapped to the reference human genome sequence (UCSC Genome

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Browser hg19). Targeted coding exons and splice junctions of known protein-coding RefSeq genes were assessed for average depth of coverage and a minimum depth of 10×. Variants were filtered as appropriate on the basis of inheritance patterns, lists of genes of interest, and phenotype and population frequencies. Resources including the Human Gene Mutation Database, 1000 Genomes, the NHLBI Exome Variant Server, ExAc, OMIM, PubMed, and ClinVar were used for evaluating genes and detecting sequence changes of interest, which were identified and confirmed in all members of the family by conventional deoxy DNA sequence analysis. Those rare functional variants that were homozygous and compound heterozygous were prioritized using the PhenoDB Variant Analysis Tool. Variants with a minor allele frequency >0.01 were excluded. At the time of the results reporting, two novel compound heterozygous variants of uncertain significance, c.245A>G (Y82C) and c.655G>A (G219R), were found in the *SLC13A5* gene in both children. Since the time of the report, the c.655G>A (G219R) mutation was also reported by Thevenon et al. (2014) and Hardies et al. (2015).

Clinical Report

We present two siblings with NaCT deficiency. Both siblings were found to be compound heterozygotes for mutations in the *SLC13A5* gene: c.245A>G (Y82C) and c.655G>A (G219R). They have similar histories and presentations, although Patient 2 has greater developmental delay.

Patient 1

Patient 1, an 8-year-old male, was born via repeat cesarean section after a full-term pregnancy. Birth weight was 7 lb, 3 oz. Apgar scores were 9 and 9. The pregnancy was notable for frequent rhythmic fetal movements in the third trimester initially attributed to “hiccups.” Seizures began within the first 24 h of life. He developed prolonged clonic seizures at 3 weeks of age, which required a phenobarbital coma to resolve. He was seizure free for approximately 10 months following that episode but then experienced recurrence of daily seizures. His semiology consisted of frequent myoclonic jerks as well as tonic seizures with arm and leg extension. Multiple electroencephalograms (EEGs) over the years have been abnormal and consistent with generalized epilepsy. The most recent EEG shows multifocal sharp waves, potentiated by sleep and absence of well-formed sleep feature. He has been treated with numerous antiseizure medications. Seizures improved at 4 years of age and now are predominantly myoclonic, occurring one to two times per month. Brain MRIs initially showed delayed myelination and subsequently have showed

no abnormal findings. Magnetic resonance spectroscopy (MRS) was normal with no lactate peak.

Patient 1 had several unrevealing CSF studies. Additional studies included normal chromosomal microarray analysis, and genetic panel for myoclonic epilepsies (including MERRF, Lafora body disease, EPM2A, EPM2B, and SCN1A). Skin biopsy revealed no inclusion bodies.

Patient 1 developed head control late and was able to sit independently between 3 and 4 years of age. At age 5, he was able to pull to stand. Now, at age 8, he is able to scoot independently and walk with a special walker. He attends school in a substantially separate classroom, where he is one of the most capable students. He is making consistent progress. He does not yet speak, but he is able to participate in classroom activities.

On physical examination at age 8, Patient 1 exhibited small stature, with height and weight less than the first percentile (−3SD) and head circumference in the first percentile. His general physical examination was otherwise normal. He was alert and interactive. Neurological examination revealed truncal hypotonia with mildly increased tone in his legs. He had hyperreflexia in his lower extremities.

Patient 2

Patient 2 is the younger sister of Patient 1, a 5-year-old female. She was born via repeat cesarean section at 38-week gestation. Her mother described no rhythmic fetal movements during the pregnancy. Apgar scores were 9 and 9. Birth weight was 7 lb. Seizures began in the first 24 h of life, consisting of lip smacking, eye rolling, and twitching of the hands. Since that time, she has continued to have daily intractable seizures resistant to multiple medications and the ketogenic diet. A vagus nerve stimulator was placed but produced no benefit.

Patient 2 had negative testing for peroxisomal disorders, unrevealing CSF studies and skin biopsy negative for inclusions.

Patient 2 has had several brain MRIs over the years, which have revealed blurring of the gray and white matter junction in the right superior frontal gyrus and at the right frontoparietal junction, including the precentral gyrus, with FLAIR/hyperintensity extending into periventricular white matter. Similar findings were seen to a lesser degree on the left side. This appearance was consistent with areas of focal cortical dysplasia. Patient 2 underwent resection of the right frontoparietal lesion at 4.5 years with resolution of left focal seizures for several weeks following the procedure. However, frequent seizures have since recurred. Her multiple EEGs have been abnormal, consistent with generalized epilepsy. Her most recent EEG demonstrates near continuous sharp waves in the right frontal area and continuous generalized slowing. Her most recent brain MRI shows postsurgical changes after right frontal lobe surgery

Fig. 1 (a) Axial T2-weighted image from Patient 2 shows blurring of the gray and white matter in the right frontal lobe and frontoparietal junction extending to the periventricular white matter. (b) Sample

short TE spectrum from a processed voxel from the left thalamus of Patient 2 shows reduced NAA peak and reduced NAA/Cr ratio (1.57) compared to normal controls (1.8–2.1)

and residual abnormality at the gray and white matter junction in the right frontoparietal region extending to the periventricular white matter (Fig. 1a). Multivoxel 3D MRS performed with both short (30 ms) and long (135 ms) echo times revealed reduced ratio of *N*-acetylaspartate to creatine (NAA/Cr) within the gray and white matter compared to a normal age-matched control and no other abnormal metabolites (Fig. 1b). Specifically, there was no evidence of elevated lactate peak at several processed voxels.

Patient 2's hearing and vision are normal. She has a history of grade II vesicoureteral reflux and is otherwise healthy.

Developmentally, Patient 2 is delayed more than her brother was at her age. She is able to roll, but she has no head control. She cannot sit unsupported. She does not have purposeful use of her hands and is unable to reach or grab objects. In spite of severe motor delay, however, Patient 2 is a very sociable child. She is able to babble. Weight and height are less than the first percentile (-5SD and -2SD) and head circumference is in the 9th percentile. On the neurologic exam, her muscle tone is increased in both upper and lower extremities with severe truncal hypotonia. She has generalized weakness and is normo-reflexic.

Discussion

In 2014, Thevenon et al. reported seven patients from three families, all of whom presented with early epileptic

encephalopathy and severe global developmental delay (2014). All of these patients developed seizures in the first few days of life; five developed seizures in the first few hours. Most had profound developmental delay. On neurological examination, they had axial hypotonia and appendicular hypertonia. None of the patients had facial dysmorphism, and all had normal head size. Metabolic and imaging studies were unrevealing. Seizures were persistent in all patients with several being in subclinical status epilepticus. All were found to have mutations in the *SLC13A5* gene. Two patients from the consanguineous family were homozygous, four patients from other families were compound heterozygotes, and one patient from the fourth family was heterozygous for c.655G>A mutation.

In 2015, Hardies et al. reported eight additional patients belonging to four different families with seven different autosomal recessive mutations in the *SLC13A5* gene (2015). All of these patients had focal clonic seizures in the first days of life. Status epilepticus was common in the course of their disease. Neurological and developmental outcome ranged from mild to severe intellectual disability. Patients had combinations of ataxia, choreoathetosis, and spasticity. All patients had teeth hypoplasia/hypodontia. This was also described by Thevenon but was not a feature in our patients. Three patients were treated with ketogenic diet for their seizures and had favorable response. The authors studied the effect of seven identified mutations in vitro, demonstrating that cells expressing mutant sodium-dependant citrate transporter had a complete loss of citrate uptake.

One of the two *SLC13A5* mutations found in our patients, c.655G>A (G219R), was present in the patients reported by Thevenon et al. (2014) and Hardies et al. (2015). This mutation causes nonconservative amino acid substitution of small, neutral, nonpolar glycine to be replaced with a large, positively charged arginine residue at a position that is highly conserved across species. In silico analysis predicts this variant to be damaging to the protein structure/function. This mutation was not observed in 6,500 individuals of European and African-American ancestry in the NHLBI Exome Sequencing Project.

The second variant found in our patients, c.245A>G (Y82C), has not been reported before. It causes a semiconservative amino acid substitution as a neutral polar tyrosine residue is replaced with neutral polar cysteine residue. The cysteine residue may impact disulfide bonding in the protein. Tyrosine is conserved at this position across species. In silico analysis predicts this variant to be pathogenic. This mutation was also not observed in 6,500 individuals in the NHLBI Exome Sequencing Project. Theoretically, this mutation may interfere with the tertiary structure of the protein as it may lead to an unwanted disulfide bridge. Alternatively, the loss of the tyrosine residue may perturb phosphorylation of the protein at that position.

It was suggested in the past that patients with SLC13A5 deficiency may benefit from a ketogenic diet for the treatment of their seizures. Three patients reported by Hardies et al. (2015) responded to the diet favorably. Patient 2, however, was placed on the ketogenic diet with no benefit. She was also found to have small cortical dysplasia/gliosis that was resected, but surgery produced no benefit, as she continues to have intractable seizures.

Brain malformations, predominantly agenesis of corpus callosum (ACC), were reported previously with disorders of energy metabolism and Krebs cycle. These conditions include pyruvate dehydrogenase (E1 α) deficiency (Shevell et al. 1994), the Amish SLC25A19 (thiamine pyrophosphate transporter) defect (Siu et al. 2010), fumarate deficiency (Mroch et al. 2012), SLC25A1 mitochondrial citrate transporter (Edvardson et al. 2013), and several others. Normal function of the citrate transporter is important in prenatal brain development, and we believe that it is possible that the cortical dysplasia found in Patient 2 may be due to SLC13A5 deficiency. Given the fact that the transporter is expressed in the liver, it is curious that none of the patients reported to date have had liver disease.

In summary, we report a novel mutation in the *SLC13A5* gene. Our patients have presentation similar to seven previously reported cases with clonic seizure onset in the first hours of life, epileptic encephalopathy with multifocal EEGs, lack of dysmorphic features, and the absence of significant medical problems. Unlike patients reported by Hardies et al. (2015), our patients do not exhibit hypodontia but rather evidence of altered enamel. While Patient 1 has

significant delay, he is more advanced in his development than most previously reported cases.

All reported cases of patients with SLC13A5 transporter deficiency presented with seizures in the first day of life. We believe that testing for this condition should be strongly considered in such cases.

Lastly, we hypothesize that triheptanoin or 1,3-Di (heptanoyloxy)propan-2-yl heptanoate may benefit patients with neuronal citrate transporter deficiency. The reasoning behind this offering is as follows. The SLC13A5-mediated transport of citrate across the plasma membrane from the extracellular space into the cytoplasm may play a role in maintaining the pool size of citrate in both the cytoplasm and mitochondrial matrix. We hypothesize that triheptanoin will increase the metabolism of odd-chain fatty acids in neuronal mitochondria and thereby increase the levels of succinyl-CoA, subsequently leading to an increase in citrate concentrations. This possibility was raised by Thevenon et al. as well (2014). The increased level of citrate in the mitochondrial matrix may lead to an increased efflux of citrate from the matrix to the cytoplasm, thus increasing the cytoplasmic pool of citrate and causing the malfunctioning citrate transporter to have less of an impact on the cytoplasmic pool size. We hypothesize that triheptanoin therapy will improve neuronal function and lead to an improvement in CNS function for patients with citrate transporter deficiency, perhaps related to improved cholinergic neurotransmission and energy metabolism.

Synopsis

We report a novel mutation in the *SLC13A5* gene, c.245A>G (Y82C), in two siblings with different phenotypic expression of the Mendelian disease.

Compliance with Ethics Guidelines

Conflict of Interest

Irina Anselm, Morgan MacCuaig, Sanjay Prabhu, and Gerard T. Berry declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by the any of the authors.

Author Contributions

Irina Anselm drafted the manuscript. Morgan MacCuaig contributed to data collection. Gerard Berry reviewed and edited the manuscript. Sanjay Prabhu contributed neuroimaging data. All authors have seen and approved this version of the manuscript.

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