HHS Public Access

Author manuscript

Eur J Med Genet. Author manuscript; available in PMC 2023 January 01.

Published in final edited form as:

Eur J Med Genet. 2022 January ; 65(1): 104403. doi:10.1016/j.ejmg.2021.104403.

Further evidence of affected females with a heterozygous variant in *FGF13* causing X-linked developmental and epileptic encephalopathy 90

Dhanya Lakshmi Narayanan[1], **Purvi Majethia**[1], **Aroor Shrikiran**[2], **Shahyan Siddiqui**[3], **Ashwin Dalal**[4], **Anju Shukla**[1]

[1]Department of Medical Genetics, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, India.

[2]Department of Pediatrics, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, India.

[3]Department of Neuroimaging and Interventional Radiology, STAR Institute of Neurosciences, STAR Hospitals, Hyderabad, India.

[4]Diagnostics Division, Center for DNA Fingerprinting and Diagnostics, Hyderabad, India.

Abstract

Developmental and epileptic encephalopathies (DEE) are a genetically heterogeneous group of disorders characterised by early onset epilepsy, epileptiform activity on electroencephalogram and associated developmental delay or neuroregression. With the advent of high throughput sequencing, novel gene-disease associations have been described for DEEs. Voltage activated sodium channels (Na_v) regulate neuronal excitability. Fibroblast growth factor homologous factors (FHFs) are proteins, which bind to the C terminal cytoplasmic tails of alpha subunits of Na_v channels and influence their function and surface expression. Gain of function hemizygous or heterozygous variants in *FGF13* (also known as *FHF2*) were recently identified as the cause for X-linked developmental and epileptic encephalopathy 90 (DEE90; MIM# 301058) in seven individuals from five families, which included one female. We report an additional female, providing further evidence for a novel *de novo* heterozygous missense variant in *FGF13*, NM_004114.5: c.14T>G p.(Ile5Ser) causing X-linked DEE90. In addition, we review the genotype and phenotype of affected individuals with DEE90.

Keywords

FHFs; sodium channels; seizure; EEG; FGFs; gain of function

Address for correspondence Dr Anju Shukla, Department of Medical Genetics, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal- 576104, Tel No.: +91 820-2923264, Fax No.: +91 820-2571934, anju.shukla@manipal.edu.

Introduction

Fibroblast growth factor homologous factors (FHFs) are proteins with different functions but related to fibroblast growth factors (FGFs) in structure and sequence (Goldfarb et al., 2007). FHFs were found to be expressed in nervous system and were postulated to play a role in nervous system development (Smallwood et al., 1996). Voltage activated sodium channels (Na_v) play a major role in regulating neuronal excitability (Kaplan, Isom, & Petrou, 2016). FHFs bind to the C-terminal cytoplasmic tails of alpha subunit of voltage activated sodium channels (Na_v) and influence their function and surface expression (Liu, Dib-Hajj, Renganathan, Cummins, & Waxman, 2003).

There are four different types of FHFs: FHF1, FHF2, FHF3 and FHF4, encoded by *FGF12, FGF13, FGF11* and *FGF14* (Goldfarb 2005). Each of the FHF genes has multiple transcription initiation sites, which generates multiple proteins with different amino acid terminal sequences (Goldfarb, 2005), (Munoz-Sanjuan, Smallwood, & Nathans, 2000). Fibroblast growth factor 13 (*FGF13*; MIM 300070), also known as *FHF2*, encodes intracellular proteins that binds to the C-terminal domain of the voltage-activated sodium channels (Na_v) and plays an essential role in its transport and function. FGF13 (FHF2) has five isoforms generated by alternate start sites of transcription and splicing of 5' exons (Munoz-Sanjuan et al., 2000). The FHF2 proteins are expressed in embryonic and adult neuronal cells. FHF2 also co-localises with voltage activated sodium channels in the nodes of Ranvier along the myelinated axons (Wittmack et al., 2004).

Gain-of-function hemizygous or heterozygous variants in *FGF13* were recently identified as the cause for X-linked developmental and epileptic encephalopathy 90 (DEE90; MIM# 301058) in seven individuals from five families, which included one female (Fry et al., 2021). We herein report an additional female, providing further evidence for a novel *de novo* heterozygous missense variant in *FGF13*, c.14T>G p.(Ile5Ser) causing X-linked DEE90. We also review the phenotype and genotype of individuals with DEE90.

Clinical report:

A one-year-four-months-old girl (IV-4), the third born of non-consanguineous parents, was evaluated for recurrent seizures and regression of milestones noticed since eight months of age (Fig. 1A). Her mother had an uneventful antenatal period. There was no history of any teratogenic exposure. She was born at term by lower segment caesarean section. She weighed 2.86kg at birth (−0.75SD). Her length at birth was 47cm (−1.14SD) and head circumference was 31.5cm (−2SD). She cried immediately and did not have any postnatal complications. She attained neck holding and social smile at three months of age. She started sitting with support at seven months and sat independently by eight months of age. She also could speak monosyllables by seven months of age. Parents had noticed that she had excessive cry since three months of age. At five months of age, she developed tonic-clonic seizures of left upper and lower limbs. She had 2–3 episodes of seizures every day, each lasting for approximately one minute. She did not have associated fever, myoclonic seizures or generalised tonic-clonic seizures. She was hospitalised and evaluation showed microcytic hypochromic anemia and elevated plasma lactate level (33.3mg/dL; ref: 4.5–19.8). Her plasma ammonia was 44.3 microgram/dL (Ref: 18.7–86) and pyruvate was 0.45

mg/dL(Ref: 0.2–0.7). Electroencephalograph (EEG) showed sleep activation of occasional left temporal spikes. Magnetic Resonance Imaging (MRI) of brain, at five months of age, showed prominent frontotemporal subarachnoid spaces, thin corpus callosum and mild delayed myelination (Fig. 1C–i). She was started on levetiracetam at 20mg/kg/day. After discharge, she continued to have focal seizures, at least three or four times a day, lasting for a minute. Hence, she was started on sodium valproate and clobazam. By eight months of age, her parents noticed that she had regression of milestones. She could no longer sit and could just roll over. She could only make cooing sounds and did not regard the mother. She was readmitted for evaluation at one year of age in view of persisting seizures and loss of acquired milestones. A repeat EEG showed focal epileptiform abnormalities over the left fronto-central region supporting the diagnosis of left fronto-central focal epilepsy. A repeat brain MRI at one year of age was also suggestive of prominent frontotemporal subarachnoid spaces without cerebral atrophy and thin corpus callosum (Fig. 1C–ii). Myelination was found to be normal (Fig. 1 C-iii-iv). On examination, her weight was 14.6kg (+5.6SD), length was 83cm (−2SD) and head circumference was 45cm (−2SD). She was alert and did not have any obvious facial dysmorphism. She had tented vermilion of upper lip, medial flaring of eyebrows and normal set ears (Fig. 1B). She had prominent finger pads. She had generalised hypotonia and her deep tendon reflexes were normal. She did not have organomegaly. Her hearing and eye evaluation were unremarkable. She was later started on lamotrigine and carbamazepine. At two years and two months, she continued to have focal seizures lasting approximately for half a minute at least 2 to 3 times per day. She had chronic constipation, which was relieved by rectal suppository. She was on physiotherapy and showed improvement in recognition of mother. She could cruise and make cooing sounds.

Methods:

Informed consent for genetic testing and publication of images was obtained from the family. The Institutional Ethics Committee approved this study. Two ml of peripheral blood sample was collected in EDTA vacutainer from the proband and her parents.

Molecular testing:

Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA; cat # 51106). Trio exome sequencing (Illumina, Inc., San Diego, California, USA) of the proband and parents was performed using Agilent SureSelect Clinical Research Exome v2 (CREv2) and Clinical Research Exome v3 (CREv3) capture kits, respectively as described earlier (Girisha et al., 2019). Annotation was performed by in-house pipeline against the GRCh38 version of the human genome and the variants were analysed and prioritised using our in-house filtering strategy. Variants with minor allele frequency <1% were filtered against population database gnomAD (v2.1.1 and v3.1.1) and in-house variant database of 1198 exomes. Exonic and splice site variants were then prioritized based on its concordance to the observed phenotype and multiple *in-silico* pathogenicity tools. Intronic and intergenic variants were excluded from further analysis. All the different inheritance patterns were considered in variant prioritisation. The variant was validated and segregated in the family members by Sanger sequencing. Human androgen

receptor gene assay (HUMARA) was carried out on the DNA sample of the proband and mother as described previously to check for X-inactivation pattern (Dalal, Sarkar, Priya, & Nandineni, 2010). The electropherograms were further analyzed using GENESCAN version 3.7.1 software (Applied Biosystems, Foster City, CA).

Results:

Molecular testing:

A novel missense variant, c.14T>G in exon 1 of *FGF13* (NM_004114.5) was observed in heterozygous state in the proband and absent in her parents. The read frequency of the reference and alternate allele was 25/37 (40%, 60%) respectively (Supplementary material Figure S1). The variant was not present in heterozygous and homozygous state in the population database gnomAD (v2.1.1 and v3.1.1) and our in-house database of 1189 exomes. Multiple *in- silico* tools (MutationTaster, SIFT, REVEL) predicted the variant to be disease-causing (Supplementary material Table S1). Sanger sequencing confirmed the *de novo* status of this variant (Fig. 1D). The variant was classified as 'likely pathogenic' using the standards and guidelines for the interpretation of sequence variants by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology criteria PS2, PM1, PM2 and PP3 (Supplementary Table S2) and has been submitted to ClinVar (Accession ID: SCV001760941). HUMARA assay showed no significant skewing of X inactivation in the proband (Supplementary material Figure S2). Her mother had normal X-inactivation pattern.

Discussion

Fibroblast growth factor 13 (*FGF13*) has been recently reported to cause DEE90 characterised by infantile-onset refractory seizures, abnormal EEG, global developmental delay, and profound to severe intellectual disability. Fry et al. described seven individuals from five families with X-linked DEE90 and hemizygous or heterozygous variant in *FGF13* (Fry et al. 2020). Only one of the previously described individuals was a female. We report a *de novo* novel missense variant, c.14T>G in exon 1 of *FGF13* in heterozygous state in a female, causing X-linked DEE90.

All the eight affected individuals with DEE90, including the proband of the study (IV-4), had developmental delay and early-onset epilepsy, not responsive to medications. A comparison of the clinical features observed in all the reported individuals is given in Table 1. Individual 4 in family 2, individual 5 in family 3 and individual 5 in family 7 and IV-4, had regression of milestones after the onset of seizures. Even though specific facial dysmorphic features were absent in the previously reported individuals, the individual we describe had medial flaring of eyebrows and tenting of vermillion of upper lip.

Individual 2 from family 1, described by Fry et al. was a 13-years-old female who had similar symptoms and carried the same variant as her elder brother. X-inactivation studies did not show any significant skewing in her blood (Fry et al., 2020). She had to undergo left anterior temporal lobectomy at the age of seven years for the control of seizures. The authors concluded that the X-inactivation studies performed on blood may not reflect the pattern

in her brain. Similarly, HUMARA assay performed in the individual we describe (IV-4), showed no significant skewing of X-inactivation. She was last followed up at two-years-two-months. She continued to have seizures in spite of multiple antiepileptic drugs.

Puranam et al. described a family with two males with genetic epilepsy with febrile seizures plus (GEFS+) and had a maternally inherited X;14 translocation (Puranam et al., 2015). The mother had a less severe phenotype and did not have epilepsy. She had simple febrile seizures in infancy. The breakpoint on the X chromosome disrupted *FGF13.* Targeted ablation of *Fgf13* in male mice resulted in embryonic lethality while heterozygous female mice showed neuronal hyperexcitability and hyperthermia induced seizures, supporting the role of FGF13 in GEFS+. The mutant mice showed decreased inhibitory and increased excitatory synaptic inputs leading to neuronal hyperexcitability.

Rigbye et al. screened 45 individuals with GEFS+ to identify variants in *FGF13.* They identified a female with a *de novo* missense variant c.638C>T p.(Thr213Met) in *FGF13* and she had facial edema, febrile seizures and malaise precipitated by intercurrent illness (Rigbye et al., 2016). Her development was normal and she never had unprovoked seizures. The authors could not ascertain the clinical significance of the variant identified in *FGF13*.

The variants described by Fry et al., c.31C>T, c.32G>C and c. 41G>C are in exon 1 of isoform A of FHF2 protein. The variant seen in IV-1 is also in exon 1. Exon 1A codes for the highly conserved N terminal domain of isoform A of FHF2 protein. FHF2 isoform A has both pro-excitatory and inhibitory effects on voltage gated sodium channels. FHF2A variants were shown to decrease the ability of FHF2A to promote inactivation of voltage gated sodium channel $Na_v1.6$. The N terminal variants have a gain-of-function effect by affecting the ability of the N-terminal inactivation particle to cause long-term inhibition of voltage gated sodium channel $Na_v1.6$ (Fry et al.2020).

In conclusion, we describe an additional female with a heterozygous novel missense variant, c.14T>G in exon 1 of *FGF13*, thus providing further evidence for X-linked DEE in females.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the family for their cooperation.

Funding

This work was supported by the National Institutes of Health (Grant number: R01HD093570-01A1).

References:

Dalal AB, Sarkar A, Priya TP, & Nandineni MR (2010). Giuffre-Tsukahara syndrome: Evidence for X-linked dominant inheritance and review. Am J Med Genet A, 152A(8), 2057–2060. doi:10.1002/ajmg.a.33505 [PubMed: 20635354]

Fry AE, Marra C, Derrick AV, Pickrell WO, Higgins AT, Te Water Naude J, … Chung SK (2021). Missense variants in the N-terminal domain of the A isoform of FHF2/FGF13 cause an X-linked

developmental and epileptic encephalopathy. Am J Hum Genet, 108(1), 176–185. doi:10.1016/j.ajhg.2020.10.017 [PubMed: 33245860]

Goldfarb M. (2005). Fibroblast growth factor homologous factors: evolution, structure, and function. Cytokine Growth Factor Rev, 16(2), 215–220. doi:10.1016/j.cytogfr.2005.02.002 [PubMed: 15863036]

Goldfarb M, Schoorlemmer J, Williams A, Diwakar S, Wang Q, Huang X, … D'Angelo E. (2007). Fibroblast growth factor homologous factors control neuronal excitability through modulation of voltage-gated sodium channels. Neuron, 55(3), 449–463. doi:10.1016/j.neuron.2007.07.006 [PubMed: 17678857]

Kaplan DI, Isom LL, & Petrou S. (2016). Role of Sodium Channels in Epilepsy. Cold Spring Harb Perspect Med, 6(6). doi:10.1101/cshperspect.a022814

Liu CJ, Dib-Hajj SD, Renganathan M, Cummins TR, & Waxman SG (2003). Modulation of the cardiac sodium channel Nav1.5 by fibroblast growth factor homologous factor 1B. J Biol Chem, 278(2), 1029–1036. doi:10.1074/jbc.M207074200 [PubMed: 12401812]

Munoz-Sanjuan I, Smallwood PM, & Nathans J. (2000). Isoform diversity among fibroblast growth factor homologous factors is generated by alternative promoter usage and differential splicing. J Biol Chem, 275(4), 2589–2597. doi:10.1074/jbc.275.4.2589 [PubMed: 10644718]

Puranam RS, He XP, Yao L, Le T, Jang W, Rehder CW, … McNamara JO (2015). Disruption of Fgf13 causes synaptic excitatory-inhibitory imbalance and genetic epilepsy and febrile seizures plus. J Neurosci, 35(23), 8866–8881. doi:10.1523/JNEUROSCI.3470-14.2015 [PubMed: 26063919]

Rigbye KA, van Hasselt PM, Burgess R, Damiano JA, Mullen SA, Petrovski S, … Hildebrand MS (2016). Is FGF13 a major contributor to genetic epilepsy with febrile seizures plus? Epilepsy Res, 128, 48–51. doi:10.1016/j.eplepsyres.2016.10.008 [PubMed: 27810516]

Smallwood PM, Munoz-Sanjuan I, Tong P, Macke JP, Hendry SH, Gilbert DJ, … Nathans J. (1996). Fibroblast growth factor (FGF) homologous factors: new members of the FGF family implicated in nervous system development. Proc Natl Acad Sci U S A, 93(18), 9850–9857. doi:10.1073/pnas.93.18.9850 [PubMed: 8790420]

Wittmack EK, Rush AM, Craner MJ, Goldfarb M, Waxman SG, & Dib-Hajj SD (2004). Fibroblast growth factor homologous factor 2B: association with Nav1.6 and selective colocalization at nodes of Ranvier of dorsal root axons. J Neurosci, 24(30), 6765–6775. doi:10.1523/JNEUROSCI.1628-04.2004 [PubMed: 15282281]

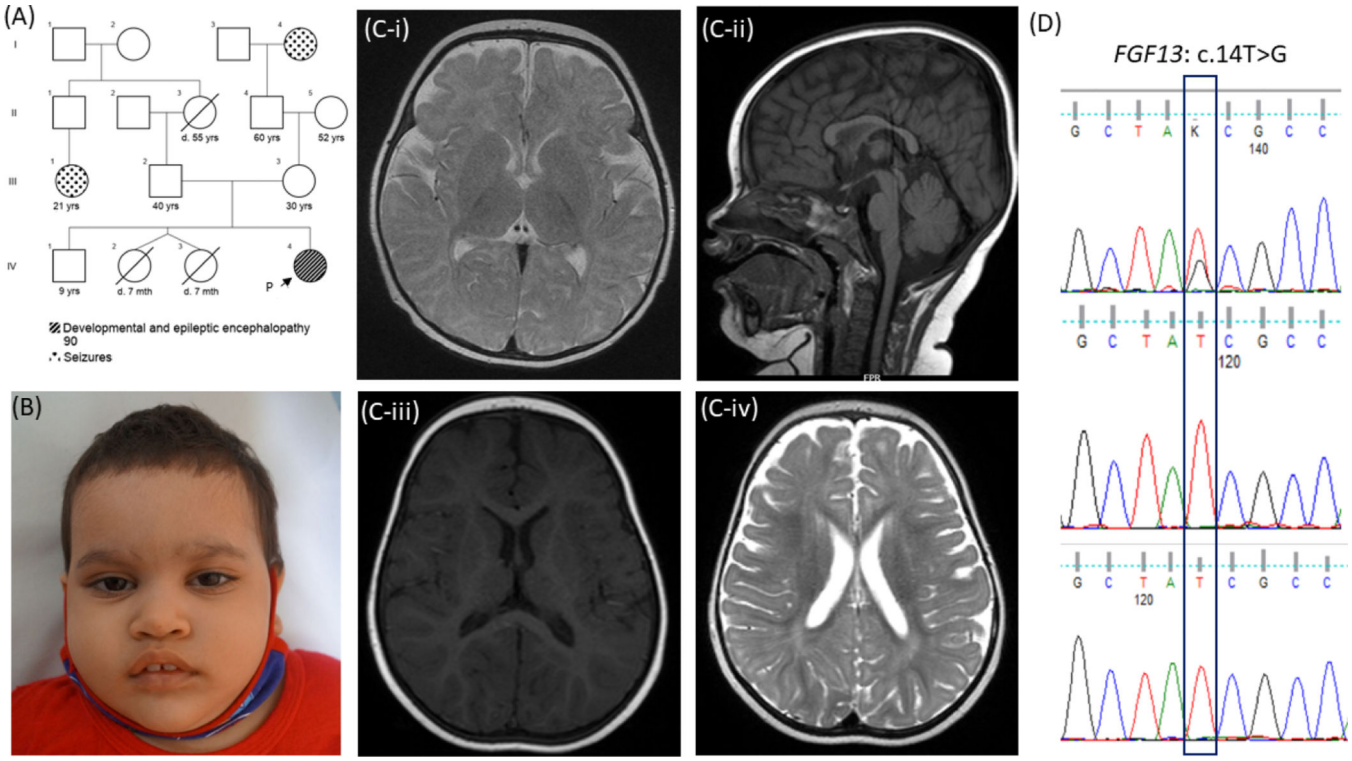

Figure 1:

(A) Pedigree of the family (B) Clinical photographs of the proband showing tented vermilion of upper lip and medical flaring of eyebrows (C) MRI brain at (i) five months showing prominent frontotemporal subarachnoid spaces, mild delayed myelination on axial T2-weighted image (ii) one year showing thin corpus callosum (iii-iv) one year showing normal myelination on T1-weighted image and T2-weighted image, respectively (D) Chromatograms showing heterozygous variant in the proband and wild type variant in the mother and father.

Table 1:

Comparison of clinical and genotypic characteristics of patients with variants in *FGF13*

Reference	**Present study**	**Fry et al., 2020**						
Family		**Family 1**		**Family 2**		**Family 3**	**Family 4**	**Family 5**
Subject	IV-4	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5	Individual 6	Individual 7
Age	1 year 4 months	15 years	13 years	19 years	12 years	2 years 3 months	5 years	5 years 8 months
Gender	Female	Male	Female	Male	Male	Male	Male	Male
Consanguinity	−	−	−	−	−	−	−	+
DD/ID	+	+	+	+	+	+	+	+
Age: milestones at last review	2 years 2 months: Could cruise and had cooing sounds	13 years: No independent walking, no talking	11 years 6 months: Used 30 words and sang nursery rhymes	19 years: Severe learning disability, walk with support, non-verbal	12 years: Walk independently, no recognisable speech	2 years 3 months: No walking, no talking	5 yeas 8 months: NA	5 years 8 months: Not able to walk or talk
Autistic features	−	−	+	+	+	+	+	−
Regression of milestones	+	−	−	−	+	+	NA	+
Seizures	+	+	+	+	+	+	+	+
Age of onset of seizures	5 months	2 months	11 months	2 months	6 months	Day 5	Day 1	6 months
Type of seizures	Focal	Focal and generalised	Focal and rarely generalised	Focal, flexor spasms	Focal, NCSE, GTCS	Focal, dystonic, myoclonic	Cyanotic spells	Focal, myoclonic, GTCS
Abnormal EEG	+	+	+	+	+	+	NA	+
Response to AEDs	Poor	Poor	Poor, underwent left anterior temporal lobectomy and partial amygdalohippocamptectomy	Poor	Poor	Poor	NA	Responded to ACTH
Chronic constipation	+	+	−	+	−	+	−	−
Birth weight in kg (SD)	2.9 (−0.75)	2.83 (−1.5)	3.13 (−0.2)	3.4 (+0.1)	NA	3.67 (+0.7)	2.6 (−1.2)	2.5 (+0.1)
Cyanotic spells	−	+	+	−	−	−	+	−
Weight in kg (SD)	14.6 (+5.6)	NA	NA	68 (−0.1)	39 (+0.1)	15.8 (+2.4)	NA	18 (−0.9)
Height/length in cm (SD)	83 (−2)	NA	NA	NA	NA	NA	NA	110 (−0.8)

Reference	Present study	Fry et al., 2020						
Family		**Family 1**		**Family 2**		**Family 3**	**Family 4**	**Family 5**
Head circumference in cm (SD)	45 (−2)	NA (−1.4) at 13 y 10 m	NA (+0.8) at 6y 9m	57 (−0.2)	53 (−1.3)	48.5 (−1.1)	49 (−2.6)	NA
Examination findings	Medial flaring of eyebrows, tenting of vermilion of upper lip, generalised hypotonia, normal deep tendon reflexes	Hypotonia, normal deep tendon reflexes,	Normal tone, normal reflexes, high palate, prognathism	Low truncal tone, gum hypertrophy, and scoliosis	Eczema, broad based gait, hypermobile joints and mild hirsutism, low truncal tone, mild scoliosis, tight hamstrings	Generalised hypotonia with no contractures or scoliosis. Normal knee reflexes, exotropia, normal fundi.	Strabismus, coarse facies	Limb hypertonia with positive Babinski sign and ankle clonus
MRI brain	Benign enlargement of subarachnoid space of infancy, thin corpus callosum, mild delayed myelination	Normal	Normal	Normal	Normal	Normal	NA	Cerebral cortex atrophy with bilaterally enlarged ventricles.
Genetic testing	Trio WES	WES,WGS	WES,WGS	WES,WGS	WES,WGS	WES	WES	Trio WGS
Variant (NM_004114.5)	c.14T>G p.(Ile5Ser)	c.31C>T (p.Arg11Cys)	c.31C>T (p.Arg11Cys)	c.31C>T (p.Arg11Cys)	c.31C>T (p.Arg11Cys)	c.31C>T (p.Arg11Cys)	c.41G>C (p.Arg14Thr)	c.32G>C (p.Arg11Pro)
Location	Exon 1	Exon 1	Exon 1	Exon 1	Exon 1	Exon1	Exon 1	Exon 1
Type of variant	Missense	Missense	Missense	Missense	Missense	Missense	Missense	Missense
Zygosity	*De novo*	Presumed maternal gonadal mosaicism	Presumed maternal gonadal mosaicism	Maternal somatic mosaicism	Maternal somatic mosaicism	*De novo*	Unknown	Mosaic

+: present, -: absent, NCSE: Non convulsive status epilepticus, GTCS: Generalised tonic clonic seizures, y: years, m: months, EEG: Electroencephalograph, MRI: Magnetic Resonance Imaging, WES: Whole Exome Sequencing, WGS: Whole Genome Sequencing, AED: Anti-epileptic drugs, DD: Developmental delay, ID: Intellectual disability, NA: Not available, ACTH: Adrenocorticotropic hormone