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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CASE REPORT

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 processes.

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

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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 cassequestrin 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.\(^5\,^6\) 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://varalt.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 UMDDPredictor (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
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
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. Indeed, supraventricular arrhythmias such as atrial fibrillation are observed in CPVT patients, especially during exercise. More recently, compound heterozygous missense variants in RPL3L gene were associated to early-onset severe form of dilated cardiomyopathy leading to neonatal heart failure. 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. 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.

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. demonstrated that this same activity is present in the heart and agrin binds
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 cytoplasmatic 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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REFERENCES
1. Priori SG, Napolitano C, Memmi M, et al. Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. Circulation. 2002;106(1):69-74.
2. Roston TM, Yuchi Z, Kannankeril PJ, et al. The clinical and genetic spectrum of catecholaminergic polymorphic ventricular tachycardia: findings from an international multicentre registry. Europace. 2018;20(3):541-547. doi:10.1093/ europace/euw389
3. Sumitomo N. Current topics in catecholaminergic polymorphic ventricular tachycardia. J Arrhythmia. 2016;32(5):344-351. doi:10.1016/j.joa.2015.09.008
4. Campuzano O. Update on genetics of catecholaminergic polymorphic ventricular tachycardia. Med Res Arch. 2018;6(12). doi:10.18103/mra.v6i12.1886. ISSN 2375-1924.
5. Dharmawan T, Nakajima T, Ohno S, et al. Identification of a novel exon3 deletion of RYR2 in a family with catecholaminergic polymorphic ventricular tachycardia. Ann Noninvasive Electrocardiol. 2019;24(3):e12623. doi:10.1111/anec.12623
6. Leong IUS, Sucich J, Frosser DO, et al. Array comparative genomic hybridization identifies a heterozygous deletion of exon 3 of the RYR2 gene. Ups J Med Sci. 2015;120(3):190-197. doi:10.3109/03009734.2015.1029101
7. Campbell MJ, Czosek RJ, Hinton RB, Miller EM. Exon 3 deletion of ryanodine receptor causes left ventricular noncompaction, worsening catecholaminergic polymorphic ventricular tachycardia, and sudden cardiac arrest. Am J Med Genet A. 2015;167A(9):2197-2200. doi:10.1002/ajmg.a.37140
8. Thorolfsdottir RB, Sveinbjornsson G, Sulem P, et al. Coding variants in RPL3L and MYZAP increase risk of atrial fibrillation. Commun Biol. 2018;1:68. doi:10.1038/s42003-018-0068-9
9. Ganapathi M, Argyriou L, Martinez-Azorín F, et al. Bi-allelic missense disease-causing variants in RPL3L associate neonatal dilated cardiomyopathy with muscle-specific ribosome biogenesis. Hum Genet. 2020;139(11):1443-1454. doi:10.1007/s00439-020-02188-6
10. Pathy L, Nikolics K. Functions of agrin and agrin-related proteins. Trends Neurosci. 1993;16(2):76-81. doi:10.1016/0166-2236(93)90021-D
11. Bezakova G, Ruegg MA. New insights into the roles of agrin. Nat Rev Mol Cell Biol. 2003;4(4):295-309. doi:10.1038/nrmm1074
12. Zong Y, Jin R. Structural mechanisms of the agrin–LRP4–MuSK signaling pathway in neuromuscular junction differentiation. Cell Mol Life Sci. 2013;70(17):3077-3088. doi:10.1007/s00018-012-1209-9
13. Maselli RA, Fernandez JM, Arredondo J, et al. LG2 agrin mutation causing severe congenital myasthenic syndrome mimics functional characteristics of non-neural (z~) agrin. Hum Genet. 2012;131(7):1123-1135. doi:10.1007/s00439-011-1132-4
14. Hilgenberg LGW, Pham B, Ortega M, et al. Agrin regulation of alpha3 sodium-potassium ATPase activity modulates cardiac myocyte contraction. J Biol Chem. 2009;284(25):16956-16965. doi:10.1074/jbc.M806855200
15. Wleklinski MJ, Kannankeril PJ, Knollmann BC. Molecular and tissue mechanisms of catecholaminergic polymorphic ventricular tachycardia. J Physiol. 2020;598(14):2817-2834. doi:10.1113/JP276757
16. Sedjid S, Heinzel FR, Walther S, et al. Na+-dependent SR Ca2+ overload induces arrhythmogenic events in mouse cardiomyocytes with a human CPVT mutation. Cardiovasc Res. 2010;87(1):50-59. doi:10.1093/cvr/cvq007
17. Bassat E, Mutlak YE, Genzelinakh A, et al. The extracellular matrix protein agrin promotes heart regeneration in mice. Nature. 2017;547(7662):179-184. doi:10.1038/nature22978

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