



Rapid whole-genome sequencing identifies a novel *GABRA1* variant associated with West syndrome

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Abstract A 9-mo-old infant was admitted with infantile spasms that improved on administration of topiramate and steroids. He also had developmental delay, esotropia, and hypsarrhythmia on interictal electroencephalogram (EEG), and normal brain magnetic resonance imaging (MRI). West syndrome is the triad of infantile spasms, interictal hypsarrhythmia, and mental retardation. Rapid trio whole-genome sequencing (WGS) revealed a novel, likely pathogenic, de novo variant in the gene encoding γ -aminobutyric acid (GABA) type A receptor, $\alpha 1$ polypeptide (*GABRA1* c.789G>A, p.Met263Ile) in the proband. *GABRA1* mutations have been associated with early infantile epileptic encephalopathy type 19 (EIEE19). We suggest that *GABRA1* p.Met263Ile is associated with a distinct West syndrome phenotype.

[Supplemental material is available for this article.]

CASE PRESENTATION

The patient was a 9-mo-old male born at term via repeat Cesarean section without complications. He had been noted to have right-sided esotropia since 4 mo of age. The patient was referred to a metabolic clinic because of elevated plasma lactate on three occasions (4.3–6.0 mmol/l [reference range <2.2 mmol/l]) at 5 mo of age, but this had normalized (1.3 mmol/l) when seen in metabolic clinic the following month. Pyruvic acid and ammonia were also normal at the time of evaluation, but creatinine kinase was slightly elevated at 150 U/l. He had neurodevelopmental delay evidenced by poor head control and inability to roll over independently at 6 mo of age. The clinician evaluating him at 6 mo of age was unable to fully extend his upper and lower extremities because of hypertonia. (See Table 1.) The patient had a normal brain magnetic resonance imaging (MRI). At 9 mo of age he was brought to the Rady Children's Hospital–San Diego emergency department because of concern over abnormal involuntary movements. He had been developing decreasing responsiveness for 10 d and developed an elevated temperature (102°F) after which the abnormal movements increased in frequency. At the time of admission he had a mildly elevated plasma lactate (3.1 mmol/l). The patient's fever resolved within 24 h without an identified infectious etiology. A video electroencephalogram (EEG) at admission to the Pediatric Intensive Care Unit showed evidence of infantile spasms (Supplemental Data 1). These were treated with high-dose intravenous methylprednisolone, and stopped after 1 d. A brain MRI with spectroscopy and spine MRI were normal. The patient's lactate normalized and he had a

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Ontology terms: developmental regression; generalized neonatal hypotonia; infantile spasms

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Table 1. Phenotypic features

Early infantile epileptic encephalopathy -19	Proband (II-1)	Comments
Onset of seizures between ages 8 and 11 months	Yes	
Hemiclonic seizures	No	
Tonic-clonic seizures	No	
Focal dyscognitive seizures	No	
Myoclonic seizures	No	
Absence seizures	No	
Atonic seizures	No	
Status epilepticus	No	
Febrile sensitivity to seizures	Yes	Not febrile seizures
Intellectual disability	Yes	Developmental delay and regression
EEG with generalized spike-wave discharges or focal discharges	Yes	
Calcified ependymal nodule on MRI	No	
Novel clinical features		
Infantile spasms	Yes	Not mentioned in OMIM, but reported in one case (Kodera et al. 2016)
Esotropia	Yes	
Infantile muscular hypertonia	Yes	Probably secondary to infantile spasms (Díaz Negrillo et al. 2011; Raviglione et al. 2016)

The list of clinical features are based on the OMIM clinical synopsis related to the GABRA1 gene (#615744; Epileptic encephalopathy, early infantile, 19). No features on the list were noted in either parent. EEG, electroencephalogram; MRI, magnetic resonance imaging; OMIM, Online Mendelian Inheritance in Man.

complete metabolic workup during admission, which was unremarkable (Supplemental Data 2). The patient and his parents underwent rapid trio whole-genome sequencing (WGS) to determine the spasm etiology. He was discharged on oral steroids and topiramate. One week later he had a repeat video EEG that showed frequent, irregular, short sets of high-voltage, biposterior, Δ waves in the awake state, in the absence of seizures.

The patient has an unaffected 6-yr-old brother. His parents are both 34 yr old. His maternal and paternal families were from India, with no known consanguinity. There were no other family members with childhood health issues.

TECHNICAL ANALYSIS AND METHODS

A chromosomal microarray (Quest Diagnostics) revealed a likely benign 61-kb interstitial deletion at Chr 2p13.3, which encompasses the genes encoding F-box protein 48 (FBX048), aprataxin (APTX), and PNKP-like factor (APLF).

Four days after consent for trio WGS, DNA was extracted and sequenced using an Illumina HiSeq X with paired 151-nt reads. Sequencing was completed 12 d after consent. Rapid alignment and nucleotide variant calling was performed using the DRAGEN (Edico Genome) hardware and software (Miller et al. 2015). The yield was 176.2, 180.8, and 161.5 Gb for the proband, mother, and father, respectively, resulting in 4,531,103, 4,888,177, and 4,868,923 distinct variant calls, respectively (Table 2).

Table 2. Sequencing data

	Proband	Mother	Father	Units	Reference range
Sex/trio order	M verified	F verified	M verified		Trio order verified
Yield: raw/bulk	176.2	180.8	161.5	Gb	>180
Percent of mapped	98.95%	98.91%	98.98%	pct	98–100
Percent of duplicates	11.21%	9.25%	10.97%	pct	<15%
Yield	154.9	162.4	142.3	Gb	>130
Insert size: mean ± SD	362 ± 128	373 ± 122	360 ± 112	bp	300–480
Average and median coverage across genome	46.0	48.0	43.0	x	>40
Average coverage over OMIM genes	47.0	48.0	44.0	x	>40
Number of OMIM genes with coverage at <10× (and list)	268	241	>292	ENST	<2% (282)
Number of OMIM genes with 100% coverage at ≥10×	98.1%	98.3%	97.9%	pct	>98%
Number of OMIM genes with 100% coverage at ≥20×	93.4%	97.0%	92.9%	pct	>94%
Number of OMIM genes with 100% coverage at ≥30×	84.1%	88.9%	75.9%	pct	>80%
Number of genes with 100% coverage at ≥40×	31.5%	37.1%	15.6%		
Variation (VCF) metrics					
Number of calls (Total)	4,853,103	4,888,177	4,868,923		2.5–6.0 × 10 ⁶
Number of PASS calls	4,760,785	4,821,561	4,772,816		2.5–6.0 × 10 ⁶
Number of calls total coding	26,917	27,093	27,282		25,000–30,000
Total number of SNVs	3,982,176 [82.05%]	4,010,837 [82.05%]	3,998,391 [82.12%]		
Total number of Indels	870,927 [17.95%]	877,340 [17.95%]	870,532 [17.88%]		
Hom/Het ratio (in coding regions)	0.58 (0.61)	0.57 (0.59)	0.58 (0.58)	Ratio	0.5–0.61
Ti/Tv ratio (in coding regions)	2.04 (2.94)	2.04 (2.92)	2.04 (2.94)	Ratio	2–2.2 (2.8–3)
Number of het calls (Number of hom calls)	3,107,938 (18,498,22)	3,205,140 (1,789,679)	3,128,433 (1,842,666)	Units	
In silico sample swap check	PASS	PASS	PASS		Mendelian test
Automated upload of VCF to Omicia	PASS	PASS	PASS		
Inform sign-out of analysis-ready state	PASS	PASS	PASS		
Detect sample analysis completion state on Omicia	PASS	PASS	PASS		

SD, standard deviation; OMIM, Online Mendelian Inheritance in Man; VCF, variant call format; SNVs, single-nucleotide variants; Hom/Het, homogeneous/heterogeneous; Ti/Tv, transition/transversion.

Variants were annotated and analyzed in Opal Clinical (Omicia) (Coonrod et al. 2013). Initially, variants were filtered to retain those with allele frequencies of <1% in the Exome Variant Server, 1000 Genomes samples, and Exome Aggregation Consortium database (<http://evs.gs.washington.edu/EVS/>. 2016; Karczewski et al. 2016). A differential diagnostic gene list was built in Phenolyzer (Yang et al. 2015) using Human Phenotype Ontology (HPO) (Köhler et al. 2016) and Systematized Nomenclature of Medicine—Clinical Terms (SNOMED-CT(SNOMED)). The following codes were used HP:0012469 (infantile spasms, SCTID: 28055006), HP:0001250 (seizures, SCTID: 91175000), HP:0002376 (developmental regression, SCTID: 248290002), HP:0000565 (esotropia, SCTID: 16596007), and HP:0008947 (infantile muscular hypotonia, SCTID: 205294008) (hypotonia was used in the initial

Table 3. Genomic findings

Gene	Genomic location	HGVS cDNA	HGVS protein	Zygosity	Parent of origin	Variant interpretation
GABRA1	NC_000005.10 (on Assembly GRCh38): g.161890983G>A	NM_000806.5: c.789G>A	NP_000797.2 p.Met263Ile	Heterozygous	De novo	Likely pathogenic

HGVS, Human Genome Variation Society.

evaluation rather than hypertonia due to the prolonged history of poor head control), yielding 1465 genes (Supplemental Data 3). Research on the optimal number of phenotypic findings to use for creation of a gene list is ongoing at RCIGM; until research is complete we are including all relevant phenotypic findings. Variants were further filtered to retain those mapping to these 1465 genes, yielding 76 proband calls (six homozygous variants, 63 heterozygous inherited variants, and seven heterozygous de novo variants) (see Supplemental Data 4). Manual curation revealed one of these as pathogenic (one strong, two moderate, and two supporting criteria) (Supplemental Data 5, 6) by American College of Medical genetics and Genomics (ACMG) guidelines (Richards et al. 2015). However, as per laboratory protocol a designation of “likely” pathogenic was reported, as this nucleotide variant had not previously been reported in any other affected individuals. (See Table 3.)

VARIANT INTERPRETATION

This patient was heterozygous for a novel likely pathogenic c.789G>A (p.Met263Ile) variant in the gene encoding the γ -aminobutyric acid (GABA) type A receptor, $\alpha 1$ polypeptide gene (GABRA1). GABA is the major inhibitory neurotransmitter in the mammalian brain. It acts at GABA-A receptors, which are ligand-gated chloride channels (Carvill et al. 2014). This gene is associated with generalized epilepsy (Lachance-Touchette et al. 2011), juvenile myoclonic epilepsy (Cossette et al. 2002), absence epilepsy (Maljevic et al. 2006), and early infantile epileptic encephalopathy type 19 (EIEE19), which is autosomal dominant, with the onset of recurrent seizures in childhood or adolescence (Epi4K Consortium et al. 2013). Affected individuals can have a wide variety of seizures, including febrile sensitivity to seizures, as in the present case. Esotropia has not previously been associated with EIEE19. GABRA1 was 538th on the 1465 Phenolyzer gene list (Score 0.002), and had a probability of match to GABRA1 of 0.016 when the same HPO terms were analyzed in Phenomizer (Köhler et al. 2009). GABRA1 had a haploinsufficiency score of 0.46 and a gene intolerance score of 0.76.

A different variant at the same codon (c.788T>C, p.Met263Thr) has been previously reported to be likely pathogenic (ClinVar Accession number RCV000187499.2). Furthermore, a missense variant at a different nucleotide in the same codon (c.789G>C), which resulted in the same amino acid change (p.Met263Ile) as the current patient, was reported as pathogenic in a patient with West syndrome (Kodera et al. 2016). The latter patient did not receive a molecular diagnosis until age 9. However, that patient had also shown hypsarrhythmia on EEG at the age of 6 mo. That patient had normal development until the age of 6 mo and then developed developmental delay. That patient also had a normal brain MRI.

The provisional diagnosis was made within 6 h of completion of the sequencing run (see Supplemental Figure 7 for current institute workflow). The research protocol under which the trio received rapid WGS was approved by the Food and Drug Administration, and the local Institutional Review Board requires confirmation by a clinically accepted standard before

reporting, except in cases of actionable diagnoses in which major morbidity or likelihood of mortality is likely during confirmatory testing. EIEE19 is an actionable diagnosis, but there was very low likelihood of major morbidity or mortality during confirmatory testing. Therefore, the clinical team was notified of the result after Sanger confirmation (day 28).

The diagnosis explained the etiology of the infant's seizures, and confirmed that the antiepileptic regimen was appropriate. The infant did well on steroids and topiramate and was gradually weaned off steroids. Several specific GABA-A receptor agonists and modulators have been developed as anticonvulsants and may represent potentially more targeted therapies for patients with refractory EIEE19 than topiramate (García-Flores and Farías 1997).

SUMMARY

GABRA1 p.Met263Ile resulting from c.789G>A (reported herein) or c.789G>C (reported previously) appears to be associated with a distinct EIEE19 phenotype that fits the definition of West syndrome (triad of infantile spasms, interictal hypsarrhythmia, and mental retardation): both patients had infantile spasms (clinical diagnosis in the present patient, previous patient with hypsarrhythmia on EEG) between 6 and 9 mo of age (Kodera et al. 2016). Both patients had normal MRIs and significant developmental delay. The rapidity of provisional diagnosis by rapid WGS has the potential to enable precision medicine for genetically heterogeneous infantile seizure disorders. A rapid diagnosis may be able to avoid further or unnecessary diagnostic steps. In this case the patient did not have a muscle biopsy to diagnose a mitochondrial disorder and the diagnostic odyssey was brief. If the patient had been referred to us before his extensive metabolic workup, extensive blood tests and laboratory work could have been avoided. The prior reported case with this same protein change had hypsarrhythmia detected at 6 mo of age, but the molecular etiology was not determined until he was 9 yr old. Although the medical therapy was not changed because of sequencing in this case, further workup for treatable causes was avoided. In addition, a firm diagnosis reassured the clinical team and family that the child was on the right therapy.

ADDITIONAL INFORMATION

Data Deposition and Access

Sequencing data has been submitted to DNAnexus (<https://platform.dnanexus.com/projects/F670ff00gp1K0Z1x4Yx84VXj/data/WestSyndrome>). Variant was submitted to ClinVar and can be found under accession number SCV000494287.1 (<https://www.ncbi.nlm.nih.gov/clinvar>).

Ethics Statement

Informed consent was obtained for all individuals in this study, both to undertake rapid whole-genome sequencing and to publish these findings. The study was approved by Institutional Review Board of the University of California at San Diego under protocol #160468 and has received nonsignificant risk status in a pre-Investigational Device Exemption submission to the Food and Drug Administration.

Author Contributions

L.F. contributed to manuscript preparation and phenotyping; S.A.N. contributed to variant interpretation and manuscript preparation; S.C. contributed to variant interpretation and manuscript preparation; J.N. contributed to clinical implementation and manuscript

preparation; D.M.D. contributed to supervision and manuscript preparation; S.B. contributed to Bioinformatics; S.F.K. contributed to supervision and manuscript preparation. RCIGM Investigators contributed to process development, infrastructure deployment, and maintenance. All authors contributed to the reviewing of the final version.

Competing Interest Statement

The authors have declared no competing interest.

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