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CASE REPORT

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Identification of two variants in *AGRN* and *RPL3L* genes in a patient with catecholaminergic polymorphic ventricular tachycardia suggesting new candidate disease genes and digenic inheritance

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Abstract

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an arrhythmogenic syndrome characterized by life-threatening arrhythmias, a normal resting electrocardiogram and the absence of overt structural heart abnormalities. Mutations in *RyR2* gene account for the large part of CPVT cases. Less frequently, mutations in *CASQ2* gene have been linked to the recessive form of the disease. Overall, approximately 35% of CPVT patients remain without a genetic etiology implying that other genes might be found causative of the disease. Here, we present a 6-year-old boy born to first-degree related parents, with a typical phenotype of CPVT and a family history of sudden cardiac death of his brother at 7 years. A trio-based whole exome sequencing was performed, and we identified a homozygous variant in *AGRN* gene and a heterozygous variant in *RPL3L* gene. We hypothesized that the presence of the homozygous variant in *AGRN* accounts for the CPVT phenotype in this family and the heterozygous variant in *RPL3L* gene may act as a modifier gene. Further studies are needed to determine the role of these genes in CPVT.

KEYWORDS

AGRN gene, CPVT, known gene-novel gene association, *RPL3L* gene, whole exome sequencing

1 | INTRODUCTION

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a life-threatening arrhythmia characterized by bidirectional or polymorphic ventricular tachycardia (VT) mostly triggered by exercise or emotional stress.[1] However, many arrhythmias and syncopal episodes occur during "wakeful rest" and normal daily activities.[2] CPVT generally occurs during the first or the second decade of life.[3] Although data on prognosis in CPVT are limited, about 40% of severely affected patients deceased within 10 years of diagnosis.[1,3] More specifically, arrhythmias events and other symptoms such as fainting and dizziness occur between 7 and 11 years.[4] As CPVT is a cardiac

disorder without overt structural heart abnormalities and ECG resting irregularities, the diagnosis remains challenging.[1,3] Thus, the genetic testing is very helpful to establish a definite diagnosis. CPVT is known to be caused mainly by mutations in sarcoplasmic reticulum genes leading to calcium mishandling and alteration of the electrical function that predispose to arrhythmias. The genetic diagnosis of CPVT is established once heterozygous pathogenic variants either in the ryanodine receptor gene (*RYR2*) or the calmodulin 1 gene (*CALM1*) or other homozygous pathogenic variants in the calsequestrin gene (*CASQ2*) or the triadin gene (*TRDN*) are identified. Other minor genes have been related to CPVT, namely *CALM2*, *CALM3*, and *TECRL*.[4] About 60% of patients with CPVT are mutated in the *RYR2* gene, and mutations in *CASQ2* account for 2 to 5% of cases.[1,2] Thus, approximately 35% of cases remain without a conclusive genetic etiology.

Herein, we report a 6-year-old boy born to first-degree related parents, with a typical phenotype of CPVT and a family history of sudden cardiac death of his brother at 7 years.

2 | MATERIALS AND METHODS

The parents provided their written informed consent to participate in this study. This work was conducted according to the principles of the Declaration of Helsinki and to the ethical guidelines of the institutions involved (Registration number: IRB00005445, FWA00010074). Informed consent was obtained from the participant for the publication.

Genomic DNA was extracted from the samples according to standard techniques. Given parental consanguinity, the entire coding region of the *CASQ2* gene was Sanger sequenced, but no mutations were detected.

Several studies have shown the implication of a large deletion of exon 3 of the RYR2 gene in severe forms of CP VT.[5(p3),6(p3),7] In order to verify that the patient does not carry this deletion, we searched for this structural variant on the data of the 715K SNP Illumina Human Omni Express array performed prior to WES. The patient and his parents did not carry any CNVs in the *RYR2* gene.

Since the *RYR2* gene comprises 105 exons (16562 bp) which make it difficult to analyze by direct sequencing and about 35% of the cases are not mutated in this gene, a MedExome was performed for this patient and his parents.

Thus, a trio-based whole exome sequencing (WES) was carried out using the NimbleGen SeqCap EZ MedExome kit and on the Illumina NextSeq 500 platform (Illumina). The MedExome is a medical WES with an enhanced coverage of genes with high clinical relevance.

Raw data were mapped to the human genome reference (hg19) using BWA 0.7.5. Variant calling and annotation was processed using GATK and ANNOVAR. WES data of the trio were analyzed and segregated simultaneously using the Variant Annotation and Filtering Tool software v.2.13 (https://varaft.eu/).

In order to identify potentially causal variants, the following filtering strategy was adopted: as a first step, variants with a Minor Allele Frequency (MAF) ≥1% in the Genome Aggregation Database (gnomAD) were removed (https://gnomad.broadinstitute.org/). As a further step, synonymous, intronic, and intergenic variants were discarded. As a final step, the obtained variants' list (including rare, nonsense, missense, splice site, and frameshift INDELs), was filtered according to the *in silico* pathogenicity prediction. Thus, variants predicted as polymorphism according to UMDPredictor (http://umd-predictor.eu/), SIFT (http://sift.jcvi.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and Mutation Taster (http://www.mutationtaster.org/) were excluded.

The full list of the identified variants is in the (Table S1).

3 | RESULTS

The patient (IV:10), a 6-year-old boy to consanguineous ostensibly healthy parents, had a structurally normal heart. At the age of 5 years, he presented signs of fainting and palpitations during playing and low-level physical activities (normal walk) as well as resting conditions.

His cardiac evaluation including exercise stress test demonstrated bidirectional VT. 24-hour Holter monitoring showed a catecholaminergic polymorphic VT in the daytime period, occurring at the acceleration of the heart rate (Figure 1). The patient is on beta blocker treatment.

Of note, his brother (IV:9) died suddenly while awakening from sleep at the age of 7 years old. Indeed, the medical history of the deceased brother (IV:9) was unremarkable, except few palpitations events under physical activities (playing) but no fainting, chest pain, or heart discomfort. Unfortunately, no DNA of the deceased child was available.

The cardiac examination of both parents (III:7 and III:8), including a 12-lead ECG and an echocardiography, was normal and they had no personal history of syncope or any other heart discomfort.

Family investigation revealed one other case of sudden death under the age of 1 year old (member IV:1). His mother (III:2) has a history of arrhythmias.

Family pedigree is shown in Figure 2.

A trio-based WES was performed for the family and the data were analyzed and segregated using VarAFT software v.2.13. We first focused on six known genes associated with CPVT (*RYR2*, *CALM1*, *CALM2*, *CALM3*, *TECRL*, *and TRDN)*. However, we did not find any

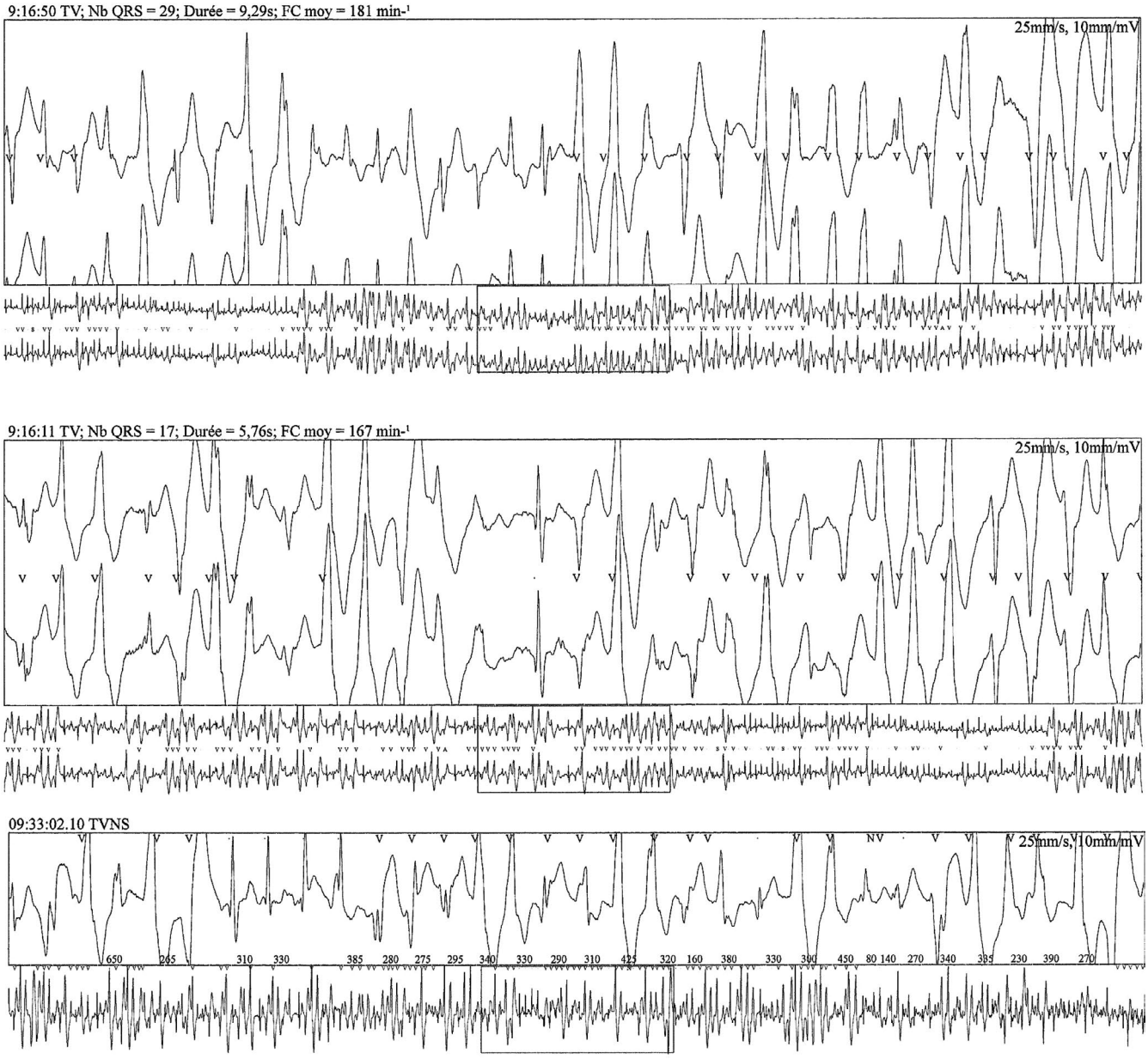

FIGURE 1 Electrocardiographic tracing taken from a 24-hour Holter monitoring of the proband

homozygous neither compound heterozygous potentially causal variant in these genes.

Thus, we performed a complete exome analysis focusing on homozygous/compound heterozygous mutations in genes expressed in the heart and/or related to the cardiovascular system. This analysis allowed us to prioritize two variants in *RPL3L* and *AGRN* genes:

A heterozygous missense variant in exon 6 of the *RPL3L* gene: c.724C>T: p.R242W, with a minor allele frequency of 0.004316 in gnomAD database (rs147972626). The *RPL3L*: p.R242W variant is predicted as damaging and disease causing according to several *in silico* prediction tools such as SIFT, Polyphen-2, Provean, Mutation assessor. This variant is shared with the proband's father.

Through an autosomal recessive model of inheritance analysis, we identified a missense variant in *AGRN*: c.874T>C: p.Y292H in exon 5. This variant is absent from public databases. Therefore, no frequency data were available. The variant is also absent from our in-house database, gathering WES data from 139 individuals (278 chromosomes).

4 | DISCUSSION

We report a 6-year-old boy with a typical phenotype of CPVT and a family history of sudden cardiac death. Whole exome sequencing of the family allowed us to

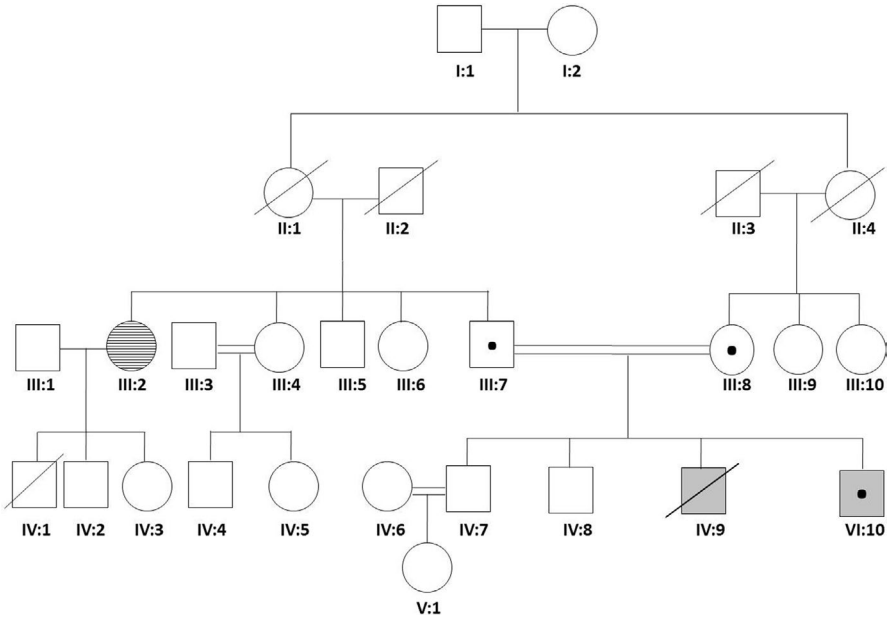

FIGURE 2 Pedigree of the family. The white symbols represent ostensibly healthy members; the filled gray symbols represent affected members. The crossed symbols represent deceased members. The individuals highlighted by black dot were genetically and clinically evaluated

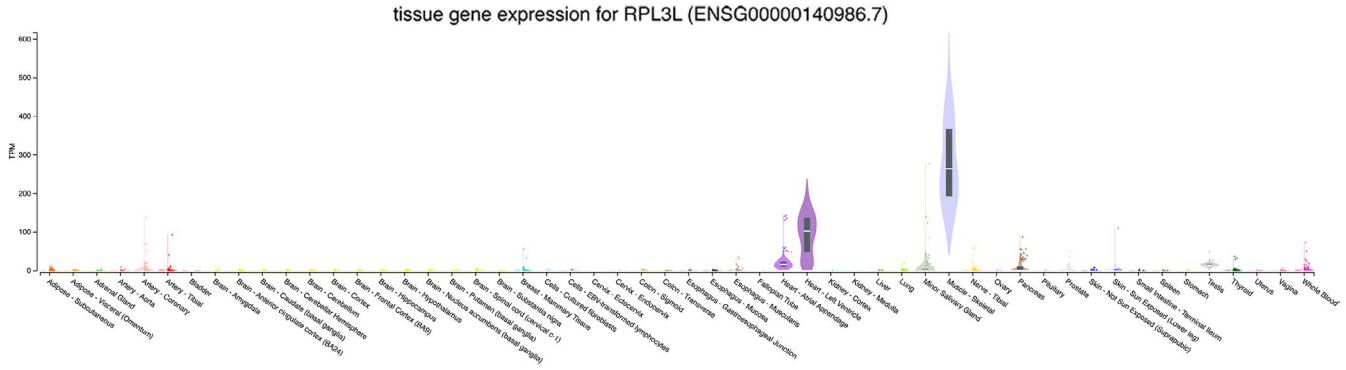

FIGURE 3 Tissue expression of *RPL3L* gene (http://www.gtexportal.org)

identify a heterozygous variant (c.724 C>T; p.R242W) in the Ribosomal Protein L3 Like gene (*RPL3L*) due to its specific tissue expression, which is restricted to the heart and the skeletal muscle (Figure 3).

A single study associates this gene to the risk of atrial fibrillation.[8] Indeed, supraventricular arrhythmias such as atrial fibrillation are observed in CPVT patients, especially during exercise.[3] More recently, compound heterozygous missense variants in *RPL3L* gene were associated to early-onset severe form of dilated cardiomyopathy leading to neonatal heart failure.[9(p3)] The role of *RPL3L* gene in heart diseases remains poorly known. Thus, further studies are needed to uncover its role in cardiac physiopathology.

We also identified a novel homozygous variant in the *AGRN* gene. Both parents were found heterozygous for the *AGRN*:c.874 T>C (p.Y292H) variant (Figure 4).

This variant is not previously described in public frequency databases and absent in our *in-house* database. The *AGRN*; p.Y292H was predicted to be deleterious by Polyphen-2 and LRT prediction, damaging by SIFT and Provean and disease causing by mutation taster.

The *AGRN* gene (OMIM number *103320) encodes agrin, an ubiquitous and large extracellular heparin sulfate proteoglycan. Of note, based on the mRNA microarray expression profiles in ProteomicsDB (https://www.proteomicsdb.org/) and by applying the robust multi-array average method (RMA), the highest agrin median mRNA expression is in the heart (RMA intensity =8.49) (Figure S1). Initially, agrin was identified as an essential regulator of neuromuscular synapse development.[10,11] *AGRN* is widely studied for its role in the neuromuscular junction development and transmission. Indeed, mutations in *AGRN* gene have been reported to cause a recessive form of congenital myasthenic syndrome.[12,13]

Thus, agrin is a key protein in the differentiation of neuromuscular junction and synaptic regeneration, which associates and regulates Na, K-ATPase activity in the human brain. Hilgenberg et al.[14], demonstrated that this same activity is present in the heart and agrin binds

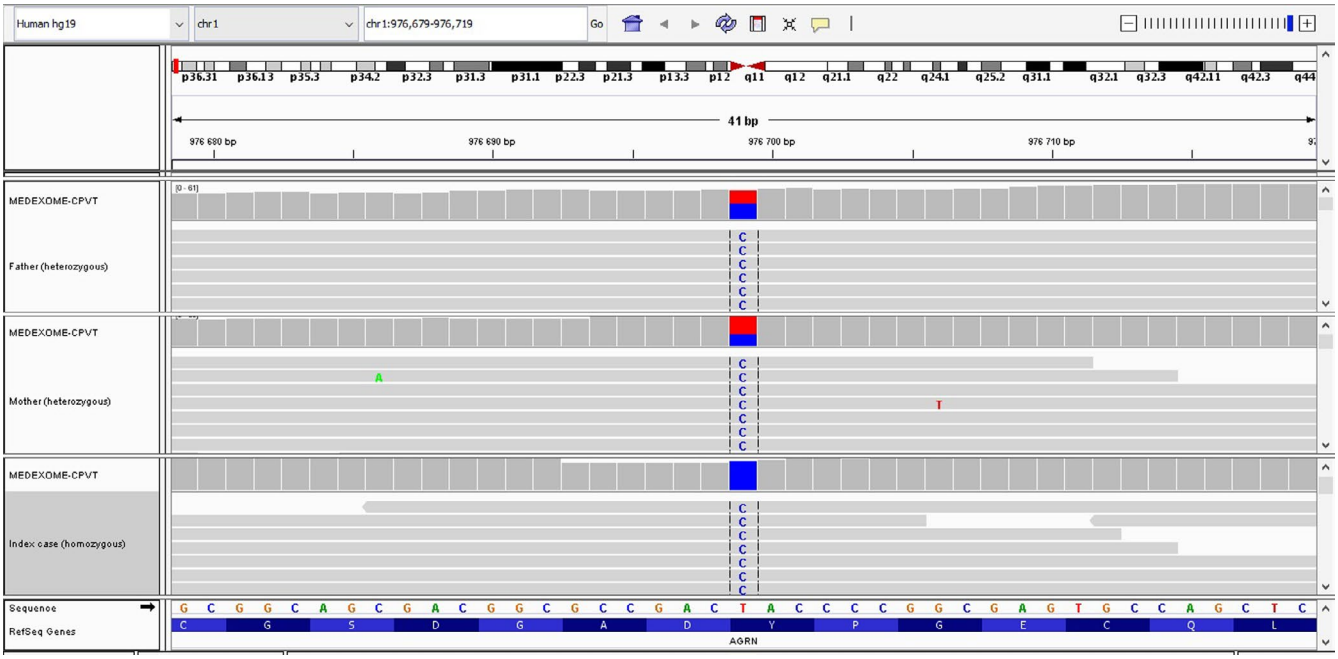

FIGURE 4 Integrative genomics viewer visualization showing the c.874 T>C substitution (p.Y292H) in the *AGRN* gene in the Trio

specifically to the cardiac α3 Na, K-ATPase. Moreover, they showed that the basal frequency of myocyte contraction depends on endogenous agrin - α3 Na, K-ATPase interaction and suggest that agrin modulation of the α3 Na, K-ATPase is important in regulating heart function. Of note, CPVT is mostly caused by functional alterations in the sarcoplasmic reticulum (SR) calcium machinery and excitation-contraction coupling.[15]

Na, K-ATPases and Na^+/Ca^{2+} exchanger are directly responsible for the initiation of cardiac contraction as they play a key role in excitation-contraction coupling. For example, the inhibition of the Na^+, K^+-ATPase lead to an increased arrhythmogenic potential in CPVT patients harboring *RyR2* mutations.[16] Indeed, agrin secreted by cardiomyocytes binds specifically to the extracellular domains of the α3 Na, K-ATPase and interact in both embryonic and adult heart muscle fibers.[14] Moreover, the suppression of agrin signaling by genetic knock-out or agrin-antagonist treatment triggers a rapid increase in cytoplasmic Na^+ in cardiomyocytes, which leads to a higher contraction frequency of cultured myocytes.[14]

This ability of agrin to regulate the activity of the α3 Na,K-ATPase and its role in Na^+ homeostasis, as an endogenous modulator of the basal cardiac contraction frequency, are highly suggestive of its potential implication in cardiac rhythmicity-associated disorders. More recently, a study identified agrin as a required protein for the full regenerative capacity of neonatal mouse hearts via the activation of the Agrin-Yes Associated Protein pathway.[17] Thus, additional studies will be needed to elucidate the role of agrin-signal pathways in the heart and cardiac myocytes function.

Of note, the *AGRN*; c.874 T>C p.Y292H variant is the only homozygous relevant variant in this family. From these data, we suggest that *AGRN* is a promising candidate gene for the CPVT.

Our study offers insights into potential genetic candidate genes for cardiac disorders. However, functional studies are required to unravel the implication of these genes in CPVT and the pathophysiological mechanisms by which they act. Genetic testing of large cohorts of CPVT cases is also warranted to increase the diagnostic yield of this life-threatening disorder.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

SA, SZ, and HJ involved in study concept and design, analysis and interpretation of data; SC involved in clinical investigation of the patient and family members; HJ and SL involved in molecular investigation; SA and SZ involved

in supervision and critical—review & editing; SA, SC, and SZ involved in validation; HJ involved in writing—original draft Preparation.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Registration number: IRB00005445, FWA00010074) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from the family members included in this study or their guardians.

CONSENT

Subjects or their legal representatives participating in this study gave written informed consent to diagnostic and research DNA testing.

DATA AVAILABILITY STATEMENT

The raw whole-exome sequencing data are not publicly available due to restrictions issued by ethical committee. Processed genetic data generated or analyzed within this study are available upon request.

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SUPPORTING INFORMATION

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