Novel variants in FZR1 consolidate its role in developmental and epileptic encephalopathies type 109

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INTRODUCTION

Developmental and epileptic encephalopathies (DEEs) are a heterogeneous group of neurologic disorders characterized by onset of seizures in infancy or early childhood and neurodevelopmental impairments. To date, pathogenic variants in over 100 genes are reported in association with DEE. De novo loss-of-function FZR1 (fizzy and cell division cycle 20-related protein 1 gene) variants were recently associated with DEE type 109, with only four reported patients (1, 2). In this study, we report novel FZR1 variants in three unrelated patients with similar phenotypes.

METHODS

Patient recruitment: Biological samples were collected from three unrelated families after obtaining informed consent from patients or their parents. DNA from three affected individuals (Patient 1-3) were subjected to whole exome sequencing and three novel variants in the FZR1 gene (NM_016263.4) were detected during analysis. These variants were further validated by Sanger analysis (Family 1 and 3) or SNP microarray analysis (Family 2). **WES:** Whole exome libraries were generated using Twist Human Core Exome kit with RefSeq and Mitochondrial Panel enrichment. The libraries were 100nt paired end sequenced on an Illumina platform to obtain 20x depth of coverage for >98% of the autosome target region and at least 300x coverage of the mitochondrial genome. Read alignment to reference genome GRCH38 and variant calling, including single nucleotide substitutions (SNVs), small insertions/deletions (Indels) and copy number variants (CNVs) with default parameters were performed using DRAGEN (version 4.2.4, Illumina). SNV and indel annotation was performed by geneyx® (https://geneyx.com). CNVs were annotated with ANNOTSV3.1 and an in-house CNV database to obtain allele frequencies. Genetic variants are described following the Human Genome Variation Society (HGVS) recommendations (www.hgvs.org). **Sanger sequencing:** The relevant region of the FZR1 was amplified via the PCR method and sequenced bidirectionally using the internal primer pairs. FinchTV version 1.4.0 was used for viewing and for analyzing trace data from Sanger DNA Sequencing (scf or ab1 file formats). (https://digitalworldbiology.com/FinchTV). **SNV Detection and Analysis:** Annovar (v.2020-06-08) and InterVar (v.2.2.1) were used to annotate detected variants. The pathogenicity of missense variants was predicted using PolyPhen-2, Combined Annotation Dependent Depletion, and MutationTaster algorithms. Only variants with depth >10 and estimated heteroplasmy level ≥10% were kept. Mitomap was used to interpret the pathogenicity of variants, and only confirmed P/LP variants were retained.

RESULTS

Clinical assessment

Three unrelated patients were studied by whole exome sequencing: **Patient 1** was a 6 months-old, Albanian boy with developmental delay and tonic-clonic seizures presented at 2.5 months of age; **Patient 2** is a Chinese boy manifested developmental delay, ataxia and seizures starting at 5 months of age; and **Patient 3** is a French boy showing developmental delay without seizure, hypotonia and feeding difficulties.

Table 1. Clinical Features of patients.

	Patient 1	Patient 2	Patient 3
Sex	Male	Male	Male
Age of diagnosis (months)	2.5	5	At birth
Ethnic origin	Albanian	Chinese	French
FZR1 variant (NM_016263.4)	c.868G>A p.Asp290Asn	c.824-2_*29dup p.?	c.1126G>A; p.Gly376Ser
Variant function	Missense	Duplication	Missense
Inheritance	De novo	De novo	UK
Gestational age at birth (week)	27-28	41	38
Head circumference at birth	30cm	normal	35.5cm
Birth weight (Kg)	1.2	3.9	2.77
Dysmorphic features	Low set ears Retro/micrognathia Prominent metopic ridge Hypertelorism-mild Depressed nasal bridge	No	UK
Seizure type at onset	Generalized, tonic-clonic	generalized, tonic-clonic	No seizure
Age of seizure at onset	2.5 month	5 month	NA
Global development	Failure to thrive Global developmental delay	Failure to thrive Global developmental delay	Failure to thrive Global developmental delay
Intellectual disability	Yes	Yes	Yes
EEG findings	NA	epileptiform abnormalities	Normal at last examination (3months)
Neurological exam	Axial hypotonia	Ataxia Ataxic gait	Axial hypotonia, moderate decreased peripheral tone
Other features	Endocrinology Feeding difficulty Recurrent infections	Feeding difficulty	Strabismus Subglottic stenosis Sleep apnea Severe gastric reflux

NA; not applicable, UK; unknown

Genetic analysis

Patient 1 was found to have a heterozygous missense variant (c.868G>A; p.Asp290Asn) in exon 10 of the FZR1 gene (NM_016263.4) with a minor allele frequency (MAF) of 0.00001681 in gnomAD (with 1 carrier). This variant has a CADD score of 30 and SIFT score of 0, indicating a potential damaging effect on protein function. Sanger analysis further confirmed the de-novo status of this variant in patient (Figure 2 a, b). This variant was classified with uncertain significance according to ACMG guidelines (PM1, PM2, PM6, PP2, PP3).

Patient 2 was found to carry a multi-exon heterozygous duplication (chr19:3531909-3534865; c.824-2_*29dup) encompassing exons 10-14 of FZR1. This variant was confirmed to be de-novo by SNP array analysis and is absent in gnomAD (Figure 2 c, d). This variant was classified with uncertain significance according to ACMG guidelines (PM2).

Patient 3 was identified with another heterozygous missense variant (c.1126G>A; p.Gly376Ser) in exon 11 of FZR1, absent in gnomAD (figure 2e). Sanger analysis further confirmed that mother is wild type for this variant. However, the de-novo status of this variant could not be confirmed as the pregnancy was obtained by medically assisted procreation with sperm donation for male infertility.

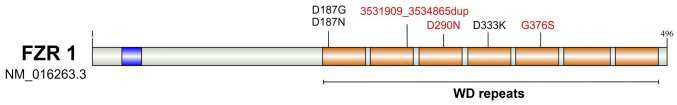

Figure 1. Schematic illustration of FZR1 protein domains and associated variants. Six variants have so far been reported on the WD domains of FZR1 in patients with DEE 109. Novel variants identified in this study are indicated in red.

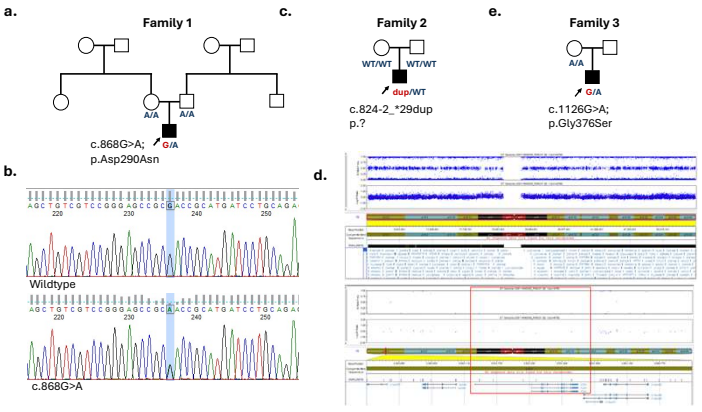

Figure 2. Family history and genetic analysis of patients.

Pedigrees for all three families with indicated index harbouring heterozygous FZR1 variants: c.868G>A (Family 1; a), c.824-2_*29dup (Family 2; c), and c.1126G>A (Family 3; e). Sanger results confirmed the presence of the missense variant c.868G>A in the index of Family 1 (b). SNP microarray analysis confirmed a de-novo duplication in 3531909_3534865 region of chromosome 19 in the patient from Family 2 (d).

CONCLUSION

- In this study, we described three novel genetic variants of FZR1 in patients with DEE type 109. In addition, we observed expanded phenotypes in one patient (patient 3) with eye complications and gastric symptoms yet without developing any type of seizure.
- Our observations, confirm previous studies that de novo heterozygous variants in FZR1 cause global developmental delay and various types of seizures in the first months or years of life (1, 2).
- With a limited number of genetic causes described so far, the underlying mechanism of the disease remains poorly understood. Future studies are required to elucidate the functional consequences of different variants on the protein structure and function, and to clarify the genotype-phenotype correlation of this disease.

Conflict of interest statement: Y. Pakdaman, A. Rad, G. Oprea and S. Kishore are current employees at Arcensus Diagnostics

References: 1. Rodriguez *et al*, Brain, 2022. 2. Manivannan *et al* , Brain, 2022.

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