Mutation in SNAP25 as a novel genetic cause of epilepsy and intellectual disability

Luis Rohena1, Julie Neidich2, Megan Truitt Cho1, Kelly DF Gonzalez2, Sha Tang2, Orrin Devinsky3, and Wendy K Chung4,*

1Children’s Hospital of New York; New York City, NY USA; 2Ambry Genetics; Aliso Viejo, CA USA; 3New York University; New York City, NY USA; 4Columbia University Medical Center; New York City, NY USA

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Abbreviations: ADHD, attention deficit hyperactivity disorder; DICIPHER, Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources; EEG, electroencephalogram; FISH, fluorescent in Situ hybridization; MERRF, myoclonic epilepsy ragged red fibers; SNAP25, synaptosomal associated protein-25kDa; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; SNP, single nucleotide polymorphisms

Whole exome sequencing using a parent-child trio design to identify de novo mutations provides an efficient method to identify novel genes for rare diseases with low reproductive fitness that are difficult to study by more classical genetic methods of linkage analysis. We describe a 15 y old female with severe static encephalopathy, intellectual disability, and generalized epilepsy. After extensive metabolic and genetic testing, whole exome sequencing identified a novel de novo variant in Synaptosomal-associated protein-25 (SNAP25), c.142G > T p.Phe48Val alteration. This variant is predicted to be damaging by all prediction algorithms. SNAP25 is part of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein complex which is involved in exocytotic release of neurotransmitters. Genetic alterations in Snap25 in animal models can cause anxiety-related behavior, ataxia and seizures. We suggest that SNAP25 mutations in humans are a novel genetic cause of intellectual disability and epilepsy.

Introduction

Many rare genetic conditions with low reproductive fitness have been difficult to genetically define using methods of linkage and association. Genetic conditions with strong negative selection and reduced reproductive fitness can in part be explained by genes with a high new mutation rate.1 Whole exome sequencing now offers the opportunity to define many of these rare genetic conditions, even within single families. Common, serious neuropsychiatric disorders like intellectual disability, schizophrenia, and autism are genetically heterogeneous and frequently caused by rare de novo mutations.1,4

Over 340 genes are associated with epilepsy, some of which are associated with other specific phenotypic features.2 Neuronal migration disorders causing lissencephaly and heterotopias have been linked to mutations in LIS1, DCX, ARX, TUBA1A, RELN, FLNA, and ARFGEF2.6 TSC1 and TSC2 mutations impair regulation of the mTOR pathway and cause tuberous sclerosis.3 Rett and atypical Rett syndromes have been linked to mutations in CSDKL5, FOXG1 and MEC2.7,8 Early onset epileptic encephalopathy has been associated with mutations in ALDH7A1, ATP6AP2, PCDH19, POLG, PNPO, SLC2A1, SLC25A22, SPTAN1, and STXBPI.5 Mutations in KCNQ2 and KCNQ3, which control voltage gated potassium channels, have been identified in patients with benign familial neonatal seizures.9 Myoclonic epilepsy has been linked to mutations in CACNB4, EFHC1, GABRA1, CSTB, EPM2A, EPM2B, and PRICKLE1.9,10 The most common familial epilepsies result from mutations in the voltage gated sodium channels genes SCN1A, SCN1B, and SCN2A.6

Whole exome sequencing was initiated on a patient with intellectual disability and epilepsy who had evaded a specific diagnosis despite significant laboratory investigations into the cause of her disorder. A mutation in a gene previously not associated with a specific human disease was identified. This analysis demonstrates the strength of whole exome sequencing as a means of molecular diagnosis for patients who have non-specific neuropsychiatric presentations.

Results

Case presentation

The proband is a 15 y old female, delivered at 41 weeks of gestation. Early prenatal ultrasounds showed bilateral choroid plexus cysts in the brain which were no longer observed by 21 weeks of gestation. APGAR scores were 8 and 9 at 1 and 5 min,
respectively. Birth weight was appropriate at 3360 g. The newborn physical exam identified hip dysplasia requiring a Pavlik harness for 4 mo.

Developmentally, she was always delayed in reaching milestones. At 6 weeks of age, the proband’s mother was concerned that she was not making eye contact. At 2.5 mo she was unable to lift her head when prone. Generalized hypotonia was noted at 4 mo. She first sat unsupported at 3 y of age. She can now ambulate with assistance, but does so infrequently. She is non-verbal. She gained weight well from 0–5 mo, maintaining her weight between the 50th–75th centiles but dropped to the 5th centile by 8 mo. Head circumference was also maintained at the 50th centile from 0–5 mo but dropped to the 5th centile by 9 mo. There was no history of developmental regression, but rather a severe delay in development from early life. To optimize nutrition and increase her caloric intake, nasogastric feeds were started at 1 y of age followed shortly thereafter with gastrostomy tube placement.

Seizures began at 5 mo of age with episodes of repetitive blinking, upward eye deviation and impaired awareness, and were later associated with bilateral kicking and arm abduction accompanied by eye deviation to either side. Video EEG monitoring revealed mild generalized slowing, 2–2.5 Hz generalized spike-and-slow wave complexes. Subsequent video EEG studies revealed (1) mild generalized slowing, (2) interictal partial and symptomatic generalized discharges, and (3) ictal partial and generalized seizures with left (95%) or right-sided (~5%) hemiconic seizures and lateralized frontocentral discharges (~90% left-sided, ~10% right sided) as well as seizures with bilateral motor features at onset, including tonic-clonic seizures, with generalized discharges. She currently averages 3–10 focal clonic seizures per hour, with an average duration of 15 s. Tonic-clonic seizures occur once every two weeks. Her most recent video EEG showed frequent epileptiform discharges but did not comprise more than 30% of her waking or sleep background. Seizures remain refractory to more than eight antiepileptic drugs (in up to three drug combinations, as well as a ketogenic diet and intravenous methylprednisolone trials.

At 5 y of age, she required bilateral varus rotation osteotomies of the proximal femurs. She required spinal fusion at 11 y of age for scoliosis.

Her current neurological examination reveals that she is nonverbal, with global developmental delays. She can intermittently follow simple commands and make intermittent, but fleeting eye contact. Eye tracking is impaired. She is microcephalic with diminished oral motor tone, excessive drooling and spastic quadriparesis.

The proband is an only child. There is no family history of birth defects, seizures, intellectual disability or consanguinity.

Brain MRI at 8 mo showed delayed myelination, but no structural abnormalities. MR spectroscopy showed increased lactate, raising concern for a mitochondrial disorder but given normal cerebrospinal fluid lactate levels it was thought that the MR spectroscopy lactate peaks were secondary to frequent seizure activity.

Metabolic screening including quantitative plasma amino acids, acylcarnitine profile, carbohydrate deficient transferrin electrophoresis, urine organic acid analysis, and cerebrospinal fluid neurotransmitters, amino acids, lactate and glucose were all normal. Sulfite oxidase testing for molybdenum cofactor deficiency was normal. Muscle biopsy pathology and electron microscopy, and mitochondrial oxidative phosphorylation enzyme activity in muscle were normal. Electromyogram was normal. Karyotype, subtelomeric FISH, and chromosome microarray were normal. Molecular genetic testing for MECP2, CDKL5, Angelman syndrome methylation, ACAT1, FOXG1, Mitochondrial DNA 3243 allele was negative, MERRF testing on muscle, 101 nuclear encoded mitochondrial genes, 53 epilepsy genes, and 64 genes associated with X linked intellectual disabilities were all normal.

Whole exome sequencing

Exome sequencing of the family trio (proband, mother and father) resulted in an average of ~16 Gb of sequence per sample (Fig. 1A). Mean coverage of captured regions was 119x per sample, with > 90% covered with at least 10x coverage, an average of > 91% base call quality of Q30 or greater, and an overall average mean quality score of < Q35. Stepwise filtering removal of common SNPs, intergenic and 3′/5′ UTR variants, non-splice-related intronic variants, and synonymous variants resulted in ~12 000 variants per sample. Family history inheritance model filtering based on de novo autosomal and X-linked dominant and recessive inheritance models of the proband, mother and father revealed 37 genes (55 unique alterations) (Table 1). Manual review of each alteration to rule out sequencing artifacts and polymorphisms along with medical interpretation to rule out genes lacking clinical overlap with the patient’s evaluated phenotype resulted in 7 genes (10 unique alterations) (Tables 1 and 2). Review of the individuals genes demonstrated that heterozygous and homozygous loss of function mutations in FAM90A1 and SGK223 were common in normal individuals in the ESP database, suggesting these are not responsible for the phenotype. PMTD1 has pseudogenes with the same 9 bp insertion suggesting this is a mapping error. Among the three possible recessive genes ABCC10 and CAPRIN2, and IGEF3, at least one of the alleles was predicted to be benign by either PolyPhen-2 or SIFT and none of the three are highly expressed within the brain, making them less likely candidates. Among these, the most likely gene with one alteration with potential clinical relevance underwent confirmation and co-segregation analysis using automated fluorescence dyeoxy sequencing: SNAP25 c.142G > T (p.V48F) (Fig. 1B).

The c.142G > T (p.V48F) alteration is located in exon 4 (3rd coding exon) of the SNAP25 gene. The V48 amino acid is highly conserved throughout vertebrates (Fig. 1C). The amino acid substitution results in changing a non-polar, neutral amino acid to a larger, non-polar, neutral amino acid and is predicted to be probably damaging by PolyPhen16 and deleterious by SIFT17 in silico analysis. Based on data from the NHLBI Exome Sequencing Project (ESP),14 no alterations were observed among 6,503 individuals tested (0.0%). Allele frequency data for this nucleotide position not currently available from the 1000 Genomes Project14 and the alteration is not currently listed in the Database of Single Nucleotide Polymorphisms (dbSNP). Confirmatory sequencing
and co-segregation analysis using automated fluorescence dideoxy sequencing revealed that the alteration was absent in the mother and father, establishing a de novo origin of the alteration.

**Discussion**

We suggest that the novel variant, c.142G > T in SNAP25, identified via whole exome sequencing is the likely cause of the proband’s epilepsy and intellectual disabilities. SNAP25 is one of the best characterized components of the SNARE protein complex which is involved in the exocytotic release of neurotransmitters during synaptic transmission by mediating synaptic vesicle fusion.\(^{19}\) SNAP25 assembles with syntaxin-1 and synaptobrevin to form the exocytotic fusion complex in neurons.\(^{20}\) Electoretinogram recordings of Snap25 null drosophila show a complete loss of synaptic transmission.\(^ {21}\)

SNAP25 has not previously been associated with any Mendelian human disease. It was discovered that blind-drunk mice (Bdr), which display ataxia and impaired gait, harbor a heterozygous single amino acid substitution (I67T) in Snap25.\(^ {22}\) Substituting serine at position 187 with alanine in Snap25 induced anxiety related behavior in the mouse.\(^ {23}\) The Coloboma mouse, which is heterozygous for 4.6Mb deletion of chromosome 2 which encompasses 12 genes including Snap25, have shown seizure predisposition.\(^ {24}\)

Snap25 exists as two isoforms.\(^ {25}\) Mice with only Snap25a develop seizures and show learning deficits and anxiety. Snap25b knockout/ Snap25a knock-in mouse mutants that only express Snap25a showed frequent episodes of tremors and seizure activity.\(^ {25}\) This experiment demonstrated that small changes in the regulation of Snap25 caused neurological problems including epilepsy.

**Figure 1.** (A) Family pedigree. Shaded shapes indicate affected individuals. (B) Next-gen sequence alignment of the SNAP25: c.142G > T (p.V48F alteration in the proband, mother, and father. (C) Sequence conservation plots at the mutated site amino acid position across different species.
| Table 1. Variant filtering based on inheritance model and interpretation |
|---------------------------------------------------------------|
| **Gene Symbol/ Model** | **Novel/ Characterized Gene** | **Variant** | **Genotype** | **PolyPhen-2** | **SIFT** | **dbSNP** | **Allele Frequency** | **Comments** |
| | | | Proband | Mother | Father | | | | |
| SNAP25 | Novel | c.142G>T p.V48F | +/- | -/- | +/- | damaging | damaging | N/A | N/A |
| FAM90A1 | Novel | c.10del p.R4VfsX12 | +/- | -/- | +/- | N/A | N/A | N/A | N/A |
| PCMTD1 | Novel | c.921_922ins9 p.K307delins4 | +/- | -/- | +/- | N/A | N/A | N/A | N/A |
| SGX223 | Novel | c.1029_1030ins8 p.C343WfsX28 | +/- | -/- | +/- | N/A | N/A | N/A | N/A |

**Autosomal Dominant Genes (Alterations)**: 22 (24) | **Manual Review**: 4 (4) | **Notable Candidate Genes**: 1 (1)

**Autosomal Recessive Genes (Alterations)**: 14 (30) | **Manual Review**: 3 (6) | **Notable Candidate Genes**: 0 (0)

**X-linked Recessive Genes (Alterations)**: 0 (0) | **Manual Review**: 0 (0) | **Notable Candidate Genes**: 0 (0)

**X-linked Dominant Genes (Alterations)**: 1 (1) | **Manual Review**: 0 (0) | **Notable Candidate Genes**: 0 (0)

**Y-linked Genes (Alterations)**: N/A | **Manual Review**: N/A | **Notable Candidate Genes**: N/A

**Total Alterations**: 37 (55) | **Manual Review**: 7 (10) | **Notable Candidate Genes**: 1 (1)

*aManual filtering involves the removal of genes unrelated to the patient’s evaluated phenotype and alterations considered benign. *bNotable Candidate Genes: Genes with disease phenotype association overlapping that of the proband.*

| Table 2. Summary of variants remaining after manual filtering |
|---------------------------------------------------------------|
| **Gene** | **Novel/ Characterized Gene** | **Variant** | **Genotype** | **PolyPhen-2** | **SIFT** | **dbSNP** | **Allele Frequency** | **Comments** |
| | | | Proband | Mother | Father | | | | |
| SNAP25 | Novel | c.142G>T p.V48F | +/- | -/- | +/- | damaging | damaging | N/A | N/A |
| FAM90A1 | Novel | c.10del p.R4VfsX12 | +/- | -/- | +/- | N/A | N/A | N/A | N/A |
| PCMTD1 | Novel | c.921_922ins9 p.K307delins4 | +/- | -/- | +/- | N/A | N/A | N/A | N/A |
| SGX223 | Novel | c.1029_1030ins8 p.C343WfsX28 | +/- | -/- | +/- | N/A | N/A | N/A | N/A |
| ABCC10 | Novel | c.1472G>A p.R491Q | +/- | +/- | +/- | benign | tolerated | rs141254177 | 0.02% | N/A |
| | | c.2696G>A p.R899Q | +/- | +/- | +/- | benign | damaging | rs142010618 | 0.02% | N/A |
| CAPRIN2 | Novel | c.1649A>G p.N550S | +/- | +/- | +/- | benign | tolerated | rs1446283389 | 0.05% | 0.09% |
| | | c.1118G>A p.R373H | +/- | +/- | +/- | benign | tolerated | rs146816559 | N/A | N/A |
| IGSF3 | Novel | c.1945C>T p.R649C | +/- | +/- | +/- | damaging | damaging | rs147792935 | 0.06% | N/A |
| | | c.1379C>A p.P460Q | +/- | +/- | +/- | benign | tolerated | rs147792935 | N/A | N/A |

N/A, not available; blank boxes indicate not applicable
variant in SNAP25. Functional in vitro analysis to further the impact of the altered gene would also substantiate these findings. We encourage others engaged in the genetic study of children with epilepsy and/or intellectual disabilities to include SNAP25 as a candidate gene in genetic studies to find additional similarly affected patients in order to better define this novel neurodevelopmental disorder.

Methods

DNA from the parents and proband were prepared using the SureSelect Target Enrichment System (Agilent Technologies).11 The exome libraries were then applied to the solid surface flow cell for clonal amplification and sequencing using paired-end, 100-cycle chemistry on the Illumina HiSeq 2000 (ILLUMINA). Sequence quality filtering script was executed with the Illumina CASAVA software (ver 1.8.2, ILLUMINA). Data yield (MBases), %PF (pass-filter), # of reads, % of raw clusters per lane, and quality scores were examined in Demultiplex_Stats.htm file. The sequence data were aligned to the reference human genome (GRCh37) and variant calls were generated using CASAVA. The exome libraries were then applied to the solid surface flow cell for clonal amplification and sequencing using paired-end, 100-cycle chemistry on the Illumina HiSeq 2000 (ILLUMINA). Sequence quality filtering script was executed with the Illumina CASAVA software (ver 1.8.2, ILLUMINA). Data yield (MBases), %PF (pass-filter), # of reads, % of raw clusters per lane, and quality scores were examined in Demultiplex_Stats.htm file. The sequence data were aligned to the reference human genome (GRCh37) and variant calls were generated using CASAVA. Exons plus at least 2 bases into the 5′ and 3′ ends of all the introns were analyzed. The Human Gene Mutation Database (HGMD),12 the Single Nucleotide Polymorphism database (dbSNP),13 1000 genomes,14 HapMap data,15 and online search engines (e.g., PubMed) were used to search for previously described gene mutations and polymorphisms. Stepwise filtering removed common SNPs, intergenic and 3′/5′ UTR variants, non-splice-related intronic variants, and synonymous variants. Variants were then filtered further based on possible recessive, X linked or de novo mutations. Data were annotated with the Ambry Variant Analyzer tool (AVA), including nucleotide and amino acid conservation, biochemical nature of amino acid substitutions, population frequency (ESP and 1000 genomes),14 and predicted functional impact (including PolyPhen16 and SIFT17 in silico prediction tools). Multiple sequence alignments were viewed using IGV (integrative genomics Viewer software).18 Identified candidate alterations were confirmed using automated fluorescence dyeoxy sequencing in the proband and parents on an ABI3730 (Life Technologies) using standard procedures.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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