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Secondary neurotransmitter deficiencies in epilepsy caused by voltage-gated sodium channelopathies: A potential treatment target?

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ABSTRACT

We describe neurotransmitter abnormalities in two patients with drug-resistant epilepsy resulting from deleterious de novo mutations in sodium channel genes. Whole exome sequencing identified a de novo *SCN2A* splice-site mutation (c.2379 + 1G>A, p.Glu717Gly.fs*30) resulting in deletion of exon 14, in a 10-year old male with early onset global developmental delay, intermittent ataxia, autism, hypotonia, epileptic encephalopathy and cerebral/cerebellar atrophy. In the cerebrospinal fluid both homovanillic acid and 5-hydroxyindoleacetic acid were significantly decreased; extensive biochemical and genetic investigations ruled out primary neurotransmitter deficiencies and other known inborn errors of metabolism. In an 8-year old female with an early onset intractable epileptic encephalopathy, developmental regression, and progressive cerebellar atrophy, a previously unreported de novo missense mutation was identified in *SCN8A* (c.5615G>A; p.Arg1872Gln), affecting a highly conserved residue located in the C-terminal of the $Na_v1.6$ protein. Aside from decreased homovanillic acid and 5-hydroxyindoleacetic acid, 5-methyltetrahydrofolate was also found to be low. We hypothesize that these channelopathies cause abnormal synaptic mono-amine metabolite secretion/uptake via impaired vesicular release and imbalance in electrochemical ion gradients, which in turn aggravate the seizures. Treatment with oral 5-hydroxytryptophan, L-Dopa/Carbidopa, and a dopa agonist resulted in mild improvement of seizure control in the male case, most likely via dopamine and serotonin receptor activated signal transduction and modulation of glutamatergic, GABA-ergic and glycinergic neurotransmission. Neurotransmitter analysis in other sodium channelopathy patients will help validate our findings, potentially yielding novel treatment opportunities.

1. Introduction

SCN2A encodes the alpha subunit of the voltage-gated type II sodium channel, and is highly expressed in the brain. It resides at the axon initial segment where it initiates an action potential in response to membrane depolarization. SCN2A is located at a crucial bottle neck for signal transduction in neurons [26]. Functional alterations may therefore have widespread consequences with respect to both excitability of neurons and the overall CNS function. This is illustrated by the clinical heterogeneity, with phenotypes varying from benign epilepsies (familial neonatal-infantile seizures, generalized epilepsy with febrile seizures plus) to a more severe epileptic encephalopathy, autism and/or intellectual disability (ID) without seizures, and rarely dystonia, hypotonia and hypersomnia [3,23,29]. *SCN8A* another voltage gated sodium channel, encodes $Na_v1.6$, a major subunit in neurons of the central and peripheral nervous system. It concentrates on the soma, axon initial segments, dendrites and nodes of Ranvier [6]. De novo *SCN8A* mutations resulting

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in persistent sodium current and incomplete channel inactivation have been reported in several patients with ID, tonic-clonic seizures, and epileptic encephalopathy [24,25]. Here we expand with a specific biochemical phenotype the spectrum of voltage gated channelopathies, by reporting significant neurotransmitter deficiencies in two patients: a nine year old boy with a novel de novo *SCN2A* splice-site mutation, and an eight year old girl with a previously unreported de novo missense mutation in *SCN8A*. The University of British Columbia Ethics Board approved the Canadian study (#H12-00067). The study in the UK was performed clinically. Parents provided written informed consent for publication of these reports.

2. Case 1

The 10 year old proband was born as the 4th child in a sibship of six to non-consanguineous, Caucasian parents with an unremarkable family history. After an uncomplicated in vitro fertilization pregnancy, he was born at term by spontaneous vaginal delivery with a birth weight of 4.7 kg (98th centile), and no dysmorphic features. There were no neonatal concerns and psychomotor development was age-appropriate during the 1st year of life, but subsequently slowed down. At 18 months he was admitted to hospital following an ataxic episode. Although he was walking by 19 months, his gait has remained broad-based and ataxic. He is non-verbal, has no use of signs, and has made minimal developmental progress, functioning at a 1.5 year level. His behavioral difficulties were most concerning to parents: oral fixation, lack of awareness of personal space, no toilet-training, and sleep disturbances. Autism was diagnosed at age 3 years. Speech, behavioral and physiotherapists have been actively involved without significant progress. Serial head MRIs at ages 2, 4, 5 and 7 years demonstrated generalized atrophy with enlargement of ventricles and extra-axial spaces (stable on age 7 scan); progressive cerebellar atrophy (more prominent in left hemisphere); T2 signal abnormality in right dentate nucleus and low NAA on MRS (stable at age 7 years).

Seizures began at 3 years of age, gradually increasing to several times per day, with brief episodes of isolated eye rolling with head drops and leg jerks. Ictal video-EEG recording revealed eye blinking and subtle eye movements associated with diffuse, high amplitude sharp waves. Interictal EEG showed diffuse, high amplitude sharp waves. Seizure frequency improved on valproic acid, but control remained incomplete despite addition of ethosuximide and levetiracetam. At age 5 years, his seizure semiology changed to current seizure type, with 30-s episodes of vocalization during sleep followed by drooling, generalized stiffness, with or without cyanosis; his interictal EEG was unchanged.

Extensive biochemical and genetic investigations, as outlined in Table 1, were performed according to published recommendations [32]. These were unremarkable, except the neurotransmitter profile showing persistently low 5-HIAA and HVA, with low neopterin and tetrahydrobiopterin at age 3.7 years (normal at age 7.5 years); biopterin loading test results were non-informative.

Treatment was initiated at age 4 with oral L-Dopa/Carbidopa, 5-hydroxytryptophan (5HTP) and BH_4 considering the possibility of a primary neurotransmitter defect. Initial improvement in focus and interaction were observed, as well as a decrease in self-stimulatory behavior. Seizures became less frequent and EEGs showed some improvement (on a stable dose of valproic acid and ethosuximide). However, after 4 months, when primary neurotransmitter deficiencies had been ruled out and speech and language development (the most relevant outcome for his parents) did not improve significantly, the medications were stopped on parental request.

As part of our TIDEX gene discovery project, whole exome sequencing (WES) was performed on unaffected parents and index, using the Agilent SureSelect kit and Illumina HiSeq 2000 (Perkin-Elmer, USA). The sequencing reads (35× average coverage) were aligned to the human reference genome version hg19 and rare variants were identified and assessed for their potential to disrupt protein function, and subsequently screened under a series of genetic models. Of the 12 candidate genes identified with rare, non-synonymous and splice site variants (0 from homozygous recessive, 8 compound heterozygous and 4 de novo), the previously unreported de novo splice-site variant (hg19 chr2:g.[166188079G>A], NM_021007.2:c.[c.2379+1G>A]) 2 affecting *SCN2A* was considered the top functional candidate (PhyloP nucleotide conservation level 4.88, conserved from human to fish; predicted damaging by software ASSEDA and CADD score) [16]. Sanger sequencing (CLIA lab) confirmed the de novo nature of the variant, as illustrated in Fig. 1A; the variant was present in the index, but absent in the parents (healthy, developmentally normal siblings were not investigated). Experimental work confirmed pathogenicity, showing that c.2379+1G>A causes a frameshift mutation in the mRNA molecule and deletion of exon 14, which in turn leads to a premature stop codon at amino acid position 717 (p.Glu717Gly.fs*30), generating a truncated SCN2A protein (deleted C-terminal 1258aa) (Fig. 1B & C). The downstream impacts include disruption of the ion transport domain, as well as the voltage-dependent channel for this gene (Interpro database). In general, truncating mutations and deletions (also of exons other than #14), like those in our case, have been reported to cause ID and autism as seen in our patient, but usually in those cases severe epilepsy is absent. The WES data was scrutinized for variants in other genes related to neurotransmitter metabolism; Sanger sequencing of another candidate gene ruled out a de novo variant in *DRD4* showing absence of segregation with disease.

At age 7.7 years, given the deteriorating seizures and persistently low CSF HVA and 5-HIAA, oral supplementation with 5HTP (1.5 mg/kg/day) and L-Dopa/Carbidopa (ratio 1:10; 7.5 mg L-Dopa/kg/day) was initiated. Other medications included topiramate, lamotrigine, and valproic acid. Epilepsy (frequency and severity of clinical seizures) was identified as primary outcome. Patient was compliant, no side-effects were noted and seizure frequency decreased from weekly to once every 5–7 weeks. EEGs performed 3 months and 10 months after onset of treatment demonstrated mild improvement in background dysrhythmia, and less epileptiform activity. The patient was also reported to be more attentive and interactive with increased vocalizations and less stereotypies. After a seizure-free period of approximately 5 months, his seizures recurred and continued on a weekly basis again despite increases in lamotrigine and oral supplements. We then introduced a dopa-agonist, pramipexole 0.625 mg TID (52 μg/kg/day), and decreased L-Dopa/Carbidopa to 60 mg TID (5 mg/kg/day), without any change in 5HTP. This strategy resulted in significant change; seizures decreased to once every 3–4 weeks; patient was described as more restless but no other side-effects were noted. At age 9.7 years, with increasing difficulties in medication administration, again parents requested to stop as many medications as possible, including these 3. Patient was noted to become less focused and communicative, showing more self-stimulatory behaviors; it was difficult to evaluate the effect on seizures given the many simultaneous changes in anti-seizure medications.

3. Case 2

This 9-year old female was born at term as first of three children to non-consanguineous parents of Caucasian descent after an uneventful pregnancy and Caesarian section for prolonged labor and mild fetal distress. Until age 3 months psychomotor development was normal; she then experienced her first seizure, tonic and self-limiting, which was treated as meningo-encephalitis with intravenous antibiotics. Subsequently tonic seizures persisted, initially twice a week with exacerbations with infections, and an escalation at 10 months with asymmetric tonic seizures up to 10×/day. There was no response to any of the following treatments: pyridoxal phosphate, vigabatrin, phenytoin, prednisolone, clobazam, valproic acid, levetiracetam, topiramate, perampanel, rufinamide, cannabidiol, and phenobarbital. There was

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