SLC7A3: In Silico Prediction of a Potential New Cause of Childhood Epilepsy

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Abstract

We report an in-depth genetic analysis in an 11-year-old boy with drug-resistant, generalized seizures and developmental disability. Three distinct variants of unknown clinical significance (VUS) were detected by whole exome sequencing (WES) but not by initial genetic analyses (microarray and epilepsy gene panel). These variants involve the SLC7A3, CACNA1H, and IGLON5 genes, which were subsequently evaluated by computational analyses using the InterVar tool and MutationTaster. While future functional studies are necessary to prove the pathogenicity of a certain VUS, segregation analyses over three generations and in silico predictions suggest the X-linked gene SLC7A3 (transmembrane solute carrier transporter) as the likely culprit gene in this patient. In addition, a search via GeneMatcher unveiled two additional patients with a VUS in SLC7A3. We propose SLC7A3 as a likely candidate gene for epilepsy and/or developmental/cognitive delay and provide an overview of the 27 SLC genes related to epilepsy by other preclinical and/or clinical studies.

Keywords

► epilepsy
► case reports
► gene panel
► whole exome sequencing
► SLC7A3

Introduction

Recent data underlines that genetic testing in childhood epilepsy can allow an accurate prognosis, impact management, likely limit the diagnostic odyssey, and possibly avoid further unnecessary testing. This is especially true in children with early-onset, drug-resistant epilepsy with neurodevelopmental disorders.[1,2]

Several genetic tests are currently available and widely used in clinic. The advantages and limitations of cytogenetics (karyotyping, microarray), gene panels, whole exome sequencing (WES), and whole genome sequencing (WGS) have recently been reviewed.[3] Gene panel sequencing (most commonly by filtering WES data and including the analysis of both parents ["Trio"]) is currently the most applied, cost-effective, and rapid test in clinic for diagnosing genetic

epilepsies.[4] This approach has a high "read depth" or "coverage," meaning that this technique achieves a relatively high number of reads at a given base position. Due to this feature, nonsynonymous single nucleotide variants (SNVs), small intragenic deletions, duplications and flanking intronic sequences, as well as somatic variants have a higher probability to be detected. Conversely, WES would miss a variant that is poorly covered but has a higher "resolution" since it targets all the genes in our genome with an acceptable coverage in over 90% of the coding sequences. As a result, WES has largely contributed to the process of disease-gene identification.[1,3] Interestingly, it has been shown that cytogenetic and sequencing analyses are complementary, and if one analysis does not yield positive results, the other one is often indicated. For example, up until now 27 genes, among those expressed in brain and belonging to the transmembrane solute carrier transporter (SLC) large family, have been related to patients with epilepsy (PWE).[5] In most epilepsy gene panels, only few of these *SLC* genes known to be involved in epilepsy are included. So, the majority of *SLC* variants would not be detected, making WES an appropriate, additional approach.

In either case, WES has entered the clinical domain,[1] and increasing research has been performed to investigate novel genes detected by WES. Even though functional studies using preclinical models are ultimately necessary to evaluate the pathogenicity of novel variants in vivo,[6–8] *in silico* computational methods and segregation analyses can contribute to elucidate the potential role of the variants of unknown clinical significance (VUS). To point out, the InterVar tool generates an automated interpretation of any VUS by using 18 criteria, validated by the American College of Medical Genetics and Genomics (ACMG),[9] and MutationTaster is able to further analyze SNVs, InDels and non-coding variants.[10] More details about InterVar and MutationTaster can be found in the ►**Supplementary Material S1** (available online only). Using the segregation analysis in three different generations and the aforementioned *in silico* prediction models, we propose that the X-linked, WES-identified *SLC7A3* variant is the possible genetic cause contributing to the etiology of epilepsy and developmental issues (motor and language) in our patient.

To reinforce the role of *SLC7A3* in neurological disease, we have used GeneMatcher[11] to unveil other patients with *SLC7A3* variants. This had led to two additional patients with *SLC7A3* variants who both show neurological problems including epilepsy and developmental problems.

Original Case Report (1)

An 11-year-old boy with a history of speech delay onset was referred to the University Hospitals Leuven at the age of 3 years due to difficult to treat epilepsy.

He was born by cesarean section at 37 weeks of uncomplicated gestation to nonconsanguineous parents. The birth weight was 3200 g. During the first years of life, there were

Table 1 Variants of unknown significance reported in our patient analyzed by five computational analyses.[9,10] Deleterious thresholds are shown between brackets in the first row[14,15]

Gene	MutationTaster (> 0.5)	CADD (> 15)	SIFT (< 0.05)	GERP++ (> 4.4)	MetaSVM (> 0.09)	Tools indicating pathogenicity
Case report 1						
ZNF335 c.3023C > G	Benign, $p = 0.999$	17.58	0.026	4.66	−1.037	3/5
ZNF335 c.3445A > G	Pathogenic, $p = 0.999$	25.1	0.001	4.86	−1.108	4/5
CACNA1H c.2264G > A	Benign, $p = 0.999$	ND	ND	ND	ND	0/1
IGLON5 c.8C > G	Benign, $p = 0.996$	14.7	0	1.24	−1.022	1/5
SLC7A3 (1) c.1229T > G	Pathogenic, $p = 0.999$	19.75	0.042	5.14	0.272	**5/5**
Case report 2						
SLC7A3 c.107A > G	Pathogenic, $p = 0.999$	27.6	3	4.84	0.906	**4/5**
ST8SIA4 c.143T > C	Pathogenic, $p = 0.999$	22.5	0.044	5.96	−1.069	**4/5**
Case report 3						
SLC7A3 c.244C > A	Pathogenic, $p = 0.999$	26.4	0.002	4.7	0.919	**5/5**

MutationTaster = conservation DNA sequence, splice site and mRNA stability prediction, protein features (p = probability); CADD = 63 distinct variant annotation retrieved from Ensemble Variant Effect Predictor, data from the ENCODE project and information from UCSC genome browser tracks; SIFT = Protein sequence conservation among homologs; GERP++ = conservation DNA sequence by multispecies sequence alignment compared with the neutral expectation; MetaSVM = Ensemble scores based on Support Vector Machine.

no medical issues despite delay in language development and psychomotor skills. At the age of 3 years, he experienced his first symmetric tonic seizure. When he turned 6 years, he also experienced clusters of tonic seizures and atypical absences. Physical examination, metabolic screening (►**Supplementary Material S1**, ►**Table S1** [available online only]), and brain imaging studies (magnetic resonance imaging [MRI]) were unremarkable. EEG analyses showed a background with high-voltage generalized spikes and spike waves bilaterally with maximum amplitude in the frontal regions. During sleep, a marked increase in epileptiform activity was shown. Although multiple distinct antiseizure medications (ASM) were used, in mono- and/or polytherapy, our patient never experienced any seizure freedom. Currently, he is on ethosuximide and levetiracetam (LEV), due to which he did not experience any tonic seizures or atypical absences since the age of 8 years. However, short series of myoclonic seizures are still occurring in the limbs when he falls to sleep (duration ± 1 second) on a daily/weekly basis. Regarding the family history, his father experienced a few seizures when he was a child. Nevertheless, he did not need ASM treatment and had a normal development and intelligence quotient (IQ).

Karyotype analysis, chromosomal microarray, investigations for GLUT1 deficiency (*SLC2A1*), myoclonic epilepsy with ragged red fibers (MERRF), and an epilepsy gene panel (IPG institute, Charleroi) were performed. The gene panel unraveled two VUS in the *ZNF335* gene: c.3023C > G (Pro1008Arg) and c.3445A > G (Ile1149Val). The first VUS was inherited from the father and is present in the European control population (ExAC, 0.0015%). The second VUS was inherited from the mother and is also present in the general control population (ExAC, 0.08%). Even as four out of five prediction tools indicate that this latter VUS could be pathogenic (►**Table 1**), compound heterozygous (or homozygous) mutations in this gene have been linked to basal ganglia abnormalities,[12] agyria with prominent extraaxial spaces, and cerebral/cerebellar hypoplasia or hypomyelination,[13] which do not overlap with our patient's phenotype.

In the context of the ERN EpiCARE network, WES revealed three VUS: that is, *CACNA1H* (c.2264G > A), *IGLON5* (c.8C > G), and *SLC7A3* (c.1229T > G). Pathogenicity prediction tools are summarized in ►**Table 1**. The *CACNA1H* variant (inherited from the father) has been reported in the control population (66/265740 alleles, gnomAD). This is a synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence or the creation of a new splice site, and the nucleotide is not highly conserved. The *IGLON5* mutation (de novo) has not been related to human disease and is reported in the control population (4/69228 alleles, gnomAD). The *SLC7A3* variant has never been reported in the control population (gnomAD). Segregation of this *SLC7A3* variant revealed that it was inherited from a healthy mother and was also present in the healthy maternal grandmother. The inheritance model of *SLC7A3* is X-linked recessive (XLR), thus females can be healthy carriers and males can be affected.[6]

Additional Case Report via GeneMatcher (2)

A 13-year-old girl was referred to the Hillerød hospital (Denmark) at the age of 14 months due to febrile seizures (FS).

One month later, she had two afebrile seizures and treatment with valproic acid (VPA) was initiated. At the age of 4 years, she was seizure-free and tapered off VPA. At the age of 8 years, seizures reoccurred (clonic seizure) during a fever episode and again at age 11 years (two afebrile generalized tonic clonic seizures), for which treatment with LEV was started. Treatment was changed to eslicarbazepine (ESL) due to LEV-induced side effects. The EEG was normal during the first years of life. In contrast, at 11 years of age, her EEG showed epileptiform activity (spike-wave complexes [3 Hz]). Improvement on the EEG was seen after ASM treatment was started, and she has currently been seizure free for 2.3 years on ESL. Physical examination was normal. Metabolic screening and brain imaging studies (MRI at age 4 and 12 years) were normal. Further clinical details can be found in the ►**Supplementary Material S1** (available online only).

Regarding the family history, the father had one FS but was never diagnosed with epilepsy or neuropsychiatric disease.

Chromosomal microarray, fragile-X analysis, and WES were performed. WES revealed one VUS in the *SLC7A3* gene (c.107A > G) and one VUS in the *ST8SIA4* gene (c.143T > C) (►**Table 1**). Both variants (de novo) have never been reported in the control population (gnomAD). We cannot assure that neither *SLC7A3* nor *ST8IA4* are causative.

Additional Case Report via GeneMatcher (3)

A 2-year-old boy with a history of laryngeal cleft, tracheomalacia, feeding difficulties, poor weight gain, and speech and fine motor delay was referred to Massachusetts General Hospital (MGH, Boston, United States) at the age of 12 months due to feeding difficulties. Further clinical details can be found in the ►**Supplementary Material S1** (available online only).

Physical examination was unremarkable. Metabolic screening showed an increase of several amino acids (including arginine), although the significance of this finding remains uncertain. MRI showed asymmetry in size of the lateral ventricles at 11 months of age (left lateral ventricle larger than right lateral ventricle, potentially related to anatomic variation). EEG analyses showed normal sleep and awake EEG and no epileptiform abnormalities.

Regarding the family history, no epilepsy or developmental delay have been reported.

Chromosomal microarray was normal. WES revealed one VUS in the *SLC7A3* gene (c.244 C > A), which was inherited from the patient's mother who never experienced any health issues (►**Table 1**).

Discussion

We report on the genetic analyses that we performed to find a possible new cause of childhood epilepsy and/or developmental issues.

In our original case report (1), gene panels and WES detected five variants possibly related to the epilepsy in this patient. Epilepsy in *ZNF335*-related disease involves biallelic recessive mutations affecting the zinc finger protein that is crucial for methylation and expression of several brain specific genes. Initial genetic studies in our patient revealed a compound heterozygous state of the *ZNF335* gene. However, computational analyses revealed that one of the two VUS are likely benign and the patient's phenotype did not overlap with those of reported PWE and *ZNF335* mutations, showing clear brain anomalies such as microcephaly and/or invisible basal ganglia.[12,13]

Subsequently, WES revealed three VUS in three genes (*IGLON5*, *SLC7A3*, and *CACNA1H*). Our *in silico* predictions showed that the *IGLON5* and *CACNA1H* variants are likely benign and also present in a healthy control population, in clear contrast to the *SLC7A3* variant (►**Table 1**). The *CACNA1H* variant was inherited from the father who experienced self-limiting seizures during childhood, thus we cannot exclude a modifier role of *CACNA1H*,[14] possibly another—yet unknown—gene with variable expression in father and son or a polygenetic cause. Whereas the *SLC7A3* variant, follows a XLR inheritance pattern,[6] which explains why female carriers in this family are not affected. However, the additional case report (2), which was found via GeneMatcher, is a girl with two VUS. The first VUS (in *ST8IA4*) is located on

chromosome 5 and might be inherited in an autosomal recessive manner. Since we did not find a VUS in the other *ST8IA4* gene, it would be more likely that the second VUS (in *SLC7A3*) contributes to the phenotype of our patient, because this gene is located on the X-chromosome, and skewed inactivation could lead to more expression of the potential pathogenic *SLC7A3* in the brain, possibly leading to epilepsy and psychiatric issues. In the same manner, other X-linked genes (e.g., *NEXMIF* or *IQSEC2*) were initially discovered in X-linked intellectual disability (XLID) families with unaffected carrier females, but later studies have shown that females could be affected as well.[15,16]

SLC7A3, or the cationic amino acid transporter (CT-3), is almost exclusively expressed in the brain and modulates arginine transportation in neurons. Defective *SLC7A3* can affect NMDA and mammalian target of rapamycin (mTOR) signaling pathways that both are crucial in neuronal development, plasticity and epilepsy. Consistently, *SLC7A3* mutations have clinically been ascribed to a few males with ASD, a neurodevelopmental disorder.[6] Thus, a role of *SLC7A3* also in epilepsy seems valid, which was present in 2 out 3 patients.

We have also summarized preclinical and/or clinical data of the 27 *SLC* genes that have linked to epilepsy (►**Table 2**).[5] One of the most prevalent epilepsies related to *SLC* genes is Glut1 deficiency syndrome (Glut1DS), even though rare

Table 2 Solute carrier transporters (SLC) and their preclinical and clinical relevance to epilepsy

SLC	Gene	Protein	Preclinical	Human (omim.org)	Gene panel
1	*SLC1A2/EAAT2*	Excitatory amino acid transporter 2 (glutamate)	Epilepsy	EE	Yes
2	*SLC1A4/ASCT1*	Alanine/serine/cysteine transporter 1	Schizophrenia	EE, developmental delay, microcephaly, severe ID	No[b]
3	*SLC2A1/GLUT1*	Glucose transporter 1	Epilepsy, metabolic dysfunction	GLUT1 deficiency syndrome, infantile seizures, ID, hypoglycorrhachia, microcephaly	Yes
4	*SLC4A10/NBCn2*	Na$^+$/HCO3- cotransporter 2	Small brain ventricles, reduced neuronal excitation	Epilepsy, ID	No[b]
5	*SLC6A1/GAT1*	GABA transport protein 1	Epilepsy	Myoclonic-atonic epilepsy	Yes
6	*SLC6A8/CrT*	Creatine transporter	Cognitive deficits	Cerebral creatine deficiency syndrome [a]	Yes
7	*SLC6A9/GlyT1*	Na$^+$/Cl$^-$ dependent glycine transporter 1	Epilepsy, schizophrenia	Glycine encephalopathy	No[b]
8	*SLC6A11/GAT3*	GABA transport protein 3	Epilepsy	Epilepsy, ID, stereotypic behavior	Yes
9	*SLC6A12/BGT1*	Betaine-GABA transporter 1	No	Epilepsy	No[b]
10[c]	*SLC7A3/CAT3*	Cationic amino acid transporter 3 (Arg, Lys, Orn)	No	ASD, epilepsy (this report)	No
11	*SLC7A5/LAT1*	L-type amino acid transporter (neutral amino acids)	Glioma	ASD	No
12	*SLC9A6/NHE6*	Na$^+$/H$^+$ exchanger 6	Christianson syndrome	ID, X-linked syndromic, Christianson type [a]	Yes

(*Continued*)

Table 2 (Continued)

SLC	Gene	Protein	Preclinical	Human (omim.org)	Gene panel
13	SLC12A2/NKCC1	Na^+, $K+$, $2Cl^-$ cotransporter 1	Epilepsy Stroke	Epilepsy	No[b]
14	SLC12A5/KCC2	$K+-Cl^-$ cotransporter 2	Epilepsy	Epilepsy	Yes
15	SLC13A5/NaCT	Na^+/citrate cotransporter	No	Kohlschütter–Tönz syndrome [a], epilepsy	Yes
16	SLC16A4/MCT4	Monocarboxylate transporter 4	Glioma	Epilepsy	No
17	SLC16A7/MCT2	Monocarboxylate transporter 2	Epilepsy	Epilepsy	No
18	SLC17A6/VGLUT2	Vesicular glutamate transporter 2	Epilepsy	Gnathodiaphyseal dysplasia, tendinosis	No
19	SLC23A3/SVCT3	Na^+-dependent vitamin C transporter 3	No	Epilepsy	No[b]
20	SLC25A12/AGC1	Aspartate glutamate carrier 1	Hypomyelination, neuronal defects	EE, early infantile, ASD	No[b]
21	SLC25A22/GC1	Mitochondrial glutamate carrier 1	No	EE, early infantile	No[b]
22	SLC25A39/CGI69	(Mitochondrial carrier variety of solutes)	No	Childhood absence epilepsy	No[b]
23	SLC25A42/MECREN	(Mitochondrial carrier variety of solutes)	No	EE, metabolic crises with variable encephalomyopathic features and neurologic regression	No
24	SLC30A1/ZNT1	Zinc transporter 1	Ischemic stroke Neonatal seizures	No	No
25	SLC32A1/VGAT	Vesicular GABA transporter	Epilepsy Anxiety Cortical dysplasia	No	No
26	SLC35A3/AMRS	(Nucleoside-sugar transporter family)	No	Epilepsy, ASD, Arthrogryposis, mental retardation, and seizures	No[b]
27	SLC55A1/LETM1	Leucine zipper-EF-hand containing transmembrane protein 1 (mitochondrial cation/proton exchangers Ca^{2+}, $K+$, H^+)	No	Epilepsy	No[b]
28	SLC62A1/ANKH	Progressive ankylosis protein homolog (ankylosis homolog) (pyrophosphate transporters)	No	Epilepsy	No[b]

Abbreviations: Arg, arginine; ASD, autism spectrum disorder; EE, epileptic encephalopathy; GABA, g-aminobutyric acid; ID, intellectual disability; Lys, lysine; Orn, Ornithine.
[a]characterized by several features such as epileptic seizures
[b]gene variants related to epilepsy though not part of the IPG epilepsy gene panel (12/27 genes, i.e., 44%)
[c]gene variant discovered in a patient with epilepsy by our case report.

(incidence of ± 4/100 000 births).[17] Glut1DS should be considered in a patient with early onset absence epilepsy (< 4 years of age), myoclonic astatic epilepsy, absence epilepsies, or any epilepsy associated with a movement disorder.[18] Early diagnosis of Glut1DS is confirmed by a low cerebrospinal fluid (CSF) glucose and a pathogenic *SLC2A1* variant. This syndrome is of high clinical importance, since a very effective treatment (ketogenic diet) can improve or even reverse symptoms, especially if started promptly after diagnosis.[17]

Overall, our study on *SLC7A3* variants shows that all patients had developmental issues—including motor skills and language development—and two out of three patients had epilepsy. Although *SLC7A3* is a potential contributor to epilepsy and developmental problems, additional functional studies on *Xenopus* oocytes quantifying the cationic amino

acid transportation are necessary to confirm the pathogenicity of *SLC7A3* mutations.[7]

Author Contributions

All authors have made substantial contributions to conception and design, data acquisition, analysis and interpretation, and drafting the article. J.S.: conceptualization, methodology, formal analysis, investigation, resources, data curation, writing, visualization; K.J.: conceptualization, writing, supervision; D.M. and R.G.: formal analysis, conceptualization, investigation, resources, writing.
T.B.H., R.S.M., N.G., and L.O.: formal analysis, resources, data curation, writing; L.L.: conceptualization, investigation, writing, supervision.

Ethical Statements and Informed Consent

We confirm that we have read the journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. Highest ethical standard was maintained during the study. Parents' informed consent was taken. All procedures performed in our study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflict of Interest

L.L. received grants, and is a consultant and/or speaker for Zogenix; LivaNova, UCB, Shire, Eisai, Novartis, Takeda/Ovid, NEL, Epihunter. L.L. has a patent for ZX008 (fenfluramine) for the treatment of Dravet syndrome and infantile epilepsies assigned to his institution and licensed to Zogenix. The remaining authors have no conflict of interest to disclose.

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