Molecular Case Study Research Report

SCN8A Mutation in Child Presenting with Seizures and Developmental Delays

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ABSTRACT

The *SCN8A* gene encodes the Nav1.6 neuronal voltage-gated sodium channel α subunit. Mutations in this gene have been associated with early infantile epileptic encephalopathy type 13. With the use of whole exome sequencing, a *de novo* missense mutation in *SCN8A* was identified in a 4-year-old female who initially exhibited symptoms of epilepsy at the age of 5-months that progressed to a severe condition with very little movement, including being unable to sit or walk on her own. Determining the molecular etiology of this proband's epileptic encephalopathy has improved her management.

INTRODUCTION

Mutations in *SCN8A* are associated with cognitive impairment with or without cerebellar ataxia (OMIM#613406) and with early infantile epileptic encephalopathy-13 (EIEE13, OMIM#613558). Loss of function mutations can be associated with cerebellar ataxia and cognitive issues, whereas gain of function mutations can underlie epileptic encephalopathy[1]. More than 60 *de novo* mutations in *SCN8A* have been discovered through genome and exome sequencing and *SCN8A* can now be evaluated with commercial epilepsy panels[2].

Epileptic encephalopathy is characterized by seizure activity that progresses to cerebral dysfunction leading to severe cognitive, motor and behavioral impairments[3]. Approximately 1% of early infantile epileptic encephalopathies are associated with missense mutations in the *SCN8A* gene and 50 cases have been described in the literature [2; 4; 5]. In a few cases the mutation was inherited from a mosaic parent, but the majority of disease-contributory mutations are *de novo* missense mutations[2]. EIEE13 has an average age of onset between 3 and 7 months, and individuals present with various types of seizures, including tonic clonic, generalized tonic, atonic, myoclonic and focal and absence seizures, while febrile seizures are rare [6]. There is often developmental regression, and movement disorders are present with 50% of affected individuals unable to sit or walk.

The *SCN8A* gene is located on chromosome 12q13 and encodes the sodium channel voltage gated type VIII α subunit protein Nav1.6, which functions in the rapid depolarization of sodium channels during generation of action potentials in neurons. Nav1.6, is one of the three major sodium channels in the brain and is involved in the regulation and propagation of firing patterns of excitatory and inhibitory neurons[7]. Nav1.6 is localized to the axonal initial segment and to nodes of Ranvier in myelinated neurons. Mutations in the related sodium channel gene *SCN1A* contribute to Dravet syndrome and mutations in *SCN2A* contribute to Ohtahara syndrome [2].

Ten patient mutations of *SCN8A* have been evaluated with functional tests[5; 7-10]. In eight of the ten cases, gain-of-function effects were observed. The most common defect is impaired channel inactivation, which leads to a persistent sodium current and increased neuronal activity. In two cases, a hyperpolarizing shift in voltage dependence of channel activation was observed, also leading to hyperactivity. This gain-of-function mechanism is opposite to the situation in Dravet syndrome, where loss-of-function mutations in *SCN1A* are most common[7; 8].

Through advances in DNA sequencing technology, DNA can now be sequenced quickly and relatively inexpensively. This study focused on the use of next generation exome sequencing to find mutations by analyzing only protein coding regions of the DNA. Although such analyses focus on approximately one percent of the human genome, this nonetheless still identifies tens of thousands of variants to analyze[11-13]. Through the use of existing and novel bioinformatics tools, we developed a protocol focused on identifying uncommon and deleterious mutations and prioritizing them by phenotypic relevance. We present here a detailed case study of a proband in which a *de novo* likely disease-contributing mutation in *SCN8A* was discovered.

RESULTS

Clinical presentation and family history

The proband is a 4-year-old female presenting with idiopathic epilepsy (10-15 seizures per day), cortical blindness, and developmental regression (**Figures 1** and **2**). Her medical records show that she has no language or motor skills, is fed through a G tube, and has recurrent fevers and ostopenia. She had acute fractures of her distal radius and distal ulna bilaterally as well as on the left distal tibia and left distal fibula while on a ketogenic diet. Table 1 outlines her phenotypic features using Human Phenotype Ontology (HPO) terms. Prior to whole exome sequencing (WES), an infantile epileptic encephalopathy panel that did not include *SCN8A* was ordered for this proband. The results showed a variant of uncertain significance in exon 3 of the *GRIN2A* gene that encodes the NMDA receptor 2A protein, resulting in an amino acid change from phenylalanine to isoleucine at position 183. Parents of the proband were tested for this variant and the unaffected father was found to carry this variant. This proband had a high resolution whole genome SNP/ copy number microarray analysis using the Cytoscan HD platform, and Chromosome Analysis Suite Software (Affymetrix) was used for analysis. The result was normal and no clinically significant copy number variants were discovered.

The only reported complication during pregnancy was a Group B Streptococcal infection in the mother. From birth to three months of age, the proband met all milestones, although the parents report she was slightly hypotonic. At three months, she

experienced her first seizure-like activity, although all medical tests revealed no abnormalities. The evening after her 5-month vaccinations including DT, HIB Hib PRP-T, IPV Polio, and PCV-13, she was admitted to the emergency department with increased seizure activity, nystagmus, hypotonia, and fever, although all medical tests revealed no pertinent abnormalities. An electroencephalogram (EEG) two weeks after the onset of severe symptoms revealed severe abnormalities, and she was diagnosed with idiopathic epileptic encephalopathy. The EEG showed the presence of frequent independent multifocal, mostly right posterior, epileptogenic abnormalities, consistent with a tendency towards partial onset seizures, and this is indicative of right hemispheric profound cerebral dysfunction. Follow up EEGs showed multifocal sharp waves. Facial features include a short, upturned nose, full cheeks, a short philtrum and a horizontal crease on the chin (**Figure 2**). Her physical manifestations include absence of all mobility and motor coordination, with daily placement in a wheelchair, and absence of a startle reflux, possibly secondary to neck weakness and poor eye control. The family history is unremarkable.

Medications that the proband is now taking include phenobarbital, carbamazepine and Charlotte's Web (Hemp extract). The addition of carbamazepine has stopped her loop seizures and prevents her from having seizures as she enters and exits sleep. She has improved awareness, more eye contact and is able to shake a rattle.

Genomic Analyses

Blood samples from the proband, as well as blood and saliva samples from parents and siblings, were sequenced at Affiliated Genetics in Salt Lake City, Utah, where genomic DNA was extracted and exons sequenced using the Life Technologies Ampliseq Exome RDY kit and the Life Technologies Proton sequencing system (see Methods). These targeted regions were sequenced using the Ion Proton sequencing system using Ion Hi-Q Chemistry with 200 bp reads. The DNA sequence was compared with the UCSC hg19 reference sequence, and several methods of analysis were applied to the sequence data (see Methods). These analyses included in-house protocols, and several commercial software packages, including Tute Genomics, Omicia Opal, and Cartagenia v4.1, along with the use of an OTG-SNP Caller pipeline (see Methods). In all analyses, we arrived at similar conclusions, but the various analyses helped to provide a more comprehensive and in-depth approach to the data.

As one example, for the OTG-SNP Caller pipeline, the final VCF spell out file for each individual contained 20,000 to 25,000 variants, of which 300-400 were found to be inherited. However, 2000-3000 variants were recognized as *de novo*, which is notably above the expected number of *de novo* mutations found in standard WES [14; 15]. In addition, even with an optimized variant calling pipeline, there were still a significant number of false

positives. The number of variants found in the proband was 28,681 and the mean depth of coverage was 101-fold.

A GEMINI query[16] selected zero autosomal recessive mutations and sixteen rare *de novo* mutations of interest. A unique *de novo* heterozygous variant in the *SCN8A* gene on chromosome 12 at position 52,093,447 was identified (c.800T>C , p.Leu267Ser) (**Figure 3a**). This mutation is not present in any frequency in public databases such as dbSNP 137, 1000 Genomes phase 1 data, NHLBI 6500 exomes, or ExAC version 0.2, which contains allelic information derived from ~60k exome sequences. The presence of the mutation was confirmed using Sanger sequencing (**Figure 3b**). This missense mutation has not been previously identified in any other patient with epilepsy, and leucine 267 is located in transmembrane segment DIS5 of the sodium channel and is highly conserved through evolution (**Figure 4**). The substitution changes the hydrophobic leucine residue to the hydrophilic serine, which is likely to alter channel function. The CADD score was 21.1, indicating that this substitution is within the 0.78% of most deleterious mutations (99.2 percentile). The SIFT[17] and Polyphen scores[18] were < 0.01 and >.999, respectively, also predicting that the mutation is deleterious. Analyses with Phenolyzer[19], wAnnovar[20], and PhenIX[21] all predict that a heterozygous missense mutation in *SCN8A* is likely to contribute to the phenotype.

The Online Mendelian Inheritance in Man (OMIM) database describes early infantile epileptic encephalopathy and cognitive impairment as two phenotypes associated with mutations in *SCN8A*. Since the proband has a similar phenotype, this is considered to be a mutation that contributes to the phenotype described here. Several studies have associated mutations in *SCN8A* with epilepsy, intellectual disability, and cranial features such as microcephaly[2-9]. Additionally, there have been several reported cases of susceptibility to fractures that accompanied mutations in this gene[8; 22], as was observed in this proband.

DISCUSSION

Overall, the various analyses employed herein were able to efficiently and accurately detect variants in this family, uncovering a deleterious SNV likely contributing to the severe phenotype and previously unidentified in clinical databases such as ClinVar and OMIM. By using existing databases to filter variants by rarity and deleteriousness, common variants and/or benign variants can be excluded from the analysis, leaving a small subset of variants to study and facilitating the analysis. Unlike other tools and protocols currently available, the described protocol efficiently examines the phenotypes associated with the genic location of a mutation by using phenotype analysis tools to efficiently provide a link between a variant and a set of phenotypes. This step prioritizes variants by phenotypic relevance quickly and efficiently, thus eliminating the need to manually research the phenotypes associated with

many genes. wANNOVAR outputs were not depicted here since this relies on Phenolyzer to determine relationships to phenotype, and was thus similar to the Phenolyzer output.

As in previous studies[23; 24], the PGM sequencing results appeared to have several erroneous calls, especially around homopolymer regions and INDELs. These errors have been attributed to the PGM 'Dark Sequencing' chemistry, which uses semiconductor chips to measure minute pH differences caused by the release of a hydrogen ion when a nucleotide attaches to a DNA template. In contrast, 'Light Sequencing' platforms such as the Illumina MiSeq and HiSeq platforms use high resolution cameras or sensors to detect wavelengths of light emitted when a reaction occurs, such as when a fluorescently labeled nucleotide attaches to a prepared DNA template[25]. Even with an optimized variant calling platform, the errors in PGM sequencing can lead to high false positive rates. It is likely that the high number of *de novo* mutations, most of which have a low quality score, were detected by the variant calling pipeline because of the small and sporadic occurrence of sequencing errors in homopolymer regions.

While mutations in *SCN8A* have been found to be associated with a similar syndrome[2], there is no entry in OMIM or ClinVar that identifies the discovered mutation as one associated with this disease. Therefore, this mutation should be added to the ClinVar database. Interestingly, mutation of the corresponding leucine residue in *SCN1A* resulted in impaired channel inactivation[26].

This study also reports the co-occurrence of an adverse reaction to vaccinations with a mutation in *SCN8A*. Mutations in *SCN1A*, a gene related to *SCN8A*, have been found to co-occur with adverse reactions to vaccinations[27; 28]. While a causal relationship has not been established, there is evidence to suggest that some vaccinations, specifically pertussis vaccinations, might trigger the onset of Dravet syndrome individuals with mutations in *SCN1A*[27; 29]. The acceleration of symptoms has been misinterpreted as 'vaccines causing autism,' leading to issues with the public's overall acceptance of inoculations[27; 28; 30]. In the future, when newborn exome and/or genome sequencing is more routine, it might be possible to recommend more astute administration of certain vaccines to children found to have these disease-contributory mutations in *SCN1A* and *SCN8A*. While there are several factors leading to a susceptibility to fractures, including a ketogenic diet often recommended for seizures[31], there appears to be a possible co-occurrence between a mutation in *SCN8A* and possible conditions involving bones. Further examining the relationship between *SCN8A* and bone tissue might uncover a previously unknown function of the gene.

METHODS

DNA ISOLATION AND SEQUENCING:

Genomic DNA was extracted using standard methods (Pure Gene, Qiagen, Valencia, CA). The Life Technologies Ampliseq Exome RDY kit (Thermo Fisher, Carlsbad, CA) was used to target the exon regions. 97% of CDCs with 5bp exon padding were amplified using 294,000 primer pairs. These products were sequenced using the Life Technologies Proton sequencing system with 200 bp reads using a P1V3 chip.

VARIANT CALLING:

The DNA sequence was aligned to the UCSC hg19 reference sequence and variants were called using the Torrent Suite software and the Torrent Variant caller. Only exonic variants and variants at the intron-exon boundary (1 or 2 nucleotides into the intron and 1 nucleotide into the exon) were reviewed. For each variant considered, depth of coverage was $\geq$10X and the quality score was $\geq$30. Ethnicity and variant frequency were considered during analysis. Analysis of the variants was conducted by two independent reviews using in-house protocols and two commercial software packages, Tute Genomics and Cartagenia v4.1. Pathogenic variants were confirmed by Sanger sequencing. ACMG reporting criteria were used to evaluate variants[32].

In additional analyses, binary alignment (BAM) files from the Ion Torrent Personal Genome Machine (PGM) platform were converted to FASTQ files. Variations in the sequenced DNA when compared to a reference genome (variants) were called for each family using the OTG-SNP Caller pipeline, which has been reported to have a substantially higher proportion of sequencing reads mapped to a reference genome, lower false positive rate, and overall lower error rate when analyzing sequences coming from the PGM platform [33]. Unlike other sequencing software and pipelines such as the Genome-Analysis-Toolkit (GATK) and Freebayes [34; 35], OTG-SNP Caller is specifically designed to take into account errors associated with PGM data, such as errors around homopolymers, thus increasing overall accuracy. Variants were aligned to the GRCh37 assembly, as several downstream analysis tools do not yet support the new GRCh38 assembly. A variant call format (VCF) file containing information about each mutation was then output [36]. After receiving this hardcoded pipeline from an author of the paper describing it, it was recoded, without any change to the function of the pipeline, in order to make it usable for this analysis. Analysis using this pipeline was then completed for the proband, his/her parents, and siblings on a computational cluster located on campus.

VARIANT SELECTION AND PRIORITIZATION:

The resulting VCF file for each individual in each family was then converted into ANNOVAR (avinput) files using ANNOVAR software, as the information is organized in a simpler manner. Avinput provides information regarding chromosome number, start position,

end position, reference nucleotide, alternate nucleotide, and quality scores for each variant [37]. All avinput files for a particular family were then loaded into a Python program, which performs set intersections using DataFrame functions from the Pandas library, and set functions using the Numpy library to identify *de novo* and autosomal recessive variants [38; 39]. Autosomal recessive variants were identified by isolating homozygous variants in the affected child, intersecting these variants with variants that were heterozygous in both parents, and subtracting variants that were homozygous in the siblings. *De novo* variants were identified by subtracting variants found in the parents and sibling from variants found in the proband, using the following set functions performed using Pandas and Numpy libraries in Python.

$$\text{Autosomal Reccessive} = [(\mathrm{M_{het}} \cap \mathrm{F_{het}}) \cap \mathrm{P_{hom}}] - \mathrm{SIB_{hom}}$$

$$\textit{de novo} = \mathrm{P_{all}} - \mathrm{M_{all}} - \mathrm{F_{all}} - \mathrm{SIB_{all}}$$

Where M refers to the mother's variants, F refers to father's variants, P refers to the proband's variants, and SIB refers to sibling'(s) variants. The subscript het refers to heterozygous variants, hom refers to homozygous variants, and all refers to all variants.

The columns examined included the chromosome number, start point, end point, and zygosity of each called variant. The resulting avinput files were then output as BED files, which contain columns providing chromosome number, start point, and end point of the mutation. This process ensured that the resulting BED files contained all autosomal recessive and *de novo* variants that could be determined from the VCF.

Using the GATK SelectVariants tool, these two BED files were intersected with the original VCF file two separate times, creating two VCF files, one containing only autosomal recessive variants, and one containing only *de novo* variants.

Both these VCF files were then annotated with the Variant Effect Predictor (VEP) software [40], which provided additional information about the variants. This annotated VCF file was then used with Genome Mining (GEMINI) software, which is a powerful, yet flexible network that allows for organization, sorting, and filtering of variants based on VEP and additional annotations. An SQL-type database was created and loaded into GEMINI. This database was then queried based on factors chosen by the author. After reviewing the GEMINI database schema to examine what annotations could be queried, factors were chosen based on three considerations: rarity, deleteriousness, and read quality. These factors were used as parameters during the GEMINI query.

Rarity was determined using the Exome Aggregation Consortum (ExAC) database, which contains population allele frequencies for exonic variants gathered from 60706 unrelated individuals with no history of 'severe pediatric disease[41]. Rare variants were considered to be variants not found in EXAC. Deleteriousness was mainly determined by Combined Annotation Dependent Depletion (CADD) scores, which encompasses 63 annotations to determine a variant's deleteriousness. CADD scores are based off PHRED quality scores; therefore a minimum CADD score of 20, corresponding to the top 1% most deleterious variants, was selected as a cutoff [42]. While the resulting quality scores from the OTG-SNP Caller pipeline did not correspond to the standard PHRED quality score, a minimum cutoff score of 120 was decided after comparing variant calls with their corresponding binary sequence alignment (BAM) files. Chromosome number, start point, and end point columns of variants that met these three requirements were queried using GEMINI, and the output was saved as a BED file.

The BED file, along with human phenotype ontology (HPO) numbers corresponding to the proband's phenotype were inputted into Phenolyzer, which aims to determine and prioritize which mutations contribute most to the phenotype by comparing the provided HPO numbers to the phenotypes attributed to the gene in which the proband's mutation is located [19]. A VCF file containing the same variants as the BED file used with Phenolyzer was then input into similar programs such as wANNOVAR and PhenIX in order to cross reference several sources [20; 21].

Additional deleteriousness scores for each variant of interest were also evaluated. Sorting Tolerant From Intolerant (SIFT) and Polymorphism Phenotyping (Polyphen) scores were evaluated for each variant, to confirm its predicted deleteriousness. Variants with a SIFT score less than 0.05 and a Polyphen Score of greater than .995 were considered deleterious.

CONFIRMATION OF VARIANTS:

Once a possible contributory mutation was identified, its location was then input into GoldenHelix GenomeBrowser, which displayed read information from the BAM files corresponding to each family. All variants of interest were researched thoroughly via Pubmed searches, and ruled out as major contributing mutations due to no association with a relevant phenotype.

The genic locations of each variant were identified using GEMINI. Initially, the known functions, phenotypes, and diseases associated with each gene, would be researched using the GeneCards online database, which contains information compiled from over 100 sources [43-45]. These results were then confirmed by researching the gene in other databases, such as NCBI, PubMed, and OMIM [46; 47]. These findings were also compared to the output of the

phenotype analysis software. No additional contributing mutations were identified in this individual. The GEMINI query selected no autosomal recessive mutations of interest and sixteen rare de novo mutations as mutations of interest.

Additional Information

Ethics Statement

Research was carried out in compliance with the Helsinki Declaration. The family was recruited to this study at the Utah Foundation for Biomedical Research (UFBR) where extensive clinical evaluation was performed. Written consent was obtained for phenotyping, use of facial photography, and whole-exome sequencing through Protocol #100 at the Utah Foundation for Biomedical Research, approved by the Independent Investigational Review Board, Inc.

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Conflict of interest

G.J.L serves on advisory boards for GenePeeks, Inc. and Omicia, Inc., and is a consultant to Good Start Genetics. K.W. is board member and shareholder of Tute Genomics, Inc. R.R. is employee, chief executive officer and shareholder of Tute Genomics, Inc.

VIDEO DESCRIPTIONS

VIDEO 1: SCN8A-Video1.mov (32 seconds) Father brings over the proband; she presents with a wide forehead (horizontally), and short forehead (vertically). She has a short, upturned nose, full cheeks, and a short philtrum. The father reports she has 10-15 seizures per day and that on some days, Leah is able to control her eyes. During the interview, she mostly stares to the side, and occasionally turns her eyes to the camera. Her mouth stays open during the interview, denoting hypotonic facies.

VIDEO 2: SCN8A-Video2.mov (12 seconds) Father describes proband's seizures usually consist of tonic for 30 seconds, and goes away, or continues with another 30 seconds of tonic/clonic seizure.

VIDEO 3: SCN8A-Video3.mov (39 seconds) Prior to video parents describe the proband met the developmental milestone of shaking a rattle, therefore the mother tries to show her how to do this. Proband is able to hold rattle but does not shake it independently. The mother reports she had two large seizures earlier that day so she may not shake the rattle on video.

VIDEO 4: SCN8A-Video4.mov (13 seconds) Proband in her stroller, she has global hypotonia and absent speech.

VIDEO 5: SCN8A-Video5.mov (31 seconds) Father of proband describes that prior to being on phenobarbital the proband would have 100s of focal seizure per day, sometimes they turn into actual seizures. Her current dose of phenobarbital is 10ml QPM, dose of carbomazopine has been reduced by half.

SUPPLEMENTARY FILES

1. VCF FILES

Brother1.vcf

Brother2.vcf

Father.vcf

Mother.vcf

Proband.vcf

Sister.vcf

2. EXCEL SPREADSHEETS ILLUSTRATING VAAST RESULTS AMONG THE VARIOUS QUADS

VAAST_Quad_K10035_SCN8A_AffectedProbandFemale_And_K10035_SCN8A_unaffected Brother1 .xlsx

VAAST_Quad_K10035_SCN8A_AffectedProbandFemale_And_K10035_SCN8A_unaffected Brother2 .xlsx

VAAST_Quad_K10035_SCN8A_AffectedProbandFemale_And_K10035_SCN8A_Unffected Sister.xlsx

3. Phenolyzer and PhenIX Outputs

FIGURES

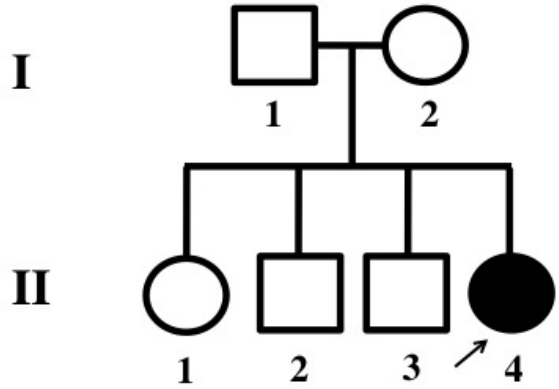

Figure 1: Pedigree: II-4, the affected proband (4-years-old), is the daughter of an unaffected, non-consanguineous couple. The proband has one older unaffected sister (14-years-old) and two older unaffected brothers (11-years-old and 7-years-old).

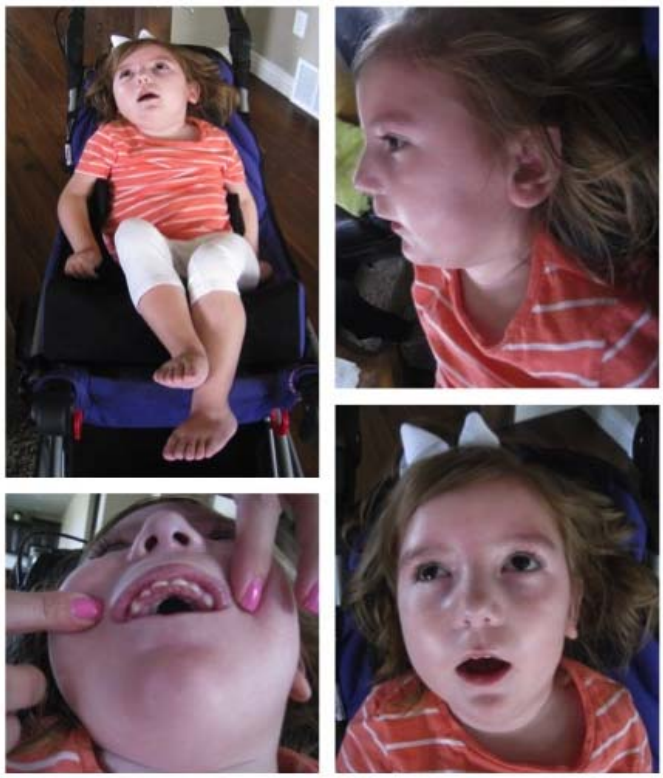

Figure 2: Pictures of phenotype of proband. Facial features include brachycephaly, broad forehead, broad nasal root, hypoplastic alae nasi, full cheeks, gingival hyperplasia, mild micrognathia and hypotonic facies.

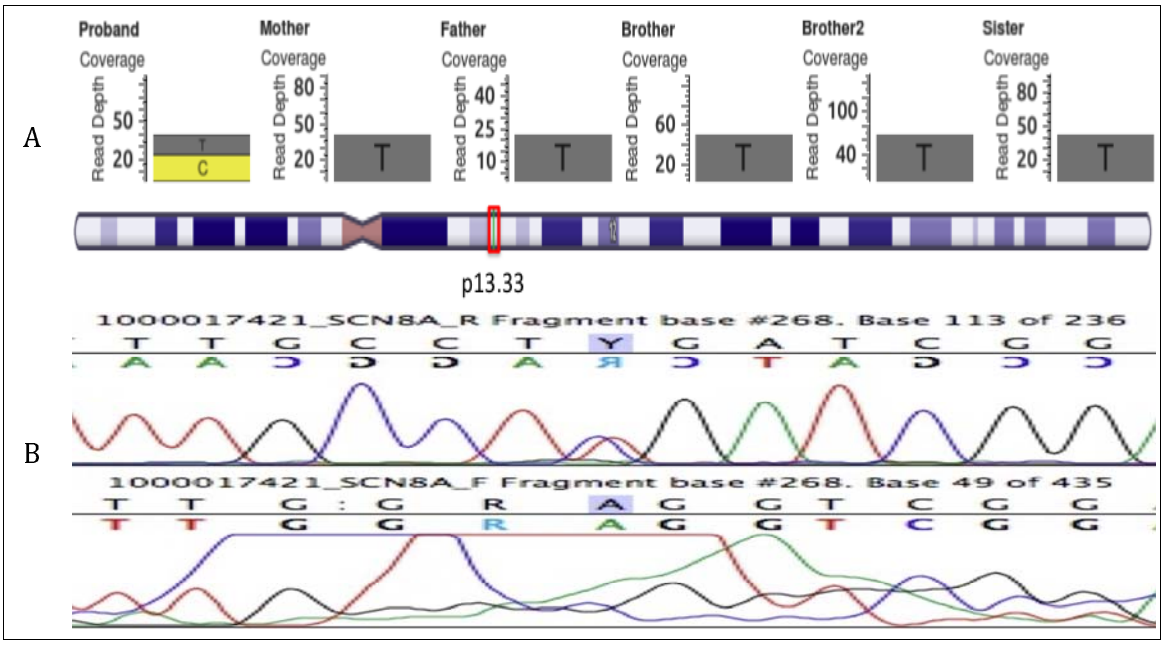

Figure 3: GenomeBrowse output for the mutation in *SCN8A.* The proband has a heterozygous C substitution in chromosome 12, position 52,093,447 in *SCN8A.* There are >20 reads covering the region, indicating that this is likely a true-positive mutation. None of the other family members appear to have this mutation, categorizing it as *de novo* (a). Sanger sequencing confirms the presence of the mutation (b).

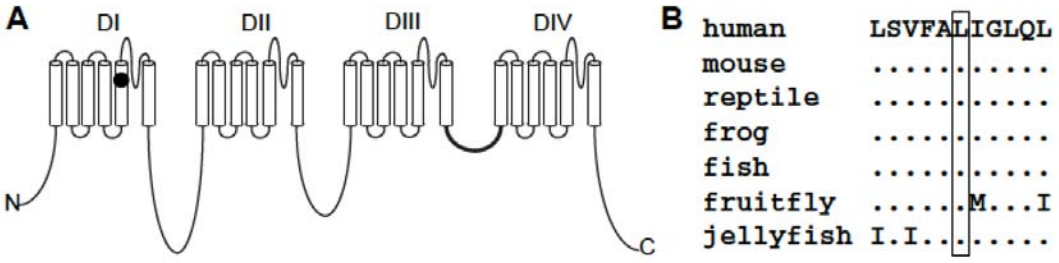

Figure 4: Mammals and invertebrate sodium channels genes also have a conserved leucine in this location of the gene. A) The site of the mutation is shown with a black circle. B) This residue is highly conserved during evolution of vertebrate and invertebrate sodium channels

Table 1. Summary of the Clinical Features of SCN8A

Features (Human Phenotype Ontology Nos.)	Proband
EPILEPSY	
Epileptic encephalopathy (HP:0200134)	+
Generalized tonic seizures (HP:0010818)	+
EEG abnormality (HP:0002353)	+
DEVELOPMENTAL/INTELLECTUAL DISABILITY	
Global developmental delay (HP:0001263)	+
Developmental stagnation at onset of seizures (HP:0006834)	+
Developmental regression (HP:0002376)	+
Absent speech (HP:0001344)	+
Intellectual disability, severe (HP:0010864)	+
Motor delay (HP:0001270)	+
NEUROLOGICAL	
Generalized hypotonia (HP:0001290)	+
Appendicular hypotonia (HP:0012389)	+
Infantile axial hypotonia (HP:0009062)	+
Neck muscle weakness (HP:0000467)	+
Rigidity (HP:0002063)	+
Spasticity (HP:0001257)	+
GROWTH/FEEDING	
Failure to thrive in infancy (HP:0001531)	+
Gastrostomy tube feeding in infancy (HP:0011471)	+
Gastroesophageal reflux (HP:0002020)	+
Dysphagia (HP:0002015)	+
RESPIRATORY	
Respiratory difficulties (HP:0002880)	+
Hypoxemia (HP:0012418)	+
Abnormality of the tonsils (HP:0100765)	+
Obstructive sleep apnea (HP:0002870)	+
DYSMORPHISM	
Brachycephaly (HP:0000248)	+
Broad forehead (HP:0000337)	+
Broad Nasal Root or Bridge (HP:0000431)	+
Hypoplastic Alae Nasi (HP:0000430)	+
Full cheeks (HP:0000293)	+
Gingival Hyperplasia (HP:0000212)	+
Micrognathia (Mild) (HP:0000347)	+
Hypotonic Facies	+
NEUROLOGICAL	
Exaggerated startle response (HP:0002267)	+
Action tremor (HP:0002345)	+
Blepharospasm (HP:0000643)	+
Bulbar palsy (HP:0001283)	+
Nystagmus (HP:0000639)	+
MISCELLANEOUS	
Hyperreexia (HP:0001347)	+
No social interaction (HP:0008763)	+

REFERENCES

1. O'Brien, J.E., and Meisler, M.H. (2013). Sodium channel SCN8A (Nav1.6): properties and de novo mutations in epileptic encephalopathy and intellectual disability. Front Genet 4, 213.
2. Wagnon, J.L., and Meisler, M.H. (2015). Recurrent and Non-Recurrent Mutations of SCN8A in Epileptic Encephalopathy. Front Neurol 6, 104.
3. Vaher, U., Noukas, M., Nikopensius, T., Kals, M., Annilo, T., Nelis, M., Ounap, K., Reimand, T., Talvik, I., Ilves, P., et al. (2014). De novo SCN8A mutation identified by whole-exome sequencing in a boy with neonatal epileptic encephalopathy, multiple congenital anomalies, and movement disorders. J Child Neurol 29, NP202-206.
4. Larsen, J., Carvill, G.L., Gardella, E., Kluger, G., Schmiedel, G., Barisic, N., Depienne, C., Brilstra, E., Mang, Y., Nielsen, J.E., et al. (2015). The phenotypic spectrum of SCN8A encephalopathy. Neurology 84, 480-489.
5. Veeramah, K.R., O'Brien, J.E., Meisler, M.H., Cheng, X., Dib-Hajj, S.D., Waxman, S.G., Talwar, D., Girirajan, S., Eichler, E.E., Restifo, L.L., et al. (2012). De novo pathogenic SCN8A mutation identified by whole-genome sequencing of a family quartet affected by infantile epileptic encephalopathy and SUDEP. Am J Hum Genet 90, 502-510.
6. Ohba, C., Kato, M., Takahashi, S., Lerman-Sagie, T., Lev, D., Terashima, H., Kubota, M., Kawawaki, H., Matsufuji, M., Kojima, Y., et al. (2014). Early onset epileptic encephalopathy caused by de novo SCN8A mutations. Epilepsia 55, 994-1000.
7. de Kovel, C.G., Meisler, M.H., Brilstra, E.H., van Berkestijn, F.M., van 't Slot, R., van Lieshout, S., Nijman, I.J., O'Brien, J.E., Hammer, M.F., Estacion, M., et al. (2014). Characterization of a de novo SCN8A mutation in a patient with epileptic encephalopathy. Epilepsy Res 108, 1511-1518.
8. Blanchard, M.G., Willemsen, M.H., Walker, J.B., Dib-Hajj, S.D., Waxman, S.G., Jongmans, M.C., Kleefstra, T., van de Warrenburg, B.P., Praamstra, P., Nicolai, J., et al. (2015). De novo gain-of-function and loss-of-function mutations of SCN8A in patients with intellectual disabilities and epilepsy. Journal of Medical Genetics, 1-8.
9. Estacion, M., O'Brien, J.E., Conravey, A., Hammer, M.F., Waxman, S.G., Dib-Hajj, S.D., and Meisler, M.H. (2014). A novel de novo mutation of SCN8A (Nav1.6) with enhanced channel activation in a child with epileptic encephalopathy. Neurobiol Dis 69, 117-123.
10. Wagnon, J.L., Barker, B.S., Hounshell, J.A., Haaxma, C.A., Shealy, A., Moss, T., Parikh, S., Messer, R.D., Patel, M.K., and Meisler, M.H. (2016). Pathogenic mechanism of recurrent mutations of SCN8A in epileptic encephalopathy. Ann Clin Transl Neurol 3, 114-123.
11. Lyon, G.J., and O'Rawe, J. (2015). Human genetics and clinical aspects of neurodevelopmental disorders. In The Genetics of Neurodevelopmental Disorders, K. Mitchell, ed. (Wiley).
12. O'Rawe, J., Jiang, T., Sun, G., Wu, Y., Wang, W., Hu, J., Bodily, P., Tian, L., Hakonarson, H., Johnson, W.E., et al. (2013). Low concordance of multiple variant-calling pipelines: practical implications for exome and genome sequencing. Genome Medicine 5, 28.
13. O'Rawe, J.A., Ferson, S., and Lyon, G.J. (2015). Accounting for uncertainty in DNA sequencing data. Trends in genetics : TIG 31, 61-66.

14. Bamshad, M.J., Ng, S.B., Bigham, A.W., Tabor, H.K., Emond, M.J., Nickerson, D.a., and Shendure, J. (2011). Exome sequencing as a tool for Mendelian disease gene discovery. Nature Reviews Genetics 12, 745-755.
15. Kong, A., Frigge, M.L., Masson, G., Besenbacher, S., Sulem, P., Magnusson, G., Gudjonsson, S.a., Sigurdsson, A., Jonasdottir, A., Jonasdottir, A., et al. (2012). Rate of de novo mutations and the importance of father's age to disease risk. Nature 488, 471-475.
16. Paila, U., Chapman, B.a., Kirchner, R., and Quinlan, A.R. (2013). GEMINI: Integrative Exploration of Genetic Variation and Genome Annotations. PLoS Computational Biology 9.
17. Ng, P.C., and Henikoff, S. (2003). SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res 31, 3812-3814.
18. Adzhubei, I., Jordan, D.M., and Sunyaev, S.R. (2013). Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet Chapter 7, Unit7 20.
19. Yang, H., Robinson, P.N., and Wang, K. (2015). Phenolyzer: phenotype-based prioritization of candidate genes for human diseases. Nat Methods 12, 841-843.
20. Chang, X., and Wang, K. (2012). wANNOVAR: annotating genetic variants for personal genomes via the web. J Med Genet 49, 433-436.
21. Zemojtel, T., Köhler, S., Mackenroth, L., Jäger, M., Hecht, J., Krawitz, P., Graul-Neumann, L., Doelken, S., Ehmke, N., and Spielmann, M. (2014). Effective diagnosis of genetic disease by computational phenotype analysis of the disease-associated genome. Science translational medicine 6, 252ra123-252ra123.
22. Dyment, D.a., Tetreault, M., Beaulieu, C.L., Hartley, T., Ferreira, P., Warman Chardon, J., Marcadier, J., Sawyer, S.L., Mosca, S.J., Micheil Innes, a., et al. (2014). Whole-exome sequencing broadens the phenotypic spectrum of rare pediatric epilepsy: A retrospective study. Clinical genetics, 34-40.
23. Salipante, S.J., Kawashima, T., Rosenthal, C., Hoogestraat, D.R., Cummings, L.A., Sengupta, D.J., Harkins, T.T., Cookson, B.T., and Hoffman, N.G. (2014). Performance comparison of Illumina and ion torrent next-generation sequencing platforms for 16S rRNA-based bacterial community profiling. Appl Environ Microbiol 80, 7583-7591.
24. Bragg, L.M., Stone, G., Butler, M.K., Hugenholtz, P., and Tyson, G.W. (2013). Shining a light on dark sequencing: characterising errors in Ion Torrent PGM data. PLoS Comput Biol 9, e1003031.
25. Bragg, L.M., Stone, G., Butler, M.K., Hugenholtz, P., and Tyson, G.W. (2013). Shining a Light on Dark Sequencing: Characterising Errors in Ion Torrent PGM Data. PLoS Computational Biology.
26. Kahlig, K.M., Rhodes, T.H., Pusch, M., Freilinger, T., Pereira-Monteiro, J.M., Ferrari, M.D., van den Maagdenberg, A.M., Dichgans, M., and George, A.L., Jr. (2008). Divergent sodium channel defects in familial hemiplegic migraine. Proc Natl Acad Sci U S A 105, 9799-9804.
27. Berkovic, S.F., Harkin, L., McMahon, J.M., Pelekanos, J.T., Zuberi, S.M., Wirrell, E.C., Gill, D.S., Iona, X., Mulley, J.C., and Scheffer, I.E. (2006). De-novo mutations of the sodium channel gene SCN1A in alleged vaccine encephalopathy: a retrospective study. Lancet Neurol 5, 488-492.
28. Verbeek, N.E., van der Maas, N.A., Jansen, F.E., van Kempen, M.J., Lindhout, D., and Brilstra, E.H. (2013). Prevalence of SCN1A-related dravet syndrome among

children reported with seizures following vaccination: a population-based ten-year cohort study. PLoS One 8, e65758.
29. Scheffer, I.E. (2015). Vaccination Triggers, Rather Than Causes, Seizures. Epilepsy Curr 15, 335-337.
30. Zamponi, N., Passamonti, C., Petrelli, C., Veggiotti, P., Baldassari, C., Verrotti, A., Capovilla, G., Viri, M., Coppola, G., and Vignoli, A. (2014). Vaccination and Occurrence of Seizures in SCN1A Mutation–Positive Patients: A Multicenter Italian Study. Pediatric neurology 50, 228-232.
31. Bergqvist, A.G., Schall, J.I., Stallings, V.A., and Zemel, B.S. (2008). Progressive bone mineral content loss in children with intractable epilepsy treated with the ketogenic diet. Am J Clin Nutr 88, 1678-1684.
32. Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W.W., Hegde, M., Lyon, E., Spector, E., et al. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17, 405-424.
33. Zhu, P., He, L., Li, Y., Huang, W., Xi, F., Lin, L., Zhi, Q., Zhang, W., Tang, Y.T., Geng, C., et al. (2014). OTG-snpcaller: an optimized pipeline based on TMAP and GATK for SNP calling from ion torrent data. PLoS One 9, e97507.
34. Garrison, E., and Marth, G. (2012). Haplotype-based variant detection from short-read sequencing. arXiv preprint arXiv:12073907.
35. McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., and Daly, M. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome research 20, 1297-1303.
36. Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., et al. (2011). The variant call format and VCFtools. Bioinformatics 27, 2156-2158.
37. Wang, K., Li, M., and Hakonarson, H. (2010). ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38, e164.
38. McKinney, W. (2010). Data structures for statistical computing in Python. In Proceedings of the 9th. pp 51-56.
39. Van Der Walt, S., Colbert, S.C., and Varoquaux, G. (2011). The NumPy array: a structure for efficient numerical computation. Computing in Science & Engineering 13, 22-30.
40. McLaren, W., Pritchard, B., Rios, D., Chen, Y., Flicek, P., and Cunningham, F. (2010). Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. Bioinformatics 26, 2069-2070.
41. Consortium, E.A., Lek, M., Karczewski, K., Minikel, E., Samocha, K., Banks, E., Fennell, T., O'Donnell-Luria, A., Ware, J., Hill, A., et al. (2015). Analysis of protein-coding genetic variation in 60,706 humans. bioRxiv.
42. Kircher, M., Witten, D.M., Jain, P., O'Roak, B.J., Cooper, G.M., and Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet 46, 310-315.
43. Dierking, A., and Schmidtke, J. (2014). The future of Clinical Utility Gene Cards in the context of next-generation sequencing diagnostic panels. Eur J Hum Genet 22, 1247.

44. Rebhan, M., Chalifa-Caspi, V., Prilusky, J., and Lancet, D. (1998). GeneCards: a novel functional genomics compendium with automated data mining and query reformulation support. Bioinformatics 14, 656-664.
45. Stelzer, G., Dalah, I., Stein, T.I., Satanower, Y., Rosen, N., Nativ, N., Oz-Levi, D., Olender, T., Belinky, F., and Bahir, I. (2011). In-silico human genomics with GeneCards. Hum Genomics 5, 709-717.
46. Brown, G.R., Hem, V., Katz, K.S., Ovetsky, M., Wallin, C., Ermolaeva, O., Tolstoy, I., Tatusova, T., Pruitt, K.D., and Maglott, D.R. (2015). Gene: a gene-centered information resource at NCBI. Nucleic acids research 43, D36-D42.
47. Hamosh, A., Scott, A.F., Amberger, J.S., Bocchini, C.A., and McKusick, V.A. (2005). Online Mendelian Inheritance in Man (OMIM), a knowledgebase of human genes and genetic disorders. Nucleic acids research 33, D514-D517.