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ORIGINAL ARTICLE

Family-Based Whole Genome Sequencing Identified Novel Variants in ABCA5 Gene in a Patient with Idiopathic Ventricular Tachycardia

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Abstract

Idiopathic ventricular tachycardia (IVT) is the major cause of sudden cardiac death. Patients with IVT were usually manifested without structural heart disease. In this present study, we performed family-based whole genome sequencing (WGS) and Sanger sequencing for a 5-year-old Chinese boy with IVT and all the unaffected family members in order to identify the candidate gene and disease-causing mutation underlying the disease phenotype. Results showed that a novel heterozygous single-nucleotide duplication (c.128dup) and a novel heterozygous missense (c.3328A > G) variant in ABCA5 gene were identified in the proband. The single-nucleotide duplication (c.128dupT), inherited from his father and patrilineal grandfather, leads to a frameshift which results into the formation of a truncated ABCA5 protein of 50 (p.Leu43Phefs*8) amino acids. Hence, it is a loss-of-function mutation. The missense (c.3328A > G) variant, inherited from his mother, leads to the replacement of isoleucine by valine at the position of 1110 (p.Ile1110Val) of the ABCA5 protein. Multiple sequence alignment showed that p.Ile1110 is evolutionarily conserved among several species indicating both the structural and functional significance of the p.Ile1110 residue in the wild-type ABCA5 protein. Quantitative RT-PCR showed that the ABCA5 mRNA expression levels were decreased in the proband. These two novel variants of ABCA5 gene were co-segregated well among all the members of this family. Our present study also strongly supports the importance of using family-based whole genome sequencing for identifying novel candidate genes associated with IVT.

Keywords Idiopathic ventricular tachycardia · *ABCA5* gene · Novel mutation · Loss-of-function mutation · Family-based whole genome sequencing

Abbreviations

IVT	Idiopathic ventricular tachycardia
WGS	Whole genome sequencing
WES	Whole exome sequencing
SNV	Single-nucleotide variant
INDEL	Insertion/deletion
CHD	Congenital heart disease
SCD	Sudden cardiac death
VF	Ventricular fibrillation
ECG	Electrocardiographic
ACMG	American college of medical genetics and genomics
VUS	Variant of uncertain significance
DCM	Dilated cardiomyopathy

Zhanhui Du, Shan Kuang and Yong Li contributed equally for this work.

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Introduction

Congenital heart disease (CHD) is the major type of birth defects, accounting for approximately one-third of all congenital anomalies worldwide [1]. The estimated incidence of CHD is 8/1000 live births and 4/1000 adults with half a million death every year globally [2, 3]. CHD is the most common and predominant cause of mortality at the perinatal and infant stage [4]. Till now, approximately 450 gene have been reported to be associated with CHD [5]. These genes and their encoded proteins are mainly involved in gene expression and regulation during development of the heart [6–8]. One of the major forms of CHD is sustained ventricular arrhythmia which leads to sudden cardiac death in 80% of all the cases [9]. Ventricular arrhythmia is usually presented with an extreme phenotypic heterogeneity, involving sustained monomorphic and polymorphic ventricular tachycardia (VT), and ventricular fibrillation (VF) [10, 11]. Ventricular arrhythmia is clinically diagnosed by electrocardiographic (ECG) test [11, 12].

Ventricular arrhythmias are mostly caused by the germline mutation of single genes and till date 76 genes have been identified to be associated with it [13, 14]. However, candidate genes and their disease-causing variants for ventricular arrythmias are not studied well due to their variable inheritance pattern, lack of penetrance, and variable expressivity [15]. Therefore, it is really a great challenge to identify the candidate gene and disease-causing variants for the patients with ventricular arrhythmia. In order to elucidate this great challenge, we performed family-based whole genome sequencing (WGS) for the proband and all the family members. Family-based WGS has been proven to be a reliable tool for identifying rare genetic variants of inherited diseases [16]. Family-based WGS also allow us to analyze the inheritance pattern of the sequence variant by co-segregational analysis [17].

ABCA5 gene is located at chromosome 17 and encodes ABCA5 protein of 1642 amino acids. It belongs to the ATP-binding cassette (ABC) transporter A subfamily of genes and structurally similar with other ABC transporter protein, consisting of a nucleotide binding domain and two sets of six transmembrane segments. ABCA5 protein is localized within lysosomes or late endosomes and highly expressed in cardiomyocytes of the heart, brain, lung, testis, and thyroid gland [18, 19]. ABCA5 gene knocked-out ($AbCA5^{-/-}$) mouse showed lysosomal diseases in heart which gradually transformed to dilated cardiomyopathy and finally caused death [18].

In the present study, we investigated a 5-year-old Chinese boy with idiopathic ventricular tachycardia (IVT), a very rare type of ventricular arrhythmia. Proband's twin sister, parents, and patrilineal grandparents are phenotypically normal. We performed family-based WGS and Sanger sequencing. WGS identified a novel heterozygous single-nucleotide duplication (c.128dupT; p.Leu43Phefs*8) and a novel heterozygous missense (c.3328A > G; p.Ile1110Val) variant in ABCA5 gene in the proband. Sanger sequencing confirmed that the proband's father and patrilineal grandfather were harboring the novel heterozygous duplication (c.128dupT, p.Leu43Phefs*8) while proband's mother was carrying the novel heterozygous missense variant (c.3328A > G, p.Ile1110Val). These two novel heterozygous mutations were not identified in proband's twin sister and patrilineal grandmother. We did not find these two variants in 100 normal healthy control individuals. Through family-based WGS, we identified compound heterozygous mutations of ABCA5 gene in a patient with IVT.

Materials and Methods

Patient and Clinical Material

Here, a Han Chinese patient with IVT was enrolled in the Division of Qingdao Women and Children's Hospital, Qingdao University. The study was approved by the ethics committee of the Qingdao Women and Children's Hospital, Qingdao University, in accordance with the recommendations of the Declaration of Helsinki. We obtained written informed consent from all the participant of this study.

Family-Based WGS

Family-based WGS was performed in BGI (Qingdao, China), based on the previously published standard procedure [20].

Genomic DNA was extracted from all the family members by using the All Prep DNA Universal kit (Qiagen) according to the manufacturer's instruction. Genomic DNA was extracted from blood and a total of 2 µg of each DNA sample was subjected to WGS by the BGISEQ-500 [20]. Sonication or fragmentase based library construction method has been used for the preparation of sequence libraries for the BGISEQ-500 platform [20]. Family-based WGS was performed using the BGISEQ-500 platform [20].

We obtained the family-based WGS data for analysis and interpretation. At first, the low-quality reads, adapters, and more than 5% of unknown bases were filtered out from the WGS data. Then, the clean reads were aligned to hg 19 reference genome sequence by using Burrows–Wheeler Alignment tool (BWA) and identified and marked the duplications by using Picard and sort bam by Samtools2 [21, 22]. GATK Best Practices Pipeline was used for calling SNP and INDEL [23, 24]. GATK Best Practices Pipeline comprises

local realignment around indels, Base Quality Score Recalibration (BQSR), haplotype caller, and Variant Quality Score Recalibration (VQSR). Then, we annotated the high-quality variants (SNPs and InDels) by using snpEff three with the following databases; dbSNP (version 147), 1000 genomes (Version August 2015), ExAC (version 3), gnomAD and dbNSFP (version 2.9) databases.

Data Interpretation

The criteria for variant filtration are as follows; (i) if the mutation type is loss of function (either a stop-gain/stop-loss or a frameshift insertion/deletion), it can pass filter directly. (ii) If the mutation type is a non-synonymous mutation, it can pass filter only when it is predicted deleterious in at least one of the three computational prediction methods (SIFT, Phlyphen-2_HDIV, MutationTaster).

Sanger Sequencing

The identified novel variants by the family-based WGS were validated by Sanger sequencing. Primers have been designed based on the NCBI-GenBank reference human genome sequences. ABI PRISM 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA) was used for the Sanger sequencing of the amplified products. Comparing and analyzing the Sanger sequencing data were performed by DNASTAR SeqMan (DNASTAR, Madison, Wisconsin, USA).

The sequences of primer pair are as follows: F1-5′-GGC GGCAGTTAAGCGTCGCGCGG-3′, R1-5′-GCCGGCAGG TTAGGACGG-3′, F2- 5′-GCGCCTTAGCGAATAGCG CGCGG-3′, R2-5′-GGCGCTAATCCGGAGCGG-3′. The reference sequence NM_018672.3 of ABCA5 was used.

In silico Analysis

Evolutionary Conservation Test

In silico analysis of the novel missense variant was performed by multiple sequence alignment. Multiple sequence alignment has been done to confirm the conservation of the wild-type amino acids of the novel missense mutation of ABCA5 gene in different species. Multiple sequence alignment has been done between human (*Homo sapiens*) (GenBank Accession: NM_018672.3), chimpanzee (*Pan troglodytes*) (GenBank Accession: XM_016932823.2), rhesus monkey (*Macaca mulatta*) (GenBank Accession: XM_015120205.1), house mouse (*Mus musculus*) (GenBank Accession: NM_147219.2), chicken (*Gallus gallus*) (GenBank Accession: XM_415695.6), zebrafish (*Danio rerio*) (GenBank Accession: NM_001099246.2), and western clawed frog (*Xenopus tropicalis*) (GenBank Accession: NM_001142127.1) using ClustalW2 [25].

Quantitative RT-PCR

Total RNA was extracted from the blood of the proband and his family. Reverse transcription was performed using superscript III reverse transcriptase (Invitrogen) according to the manufacturer's instruction. The following primers were used for qRT-PCR assays [26]: ABCA5: F: 5′-GAACCA ACTTCAGGCCAGGTATT-3′, R: 5′-CACATGTGCTGT TTGGCTTTGGGATC-3′; GAPDH: F: 5′-GGAGCGAGA TCCCTCCAAAAT-3′, R: 5′- GGCTGTTGTCATACTTCT CATGG-3'.

Data Availability and Statistical Analysis

All data used for the analysis in this study are available in the CNGB Nucleotide Sequence Archive (CNSA: https://db.cngb.org/cnsa). Accession number: CNP0000382. The statistical analysis was performed using GraphPad Prism Software (Version 6.0). A Student unpaired *p* test was used to determine statistical significance in qRT-PCR experiments with a *p* value of 0.05 as the cutoff value for significant difference.

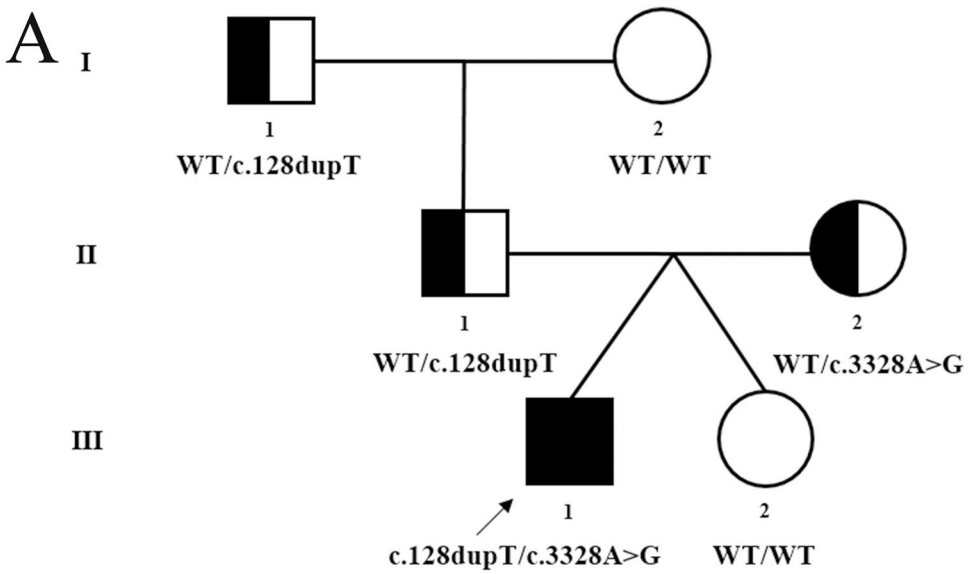
Results

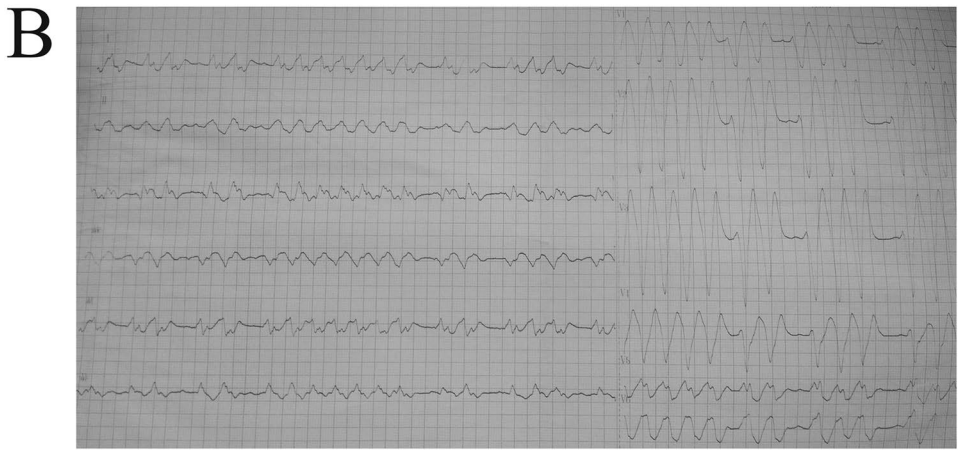
Human Subjects

In the present study, we investigated a 5-year-old Chinese boy with idiopathic VT. The proband belongs to a non-consanguineous Chinese family (Fig. 1a). The proband was born through a full-term uneventful delivery with birth weight of 3.2 kg. The parents of the proband are phenotypically normal. Proband's twin sister and patrilineal grandparents are also phenotypically normal.

In March' 2014, the proband was first admitted in our hospital for "paleness and tachycardia" at his age of 1-year and 3 months. Physical examination revealed that the proband presented with poor mental reaction, paleness with a heart rate of 111 bpm. Echocardiographic test found no abnormalities in heart structure and function. No abnormality was found in myocardial zymogram. Hence, we clinically diagnosed the patient with outbreak-type myocarditis and VT.

In September' 2014, he was again admitted to our hospital with fever and tachypnea at his age of 1-year and 9 months. Physical examination revealed that the highest body temperature was 39.0℃ with poor mental response. ECG test revealed that the heart rate was 105 bpm (Fig. 1b). Hence, we clinically diagnosed the patient with arrhythmia and ventricular tachycardia. The myocardial enzymes and





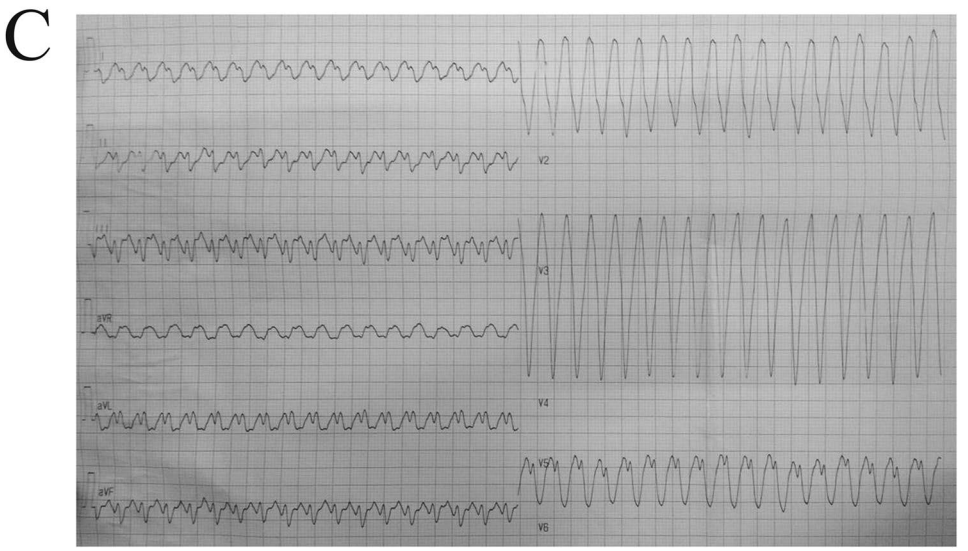


Fig. 1 **a** Pedigree of the family. The filled symbol indicates the patient (proband), and the half-filled symbols show the carrier parents, who were heterozygous carriers but were unaffected. The arrow points to the proband. **b**-**c**. Clinical details. **b** ECG showed the heart rate was 105 bpm and ventricular tachycardia. **c** ECG showed the heart rate was 200 bpm and ventricular tachycardia

related antibodies were absolutely normal. The antiarrhythmic medicine such as propafenone, lidocaine, and amiodarone could not maintain the normal rhythm, so, dobutamine continuous pump was given.

In January' 2018, he was again admitted to our hospital with tachycardia at his age of 5 years. Physical examination of the patient revealed that, body temperature: 37.3° C, pulse rate: 29 beats/min, body weight: 9 kg, blood pressure: 101/51 mmHg. Physical examination of the patient was also identified with general mental reaction, pale face, and slightly increased breathing. Proband's heart rate was 218 bpm, but no heart murmur was found. ECG test of the proband showed that the heart rate was 200 bpm with VT (Fig. 1c). Chest X-ray of the proband found that a ratio of heart to chest was about 0.56. No abnormality was identified in myocardial enzymes. Blood coagulation was normal. We also found that the value of the NT-ProBNP was 3813.00 pg/ml. Electroencephalograph showed abnormal electroencephalogram and brain topographic map of the proband. Brain MRI scan showed no obvious abnormalities.

In May' 2018, he was again admitted to our hospital again with ventricular arrhythmia and idiopathic VT. Growth and development of the proband were found normal. No abnormalities were found in blood routine test and biochemical examination in the proband. ECG test revealed sinus rhythm and the heart rate was 77 bpm with QTc of 0.460 s. Hence, we clinically diagnosed the patient with arrhythmia paroxysmal idiopathic VT.

Family-based WGS Data Interpretation

All the identified sequence variants by family-based WGS were interpreted through several steps to identify the candidate gene and the disease-causing variants underlying the disease phenotype in the patient. The comprehensive and detailed variant interpretation process were described as follows; All the variants (SNPs and InDels) were selected for next step of analysis if their minor allele frequency is < 0.01 in these population databases (ExAC, gnomAD, dbSNP, 1000 Genomes, and BGI's in-house database of ~ 50,000 Chinese people). Then, we functionally analyze all the variants. All the variants were divided into two groups; (i) exonic and splice-site, (ii) intronic followed by analysis. We keep all the exonic or coding variants and canonical splice-site variants because these variants could affect the structure or function of the protein. So, we discarded all the intronic variants. In order to understand the functional effect of all the intronic variants, we used Human Splice Finder (https://www.umd.be/HSF3/HSF.shtml) but we have not identified any intronic variants which could negatively affect on mRNA splicing. So, we discarded all intronic variants. Next, we categorized the remaining variants as "pathogenic" or "likely pathogenic" or variant of uncertain significance (VUS) based on the variant interpretation guidelines of American College of Medical Genetics and Genomics (ACMG) [27]. In silico analysis and prediction of the variants have been done by softwares, i.e., SIFT, Polyphen-2, and Mutation Taster [28, 29]. Then, we analyzed the co-segregation of variants among all the family members to confirm the inheritance pattern. After that, we compare the remaining variants with OMIM and CGD databases in order to understand the correlation between the candidate genes and the disease phenotype. Lastly, functional evaluation of the candidate genes has been done by the information from the well-updated databases and literature review [30]. The comprehensive and detailed variant interpretation pipeline are schematically presented in Fig. 2. The quality control data of this family-based WGS are given in Table 1. The total number of variants and step-wise interpretation process has been described in Table 2. The supplementary file S1 comprises scores, frequency, inheritance of all the variants at step 6 of Table 2.

Family-based WGS and Sanger Sequencing Identified Two Novel Variants in ABCA5

Family-based WGS identified two novel heterozygous variants (c.128dupT, p.Leu43Phefs*8) and (c.3328A > G; p.Ile1110Val) in the ABCA5 gene in the proband (Fig. 3). The novel heterozygous duplication (c.128dupT) leads to a frameshift which finally results in the formation of a truncated ABCA5 protein of 50 amino acids (p.Leu43Phefs*8) instead of the wild-type ABCA5 protein consisting of 1642 amino acids. Hence, it is a loss-of-function mutation. Sanger sequencing confirmed that the proband was inherited by this novel heterozygous duplication (c.128dupT) from his patrilineal grandfather through his father (Fig. 3). The novel heterozygous missense variant (c.3328A > G) leads to the replacement of isoleucine by valine in the position of 1110 (p.Ile1110Val) of the ABCA5 protein. The residue p.Ile1110 of ABCA5 protein is evolutionarily highly conserved among different species (Fig. 4), indicating the significance of the p.Ile1110 residue in both the structure as well as in the functions of the wild-type ABCA5 protein. Sanger sequencing confirmed that the proband was inherited by this novel heterozygous missense variant (c.3328A > G) from his mother (Fig. 3). None of these variants were present in proband's twin sister and patrilineal grandmother. These two novel heterozygous variants were not found in 100 normal healthy control individuals. These two novel heterozygous variants were also not present in the Human Gene Mutation database (HGMD, www.hgmd.cf.ac.uk/), Online Mendelian Inheritance in Man (MIM, https://www.omim.org), ExAC, gnomAD, dbSNP,000 Genome Database, and BGI's in-house database of ~ 50,000 Han Chinese samples.

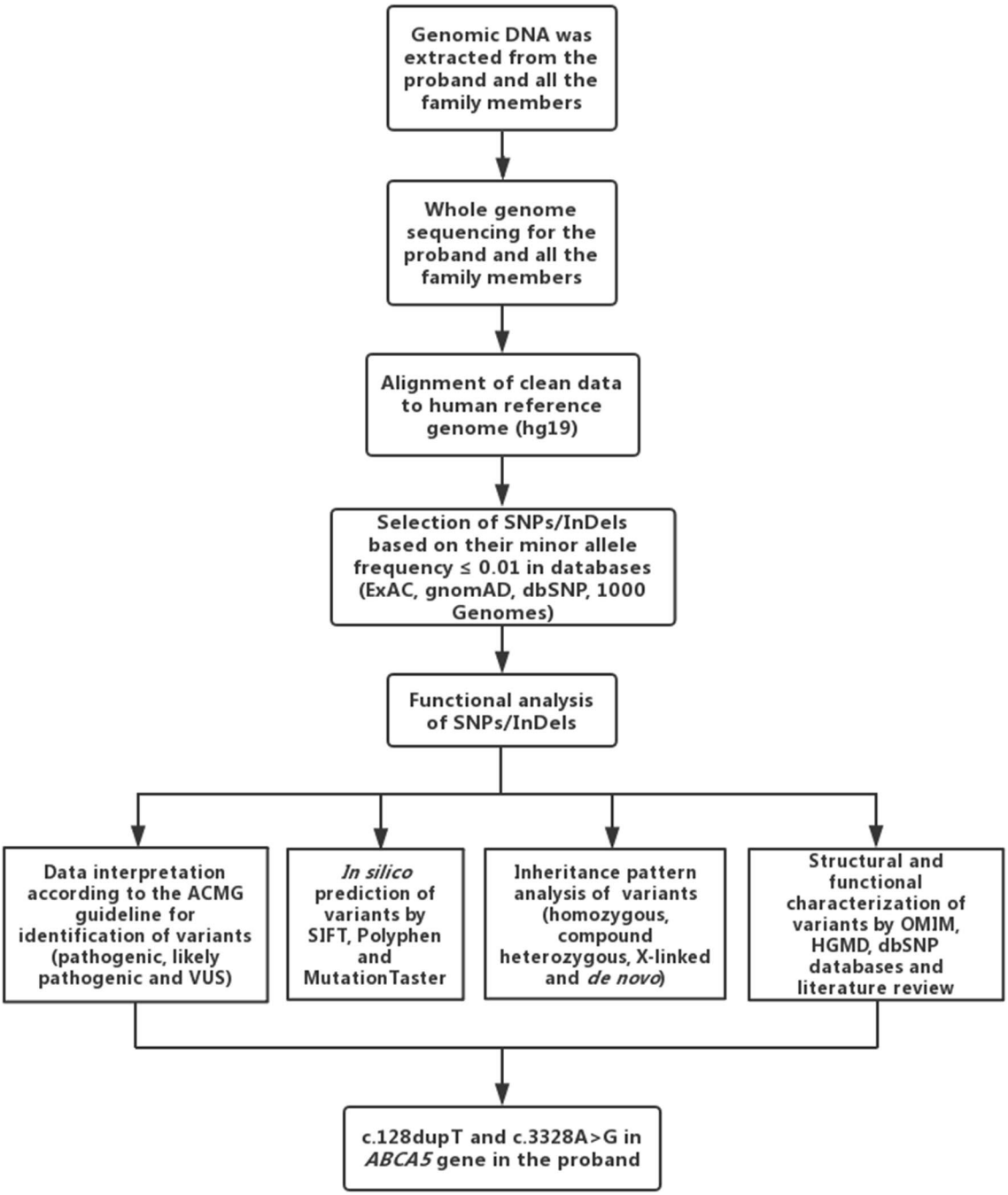


Fig. 2 Schematic presentation of detailed and comprehensive pipeline for family-based whole genome sequencing data analysis and interpretation

ABCA5 mRNA Levels are Dramatically Reduced in the Proband

To test whether the two heterozygous variants affect the transcription levels of ABCA5 gene. We performed qRT-PCR in blood from the proband compared to control, and observed significantly lower ABCA5 mRNA levels in proband than that in the proband's parents and grandparents (Fig. 5), suggesting the two variants in splice-site induced a downregulation of the gene.

Table 1 The quality control data of family-based whole genome sequencing

Sample	Proband	Proband's sister	Proband's father	Proband's mother	Proband's grandmother	Proband's grandfather
Raw reads	1,431,452,690	1,490,890,996	1,442,643,360	1,530,393,668	1,568,089,058	1,465,166,066
Raw bases (Mb)	150,302.53	149,089.1	144,264.34	153,039.37	156,808.91	146,516.61
Clean reads	1,427,507,922	1,483,815,480	1,432,371,532	1,515,838,676	1,563,317,384	1,456,326,322
Clean bases (Mb)	149,888.33	148,381.55	143,237.15	151,583.87	156,331.74	145,632.63
Mapping rate (%)	97.98	99.87	99.81	99.69	99.81	94.84
Unique rate (%)	90.50	92.68	92.94	92.75	92.51	89.05
Duplicate rate (%)	4.25	3.81	3.38	3.74	4.13	2.86
Mismatch rate (%)	0.40	0.41	0.48	0.52	0.34	0.34
Average sequencing depth (X)	44.81	48.59	47.16	49.00	51.07	43.42
Coverage (%)	96.74	99.13	99.77	99.14	99.15	93.61
Coverage at least 4X (%)	96.63	98.79	99.47	98.80	98.84	93.50
Coverage at least 10X (%)	96.22	97.98	98.63	98.00	98.07	93.19
Coverage at least 20X (%)	93.06	96.83	96.12	97.00	97.15	91.36
Clean data rate (%)	99.72	99.53	99.29	99.05	99.70	99.40
Clean read1 Q20 (%)	97.67	97.99	98.10	96.23	98.94	98.70
Clean read2 Q20 (%)	91.36	96.14	95.79	96.53	96.56	95.34
Clean read1 Q30 (%)	90.38	91.27	91.79	89.58	93.90	93.18
Clean read2 Q30 (%)	80.93	87.36	86.96	88.89	88.21	85.78
GC content (%)	42.30	41.56	41.28	41.43	40.61	40.61

Discussion

In our present study, we describe a 5-year-old Chinese boy manifested with IVT. The proband was the only affected member in this family. Proband's twin sister, parents, and patrilineal grandparents are phenotypically normal. The proband was clinically diagnosed with IVT in our hospital. In order to understand the molecular genetic cause underlying the disease phenotype in the proband, we performed family-based WGS and identified two novel heterozygous variants (c.128dupT, p.Leu43Phefs*8) and (c.3328A > G; p.Ile1110Val) in the ABCA5 gene in the proband. Sanger sequencing confirmed that proband was inherited these two novel heterozygous variants from his grandfather through father (c.128dupT, p.Leu43Phefs*8) and mother (c.3328A > G; p.Ile1110Val), respectively. This is the first report of identifying germline mutations of ABCA5 gene in a patient with IVT.

Recently, cardiovascular disease become one of the most common and leading causes of sudden cardiac death (SCD) which approximately affects 3 million people each year, world-wide [31, 32]. However, sustained ventricular arrhythmias are approximately share the half of all SCD cases [33, 34]. It has been reported that almost 5085% of SCDs occur due to ventricular arrhythmias, more precisely, VT or VF [34, 35]. Idiopathic VT, a term used for VT with no clinically apparent structural heart disease, accounts for about 10% of all the cases of VT [36]. Moreover, young patients are also presented with idiopathic VT, mostly due to myocarditis, electrolyte disturbances, and effects of anesthesia [36]. Also, it is reported that 6.8% of all the IVT patients (~ 1 in every 15 patients) have tachycardia-induced cardiomyopathy [37]. So, cardiomyopathy can both be a cause or phenotype of IVT. Although IVT is believed to carry a favourable prognosis, yet it still has a mortality rate of 7% [36–38]. In order to make an accurate, cost-effective, and timely clinical diagnosis of patients with IVT, understanding the genetic molecular basis underlying the occurrence of IVT is the most significant approach.

WGS and whole exome sequencing (WES) have facilitated single-base-resolution genetic test at substantially lower cost which provides a very powerful strategy for detecting single-nucleotide variant (SNV) as well as small insertion/deletion (INDEL). Although WES is more cost effective than WGS, WES cannot detect mutations in non-coding regions as well as large structural changes. WGS enables the identification of all classes and sizes of mutations, many of which will be missed by WES. Moreover, it is already demonstrated that WGS provides more uniform coverage of the coding regions of the genome than WES, so, WGS can increase the detection rate of rare variants [39, 40]. Family-based WGS is a powerful method to identify

Table 2 The comprehensive and detailed variant interpretation

Data filtration pipeline	Proband		Father		Mother		Grandfather		Grandmother		Sister	
	SNP	InDel	SNP	InDel	SNP	InDel	SNP	InDel	SNP	InDel	SNP	InDel
1. Alignment to human genome	3,489,402	882,633	3,302,406	846,865	3,422,964	894,822	3,413,690	897,590	3,445,963	903,176	3,455,612	918,017
2. Filtration by minor allele frequency ≤ 0.01	106,789	118,023	100,973	118,629	88,598	123,379	92,776	126,954	98,025	125,461	90,238	129,210
3. Functional characterization of variants	105,950	117,835	100,211	118,445	87,882	123,185	92,066	126,769	97,223	125,264	89,520	120,018
(1) Exonic and splice-site	541	71	528	58	537	57	531	49	537	56	528	80
(2) Intronic	105,409	117,764	99,683	118,387	87,345	123,128	91,535	126,720	96,686	125,208	88,992	128,938
4. ACMG guideline (all VUS)	514	67	501	57	517	52	506	48	509	53	502	76
5. In silico prediction	432	67	336	57	361	52	332	48	353	53	347	76
6. Inheritance pattern of all the variants	Homozygous (1), compound heterozygous (2), X-linked (5), de novo (17)											
7. Gene function and literature review	Homozygous (0), compound heterozygous (1), X-linked (0), de novo (0)											

the candidate gene and disease-causing mutation according to the co-segregation of the mutation among all the family members with inheritance pattern. Identification of inheritance patterns by family-based WGS decreases about 70% of sequencing errors and greatly reduces the search space for disease-causing variants [40].

The ABCA5 gene is located in chromosome 17 and encodes ABCA5 protein of 1642 amino acids and the sequence homology between ABCA5 protein with other proteins of ABCA family is very low, which indicates that structurally and functionally ABCA5 protein is quite unique than that of other members of the ABCA family. ABCA5 protein has been reported to participate in cholesterol trafficking, which stimulates cholesterol efflux in both macrophages and neurons [41, 42]. In both rats and mice, ABCA5 protein was reported to localize in Leydig cells, a primary site for cholesterol processing [19, 26]. Therefore, cholesterol is probably a potential allocrite for ABCA5. It has been proven that elevated plasma cholesterol is closely related with cardiac arrhythmias [43]. Therefore, changes in cholesterol trafficking and distribution caused by the loss of function of the *ABCA5* gene may affect its normal rhythm.

Kubo et al. reported that homozygous ABCA5 gene knocked-out mice (ABCA5 $^{-/-}$) developed a dilated cardiomyopathy (DCM)-like heart after reaching adulthood and died because of dysfunction of heart. The organization in thrombi of heart in ABCA5 −/− mice indicated the depression of the heart function is a chronic symptom [18]. Since our proband is a 5-year-old VT patient without structural heart diseases, it is well known that DCM patients may have VT early in the disease course, which is unrelated to the severity of left ventricular dysfunction [44]. It is not hard to reminiscent of the similarities between the symptoms of our proband and the symptoms of ABCA5 −/− mice. DeStefano et al. reported that a homozygous splice-site mutation in ABCA5 (c.4320 + 1G > C) is associated with inherited hypertrichosis [26]. DeStefano et al. also showed that homozygous loss-of-function mutation of ABCA5 leads to reduced lysosomal function, followed by intra-endo-lysosomal accumulation of cholesterol in keratinocytes [26]. Moreover, short deletions affecting the ABCA8ABCA9ABCA6-ABCA10ABCA5 gene cluster have been described in several patients with cardiovascular diseases [45–47].

Germline variants of *ABCA5* gene have never been reported to cause IVT. However, in this present study, we identified two novel variants in ABCA5 gene in a patient with IVT. We have not identified any pathogenic or likely pathogenic variant in this patient. Whole genome sequencing study identified a lot of variants but after data analysis and interpretation, we only found these two variants of ABCA5 gene which might have interesting possibility to be associated with IVT. Identification of a new disease-causing gene and functional characterization of mutation need functional

Fig. 3 Partial DNA sequences in the ABCA5 by Sanger sequencing of the family. Arrows point to the mutations. The reference sequence NM_018672.3 of ABCA5 was used

Human (*Homo sapiens*)	T	V	K	F	L	A	V	V	F	C	L	I	G	Y	V	P	S	V	I	L	F	T	Y
Chimpanzee (*Pan troglodytes*)	T	V	K	F	L	A	V	V	F	C	L	I	G	Y	V	P	S	V	I	L	F	T	Y
Monkey (*Macaca mulatta*)	T	V	K	F	L	A	V	V	F	C	L	I	G	Y	V	P	S	L	I	L	F	T	Y
Mouse (*Mus musculus*)	P	A	K	F	L	A	V	V	F	C	L	I	A	Y	V	P	S	V	I	L	F	T	Y
Chicken (*Gallus gallus*)	A	G	K	F	M	A	V	L	F	C	L	I	G	Y	V	P	S	V	V	L	F	T	Y
Zebrafish (*Danio rerio*)	S	H	N	I	L	S	V	A	L	C	L	I	G	F	S	P	A	M	V	L	F	T	Y
Frog (*Xenopus tropicalis*)	A	G	T	V	L	A	M	I	F	C	L	I	G	Y	V	P	A	V	V	L	L	T	Y

Fig. 4 Evolutionary conservation test was done by multiple sequence alignment

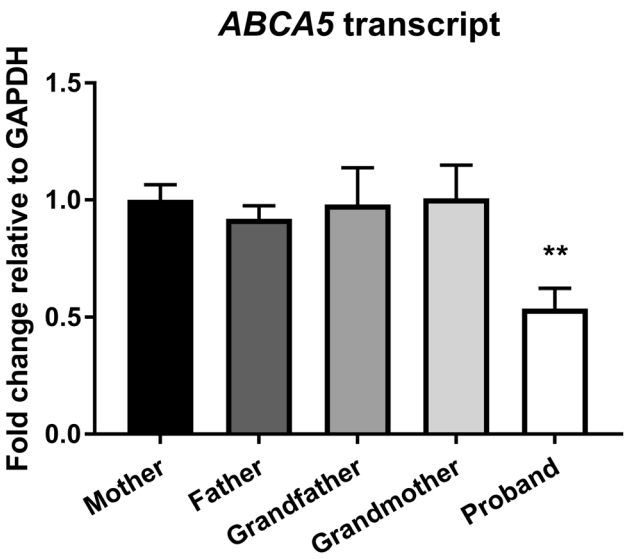


Fig. 5 Expression levels of ABCA5 in blood of the whole family. The relative mRNA of ABCA5 was normalized with reference gene GAPDH and showed a significant decrease in proband compared to his mother. $^{**}p < 0.01$, Student unpaired t test; $n = 3$

study by both in vitro and in vivo study. Further analysis of these novel variants by animal modelling will be valuable. However, in our present study, we identified two novel variants in a patient with IVT.

Concluding Remarks

In conclusion, we describe a 5-year-old Chinese boy manifested with IVT. Proband's twin sister, parents, and patrilineal grandparents are phenotypically normal. We performed family-based WGS and identified two novel heterozygous variants in the ABCA5 gene in the proband. These two novel variants of ABCA5 gene are well segregated among the members of this family. This is the first report of identifying germline mutations of ABCA5 gene in a patient with IVT.

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Author Contributions SB and SP designed the study. Z conducted acquisition and analysis of all the clinical data. YH, PH, and YG made WES pipeline. SB, SK, YL, JL, and ZW analyzed the data. SB, SK, and YL wrote the manuscript. XL and XX supervised manuscript preparation and edited the manuscript.

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Compliance with Ethical Standards

Conflict of Interest The authors confirm that there are no conflicts of interest.

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