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Supplemental Data

De Novo Mutations in *SIK1* Cause a Spectrum of Developmental Epilepsies

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Supplemental Notes: Case Reports

DB13-001

This was an infant boy born at term after an uncomplicated pregnancy. Delivery was via caesarean section after failure to progress. Apgar scores were 8 and 9 at one and five minutes. Birth weight was 3340 g. At 20 minutes of age he was noted to be dusky and grunting, and was transferred to the NICU for observation. At 80 minutes of life he had repeated episodes of periodic breathing and oxygen desaturations. These episodes continued. Metabolic evaluation and infectious workup, including cerebrospinal fluid studies, were normal. An EEG on day of life 2 showed discontinuous background with burst suppression. He developed intermittent myoclonic jerking movements and repeat EEG on day of life 14 showed continuous high amplitude burst activity alternating with periods of voltage suppression. Brain MRI was normal. Pyridoxine challenge was performed without changes in the EEG after administration. He was started on phenobarbital, biotin, and clonazepam, and was discharged home. Clinical sequencing of a 70 gene comprehensive epilepsy panel was normal, as was chromosomal microarray. He continued to have frequent myoclonic seizures, worsening at 6 months of life. He had very limited development, and at 8 months of age had no head control, did not roll over, was unable to sit, did not bring hands to midline, and did not track objects or attend to voices. He required a gastrostomy tube for feeding, and was on supplemental home oxygen. Repeat EEG at 8 months of age showed highly discontinuous, mildly asynchronous background with no state change, reactivity, or normal waking or sleep patterns, consistent with burst suppression pattern. He also had multifocal epileptiform

discharges within the bursts, many of which correlated with short random jerks of arms, body and legs. He died after complications of a respiratory illness at 10 months of age.

DB14-013

This was an infant boy born at term after an uncomplicated pregnancy. Myoclonic seizures began minutes after an uncomplicated vaginal delivery. Apgar scores were 8 and 9 at 1 and 5 minutes. Birth weight was 3235 g and head circumference was 37 cm. He remained in a phenobarbital coma in the NICU for three months, and continued to have breakthrough myoclonic seizures despite therapeutic phenobarbital levels. EEGs demonstrated burst suppression pattern consistent with early myoclonic encephalopathy and/or Ohtahara syndrome with multifocal spikes and generalized high amplitude multispikes correlating with myoclonic activity. No recognizable background rhythms were noted on repeat EEGs. Pyridoxine did not change the EEG features. Extensive metabolic and infectious evaluations were negative. Muscle, nerve, and skin biopsies were normal. Cerebrospinal fluid neurotransmitters were normal. Brain MRI was normal. Tracheostomy was performed and gastrostomy tube was placed. He continued to have breakthrough seizures with bradycardia and hypoxia requiring increasing ventilator support. The parents elected to remove ventilator support and the infant died at 3 months of age. On autopsy, the cerebral convolutions and sulci were well developed and without malformation. The brain weight was 584 g (average is 516 g).

EP-055

This male subject was born at term via an uneventful spontaneous vaginal delivery after a pregnancy complicated by oligohydramnios. His birth weight was 3.2 Kg, birth length 51 cm, and head circumference was 34 cm. At 4 months of age he began having infantile

spasms and was treated with adrenocorticotropic hormone (ACTH). He then developed generalized tonic-clonic seizures, and was started on valproic acid, zonisamide, and clorazepate. He was also noted to have an anomalous left circumflex artery arising from his pulmonary artery that was reimplanted at 9 months of age, after which he was asymptomatic. He had global developmental delay, began walking at 5 years of age, is unable to feed himself, and did not develop verbal language. Brain MRI was reportedly normal. An EEG at 5 years of age showed generalized slowing. The proband exhibited behavioral problems, aggressiveness, and self-injurious behaviors for which he received guaifenesin, risperidone, and olanzpine. At 11 years of age, his weight was at the 60th percentile, height was at the 25th percentile, and head circumference was at the 25th percentile. He exhibited deep set eyes, short nose and philtrum, and thin upper lip. He had repetitive movements including head-banging, biting, and other self-injurious behaviors.

IS13-013

This female subject was born at term after an uncomplicated pregnancy and delivery. Birth weight was 7 pounds ½ ounce. Head circumference was normal at birth. At 4 months of age she had onset of infantile spasms, was initially treated with phenobarbital, and then ACTH. She did not respond to ACTH, and her longest seizure-free interval was 5 weeks. She continued to have a variety of seizure types, the most common were epileptic spasms identical to previous infantile spasms, and occurring in 1-2 clusters a month. She also had atonic seizures with head drops that were refractory to multiple anti-seizure medications. She had global developmental delay, and rolled at 10 months, sat alone at 1 year, and walked at 3 years of age. At 6 ½ years her head circumference was -2

SD below the mean. She had a normal karyotype, normal chromosomal microarray, and normal sequencing of *MECP2* and *PCDH19*. At 15 years of age she had no clear words, appeared to understand some speech. She has repetitive movements such as bruxism and hand-flapping, especially when excited. She has never slept through the night, and has great difficulty with sleep onset. She began having breath-holding spells at age 7 years, which do not have EEG correlate. She has had self-injurious behaviors such as biting. Vagal nerve stimulator was placed at 5 years of age, and gastrostomy tube was placed at 6 years of age. She has a history of scoliosis with a 43 degree curve.

IS09-018

This female patient was born at term after an uncomplicated pregnancy. She developed seizures at 2 months of age and was diagnosed with infantile spasms at 8 months of age. She was treated with levetiracetam and clobazam, but continues to have myoclonic and tonic-clonic generalized seizures. Brain MRI was normal. At 8 years of age, she has no verbal language, is fed through a gastrostomy tube, is nonambulatory, and is dependent on others for care. She occasionally fixes visually on her parents, but does not respond to other visual or auditory stimuli. She has multiple involuntary movements of the extremities.

LR05-086

This female patient was born at term after an uncomplicated pregnancy. She had myoclonic jerks at birth and tonic seizures starting at 4 hours of life. An EEG at 8 days showed burst-suppression pattern, and she was diagnosed with Ohtahara syndrome. At 6 months of age she developed infantile spasms with EEGs at 6 months, 1 year, and 2 years showing hypsarrhythmia. Infantile spasms did not respond to ACTH. She has been

treated with phenobarbital, phenytoin, zonisamide, levetiracetam, vigabatrin, rufinamide, and valproic acid. A gastrostomy tube was placed at 6 months of age. A tracheostomy was placed at age 3 years after complications of aspiration pneumonia. Major illnesses have been complicated by diabetes insipidus. Scoliosis surgery was performed at age 4 years. At last follow-up at 10 years of age she continues to have daily myoclonic seizures. She requires mechanical ventilation at night. Developmentally, she makes eye contact and regards when spoken to, and has no head control and few spontaneous movements.

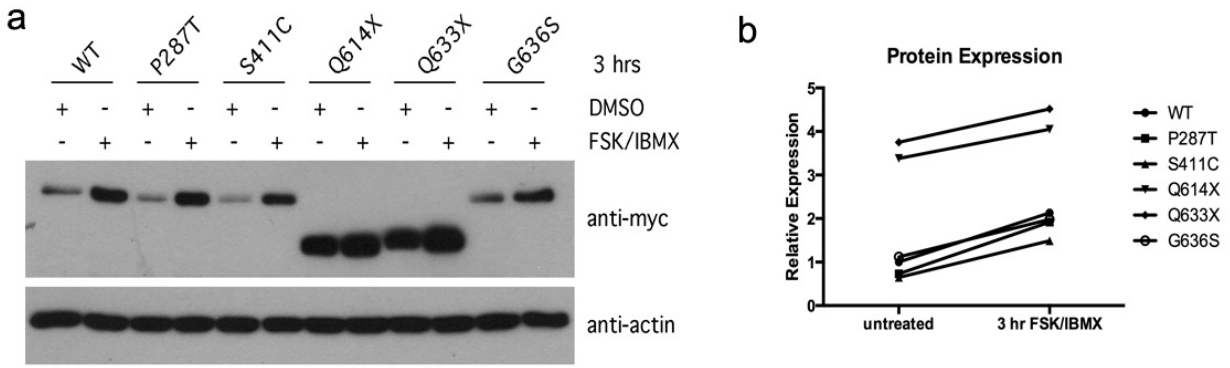

Figure S1: The expression of the p.Gln614* and p.Gln633* proteins increased under Fsk/IBMX treatment and their overall expression was higher than that of the wild-type protein or the missense variants.

Variant	Status from Exome Analysis	Notes
CYP2A13 (NM_000766) c.884C>A p.T295N	*De novo*	Cytochrome P450 family member. Ubiquitously expressed in many tissues.
GOLGA6B (NM_018652) c.623G>A p.R208Q	*De novo*	Possible pseudogene. Not brain expressed.
MAGEL2 (NM_019066) c.509C>T p.A170V	*De novo*	Identified as *de novo* from exome analysis, but found to be paternally inherited by Sanger sequencing.
PSME1 (NM_006263) c.460G>A p.E154K	*De novo*	Implicated in immunoproteasome assembly and required for efficient antigen processing. Not brain expressed.
PRRC2C (NM_015172) c.2161A>G p.M721V c.3875G>A p.R1292Q	AR	Proline-rich coiled coil 2C Evidence for brain expression in Allen Brain Atlas. The c.2161A>G is present in EVS (rs 376665075), predicted benign by PolyPhen2. The c.3875G>A variant is not present in EVS, but SIFT/PolyPhen give "unknown" prediction of effect.
POLRMT (NM_005035) c.2570T>C p.L857P c.526G>A p.E176K	AR	Mitochondrial polymerase. No specific evidence for brain expression in Allen Brain Atlas. The c.2570T>C variant is predicted deleterious (0) by SIFT and probably damaging (0.999) by PolyPhen. However, the c.526G>A variant is present in the EVS (rs367986983), and is predicted to be benign.

Table S1: Other *de novo* and autosomal recessive variants identified by whole exome sequencing of subject DB13-001 that were not considered potentially causative after further analysis. No indels or X-linked variants were identified that could be plausibly pathogenic.

Exon	Forward	Reverse
2	GACAGGCGTGGGAGCAG	GACAATCAAGCCTGGAGCAG
3	TGTGTAAATTAGGTTGAGGGTTTC	AAAAGGGAAGCCACCTTCAG
4-5	TTTGTAGCGCTGCCTTGTG	AGCTTGATGTCCCTGCTCC
6-7	AGCCCCTTGCCATTTGC	TCCCAGACTCAACAAGGACTC
8	CCTGGCGTGTCAAACCAC	GACAGCTGCCTAGCCCC
9	AAGAATAACCTGGGATCGGG	GGTGCCTGACAGGGGAG
10-11	TGTTTCTTCTCTTGATGGCG	ACGTCGACCGTAAGCCTATC
12	GGGGAGCCTATTCCTTTGC	CGGACAAAGTCACGTACTGC
13	TGGAAACCCCTTAAGCCAAG	AGGAGATAACCTGCCGTGTG
14	CACTCGGAAACCCGAGAAC	AGGCCAGATGTCTTCACCTC

Table S2: Primers used for Sanger sequencing of *SIK1* (NM_173354.3).

Human SIK1 was amplified by PCR (Phusion Polymerase, NEB) from a cDNA construct (Thermo Scientific, Clone ID: 4831049, Accession: BC038504) using the following primers: 5’-gcggctagcatggttatcatgtcgg-3’ and 5’- taaggatccgcggccgctcactgcaccaggac-3’.
Myc sequence was introduced by annealing complementary primers 5’-tcgagaccatggaacaaaaactcatctcagaagaagatgcgg-3’ and 5’-ctagccgcatcttcttctgagatgagttttgttccatggtc-3’.

Table S3: Primers used for SIK1 expression constructs.