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# Putative role of Brugada syndrome genes in familial atrial fibrillation

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**Abstract. – OBJECTIVE: Familial atrial fibrillation (FAF), a not uncommon arrhythmia of the atrium, is characterized by heritability, early onset and absence of other heart defects. The molecular and genetic basis is still not completely clear and genetic diagnosis cannot be achieved in about 90% of patients. In this study, we present the results of genetic screening by next generation sequencing in affected Russian families.**

**PATIENTS AND METHODS: Sixty subjects (18 probands and 42 relatives) with a clinical diagnosis of FAF were enrolled in the study. Since AF frequently associates with other cardiomyopathies, we included all genes that were known to be associated with these disorders at the time of our study. All probands were therefore systematically screened for 47 genes selected from the literature.**

**RESULTS: Our study revealed that seven variants co-segregated with the clinical phenotype in seven families. Interestingly, four out of six genes and three out of seven variants have already been associated with Brugada syndrome in the literature.**

**CONCLUSIONS: To our knowledge, this is the first report of association of the *CACNA1C, CTNNA3, PKP2, ANK2* and *SCN10A* genes with FAF; it is also the first study in Russian families.**

*Key Words:*
FAF, Cardiology, Channelopathy, Next generation sequencing.

## Introduction

Atrial fibrillation (AF) is the most common arrhythmia encountered in clinical practice mostly because it is typically associated with various disorders and other genetic cardiomyopathies[1]. By contrast, familial AF (FAF), also known as lone AF, is not associated with other cardiovascular risk factors, affects more than one family member with early onset[2] and is therefore considered a heritable nosological entity in its own right. Familial AF is not uncommon and is also genetically heterogeneous: 16 genes and 4 loci are currently known to be associated with the condition, as listed in the Online Mendelian Inheritance in Man database (OMIM, http://omim.org/). In a cohort of 192 individuals (384 alleles) and considering 14 AF-associated genes, Olesen et al[3] demonstrated that only 7.6% of alleles harboured a very rare variant that could justify a Mendelian hereditary pattern of the condition.

Since an increasing number of genes are associated with AF, the most time- and cost-effective strategy is next generation sequencing (NGS), which however is not currently performed for routine genetic testing.

In the present study, we report the genetic characterization of 18 Russian probands with FAF and their relatives by NGS. Given the low positivity rate to genetic testing and considering that AF can be associated with other conditions, we also extended the analysis to all genes known to be involved in other cardiomyopathies, such as Brugada syndrome (BS), long-QT syndrome (LQTS) and arrhythmogenic right ventricular dysplasia (ARRVD). We therefore considered a total of 47 genes.

Since AF is associated with increased mortality, the genetic characterization of all family


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members is useful to identify individuals at risk and to decide appropriate clinical follow-up to prevent serious complications.

## Patients and Methods

A total of 60 subjects, 18 probands and their families, were examined in the clinics of Krasnoyarsk, Russia. All patients and healthy subjects underwent electrocardiogram (ECG), Holter monitoring and blood sampling at the Federal Cardiological and Vascular Center (Krasnoyarsk) and at the Department of Internal Medicine I and Pediatrics IPO, Krasnoyarsk State Medical University "V.F. Voino-Yasenetsky". Enrolment criteria included:

1 **ECG characteristics:** absence of P waves; irregular R-R intervals;
2. **Clinical presentation:** AF as major clinical manifestation (phenotype) with early onset (before age 60)[4];
3. **Family history:** at least one affected first or second-degree family member.

All subjects underwent genetic counselling in which the risks and benefits of genetic testing were explained. They signed specific consent to use of their clinical and genetic data for research and publication.

The work described in this paper was approved by the Krasnoyarsk State Medical University Ethics Committee (protocol no. 54/2014) and carried out in accordance with the Declaration of Helsinki.

Genetic testing protocols were developed at MAGI's Laboratories (MAGI's Lab, Rovereto and MAGI Euregio, Bolzano, Italy). Genetic studies were performed at the Russian-Italian Laboratory of Medical Genetics (Krasnoyarsk, Russia) where a total of 60 blood samples were sent for DNA extraction.

Targeted resequencing was performed using the Illumina commercial kit "TruSight One sequencing panel" on the MiSeq platform (Illumina, San Diego, CA, USA). This kit makes it possible to perform enrichment and final analysis of a panel of approximately 4800 genes (http://www.illumina.com/ products/trusight-one-sequencing-panel.ilmn).

In-solution target enrichment was performed according to the "TruSight One sequencing panel" manufacturer's instructions. For the quantification and validation of the genomic library, we used the Qubit® 2.0 Fluorometer system (Life Technologies, Carlsbad, CA, USA) and a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The analysis of raw read data in fastq format was performed using an in-house bioinformatics pipeline as described in our previous paper on long-QT syndrome (LQTS) in Russian families[5]. The analysis was performed on the coding exons and 15 bp flanking sequence of a list of 47 genes that were reported to be linked to hereditary cardiomyopathies at the time of analysis (Table I).

Variants were selected for further study on the basis of previously described criteria[5].

DNA samples from probands were analyzed by this method and all genetic variants were validated by Sanger sequencing using a Beckman Coulter CEQ 8000 sequencer (Beckmann Coulter, Milan, Italy). The genotype-phenotype correlation of each variant was evaluated by family segregation study using the same method.

The electropherograms of amplified fragments were analyzed using ChromasPro 1.5 (Technelysium Pty. Ltd., Brisbane, Queensland, Australia) and Sequencher 5.0 (Gene Codes®; Ann Arbor, MI, USA) software and compared to GenBank reference sequences with the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov). Nonsense, frameshift and splice site variants were considered disease-causing.

We consulted the Human Gene Mutation Professional Database (HGMD) (http://www.biobase-international.com/product/hgmd), the Exome Aggregation Consortium (ExAC) database (exac.broadinstitute.org/), the Exome Variant Server (EVS) database (http://evs.gs.washington.edu/EVS/) and the public database of single nucleotide variants (dbSNP, www.ncbi.nlm.nih.gov/SNP/) in order to identify genetic variants previously reported as pathogenic and to check for allele frequencies.

Nucleotide variants were assessed for pathogenicity using the PolyPhen 2 and SIFT algorithms[6,7] via the Variant Effect Predictor tool (http://www.ensembl.org/info/docs/tools/vep/index.html) and MutationTaster (http://www.mutationtaster.org)[8]. Intronic variants were checked for their potential to affect splicing using Human Splicing Finder software Version 3 (http://www.umd.be/HSF3/HSF.html).

Genetic variants were classified according to the criteria of ACMG Standards and Guidelines[9].

**Table I.** Genes involved in hereditary cardiomyopathies.

| Gene (OMIM ID:) | Protein name | Phenotype (OMIM ID:) | Inheritance |
|---|---|---|---|
| *ABCC9* (601439) | ATP-binding cassette sub-family C member 9 | Atrial fibrillation, familial, 12 (614050)<br>Cardiomyopathy, dilated, 1O (608569)<br>Hypertrichotic osteochondrodysplasia (239850) | AD<br>NA<br>AD |
| *AKAP9* (604001) | A-kinase anchor protein 9 | Long QT syndrome-11 (611820) | AD |
| *ANK2* (106410) | Ankyrin B | Long QT syndrome 4 (600919)<br>Cardiac arrhythmia, ankyrin-B-related (600919) | AD<br>AD |
| *CACNA1C* (114205) | Calcium channel, L type, alpha 1 polypeptide isoform | Brugada syndrome 3 (611875)<br>Timothy syndrome (601005)a | AD<br>AD |
| *CACNA2D1* (114204) | Voltage-dependent calcium channel subunit alpha-2/delta-1 | Brugada syndrome (NA) | AD |
| *CACNB2* (600003) | Voltage-dependent L-type calcium channel subunit beta-2 | Brugada syndrome 4 (611876) | AD |
| *CALM1* (114180) | Calmodulin 1 - calcium-modulated protein | Long QT syndrome 14 (616247)<br>Ventricular tachycardia, catecholaminergic polymorphic, 4 (614916) | AD<br>AD |
| *CASQ2* (114251) | Calsequestrin-2 | Ventricular tachycardia, catecholaminergic polymorphic, 2 (611938) | AR |
| *CAV3* (601253) | Caveolin 3 | Cardiomyopathy, familial hypertrophic (192600)<br>Creatine phosphokinase, elevated serum (123320)<br>Long QT syndrome 9 (611818)<br>Myopathy, distal, Tateyama type (614321)<br>Rippling muscle disease 2 (606072) | AD<br>AD<br>AD<br>AD<br>AD |
| *CTNNA3* (607667) | Catenin alpha-3 | Arrhythmogenic right ventricular dysplasia, familial, 13 (615616) | AD |
| *DPP6* (126141) | Dipeptidyl aminopeptidase-like protein 6 | Ventricular fibrillation, paroxysmal familial, 2 (612956) | AD |
| *DSC2* (125645) | Desmocollin-2 | Arrhythmogenic right ventricular dysplasia 11 (610476) | AD, AR |
| *DSG2* (125671) | Desmoglein-2 | Arrhythmogenic right ventricular dysplasia 10 (610193)<br>Cardiomyopathy, dilated, 1BB (612877) | AD<br>NA |
| *DSP* (125647) | Desmoplakin | Arrhythmogenic right ventricular dysplasia 8 (607450)<br>Cardiomyopathy, dilated, with woolly hair and keratoderma (605676)<br>Dilated cardiomyopathy with woolly hair, keratoderma, and tooth agenesis (615821) | AD<br>AR<br>AD |
| *GJA5* (121013) | Gap junction alpha-5 protein | Atrial fibrillation, familial, 11 (614049)<br>Atrial standstill, digenic (GJA5/SCN5A) (108770) | AD<br>AD |
| *GNAI2* (139360) | Guanine nucleotide-binding protein G(i) subunit alpha-2 | Ventricular tachycardia, idiopathic (192605) | AD |
| *GPD1L* (611778) | Glycerol-3-phosphate dehydrogenase 1-like protein | Brugada syndrome 2 (611777) | AD |
| *HCN4* (605206) | Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4 | Brugada syndrome 8 (613123)<br>Sick sinus syndrome 2 (163800) | AD<br>AD |
| *JUP* (173325) | Junction plakoglobin | Arrhythmogenic right ventricular dysplasia 12 (611528)<br>Naxos disease (601214) | AD<br>AR |
| *KCNA5* (176267) | Potassium voltage-gated channel subfamily A member 5 | Atrial fibrillation, familial, 7 (612240) | AD |
| *KCND3* (605411) | Potassium voltage-gated channel subfamily D member 3 | Brugada syndrome 9 (616399) | AD |
| *KCNE1* (176261) | Voltage-gated potassium channel, Isk related subfamily, member 1 | Jervell and Lange-Nielsen syndrome 2 (612347)<br>Long QT syndrome 5 (613695) | AR<br>AD |

*Table continued*

**Table I *(Continued)*.** Genes involved in hereditary cardiomyopathies.

| Gene (OMIM ID:) | Protein name | Phenotype (OMIM ID:) | Inheritance |
|---|---|---|---|
| *KCNE2* (603796) | Voltage-gated potassium channel, Isk related subfamily, member 2 | Atrial fibrillation, familial, 4 (611493)<br>Long QT syndrome 6 (613693) | AD<br>AD |
| *KCNE3* (604433) | Potassium voltage-gated channel subfamily E member 3 | Brugada syndrome 6 (613119) | AD |
| *KCNH2* (152427) | Potassium channel, voltage-gated, H2 | Long QT syndrome 2 (613688)<br>Short QT syndrome 1 (609620)<br>Long QT syndrome 2, acquired, susceptibility to (613688) | AD<br>AD<br>AD |
| *KCNJ2* (600681) | Inwardly rectifying potassium channel | Andersen syndrome (170390)<br>Atrial fibrillation, familial, 9 (613980)<br>Short QT syndrome 3 (609622) | AD<br>AD<br>AD |
| *KCNJ5* (600734) | Potassium inwardly-rectifying channel, subfamily J, member 5 | Hyperaldosteronism, familial, type III (613677)<br>Long QT syndrome 13 (613485) | AD<br>AD |
| *KCNJ8* (600935) | ATP-sensitive inward rectifier potassium channel 8 | Sudden infant death syndrome (NA) | AD |
| *KCNQ1* (607542) | KQT-like voltage-gated potassium channel 1 | Atrial fibrillation, familial, 3 (607554)<br>Jervell and Lange-Nielsen syndrome (220400)<br>Long QT syndrome 1 (192500)<br>Short QT syndrome 2 (609621)<br>Long QT syndrome 1, acquired, susceptibility to (192500) | AD<br>AR<br>AD<br>AD<br>AD |
| *LMNA* (150330) | Prelamin-A/C | Cardiomyopathy, dilated, 1A (115200)<br>Emery-Dreifuss muscular dystrophy 2, AD (181350)<br>Emery-Dreifuss muscular dystrophy 3, AR (616516)<br>Heart-hand syndrome, Slovenian type (610140)<br>Lipodystrophy, familial partial, type 2 (151660)<br>Malouf syndrome (212112)<br>Muscular dystrophy, congenital (613205)<br>Restrictive dermopathy, lethal (275210) | AD<br>AD<br>AR<br>AD<br>AD<br>AD<br>AD<br>AR |
| *NPPA* (108780) | Natriuretic peptides A | Atrial fibrillation, familial, 6 (612201)<br>Atrial standstill 2 (615745) | AD<br>AR |
| *NUP155* (606694) | Nuclear pore complex protein Nup155 | Atrial fibrillation 15 (615770) | AR |
| *PKP2* (602861) | Plakophilin-2 | Arrhythmogenic right ventricular dysplasia 9 (609040) | AD |
| *PRKAG2* (602743) | 5'-AMP-activated protein kinase subunit gamma-2 | Cardiomyopathy, hypertrophic 6 (600858)<br>Glycogen storage disease of heart, lethal congenital (261740)<br>Wolff-Parkinson-White syndrome (194200) | AD<br>AD<br>AD |
| *RYR2* (180902) | Ryanodine receptor 2 | Arrhythmogenic right ventricular dysplasia 2 (600996)<br>Ventricular tachycardia, catecholaminergic polymorphic, 1 (604772) | AD<br>AD |
| *SCN10A* (108980) | Sodium channel protein type 10 subunit alpha | Brugada syndrome (NA)<br>Atrial fibrillation (NA)<br>Long QT syndrome (NA) | AD<br>AD<br>AD |
| *SCN1B* (600235) | Sodium channel subunit beta-1 | Atrial fibrillation, familial, 13 (615377)<br>Brugada syndrome 5 (612838)<br>Cardiac conduction defect, nonspecific (612838) | AD<br>AD<br>AD |
| *SCN2B* (601327) | Sodium channel subunit beta-2 | Atrial fibrillation, familial, 14 (615378) | AD |
| *SCN3B* (608214) | Sodium channel subunit beta-3 | Sodium channel subunit beta-3 (613120)<br>Brugada syndrome 7 (613120) | AD<br>AD |
| *SCN4B* (608256) | Sodium channel, voltage-gated, type IV beta subunit | Atrial fibrillation, familial, 17 (611819)<br>Long QT syndrome-10 (611819) | AD<br>AD |

*Table continued*

**Table I *(Continued)*.** Genes involved in hereditary cardiomyopathies.

| Gene (OMIM ID:) | Protein name | Phenotype (OMIM ID:) | Inheritance |
|---|---|---|---|
| *SCN5A* (600163) | Alfa polypeptide of voltage-gated sodium channel type V | Atrial fibrillation, familial, 10 (614022)<br>Brugada syndrome 1 (601144)<br>Cardiomyopathy, dilated, 1E (601154)<br>Heart block, nonprogressive (113900)<br>Heart block, progressive, type IA (113900)<br>Long QT syndrome-3 (603830)<br>Sick sinus syndrome 1 (608567)<br>Ventricular fibrillation, familial, 1 (603829)<br>Sudden infant death syndrome, susceptibility to (272120) | AD<br>AD<br>AD<br>AD<br>AD<br>AD<br>AR<br>AD<br>AR |
| *SNTA1* (601017) | Syntrophin, alpha 1 | Long QT syndrome 12 (612955) | AD |
| *TGFB3* (606237) | Transforming growth factor beta-3 proprotein | Arrhythmogenic right ventricular dysplasia 1 (107970)<br>Loeys-Dietz syndrome 5 (615582) | AD<br>AD |
| *TMEM43* (612048) | Transmembrane protein 43 | Arrhythmogenic right ventricular dysplasia 5 (604400)<br>Emery-Dreifuss muscular dystrophy 7, AD (614302) | AD<br>AD |
| *TRDN* (603283) | Triadin | Ventricular tachycardia, catecholaminergic polymorphic, 5, with or without muscle weakness (615441) | AR |
| *TRPM4* (606936) | Transient receptor potential cation channel subfamily M member 4 | Progressive familial heart block, type IB (604559) | AD |
| *TTN* (188840) | Titin | Cardiomyopathy, dilated, 1G (604145)<br>Cardiomyopathy, familial hypertrophic, 9 (613765)<br>Myopathy, myofibrillar, 9, with early respiratory failure (603689)<br>Salih myopathy (611705) | AD<br>AD<br>AD<br>AR |

AD: autosomal dominant; AR: autosomal recessive; NA: not available; OMIM: Online Mendelian Inheritance in Man.

## Results

Next generation sequencing analysis was performed for the 47 known hereditary cardiomyopathy-related genes using a TruSight One sequencing panel (Illumina). The average number of mappable reads per sample was 10 M. On average, 97% of the target bases of gene-disease subpanels were covered at least 10×, with a mean coverage of 120× per sample.

The NGS analysis revealed that 17 out of 18 probands had a genetic variant in one of the genes included in the panel (Table II); the segregation study in these families (40 relatives) led us to identify seven families with variants (39%, 7/18 probands) probably related to the phenotype (Figure 1). Five variants found in four families had already been described in the literature, while two, found in three unrelated probands and their affected relatives, were new. In three families, rare genetic variants were found in the *CACNA1C* gene. The other four families showed variants in the *CTNNA3*, *PKP2*, *SCN10A*, *ANK2* and *SCN5A* genes. Electropherograms from Sanger sequencing of these six variants are shown in Figure 2A. Results from the genotype-phenotype segregation study and a summary of clinical features of probands and relatives are shown in Figure 1 and Table III, respectively. All the remaining 24 variants listed in Table II did not segregate with the disease (data not shown).

**Family 2:** The proband (R585) is a 30-year-old male who suffered episodes of AF once every two months and also complained of continuous weakness and dizziness. Symptoms began at age 18 years. In his family, only the maternal grandmother (R619) shows similar cardiological features; she suffered AF attacks once a month for more than 20 years. She also complains of irregular heartbeat, weakness, dizziness and shortness of breath. Her moderately abnormal[10] left atrial diameter (Table III) can be attributed to age and her long history of arrhythmia[11].

**Family 3:** The male proband (R586), 33 years of age with onset at 21 years, complained of AF episodes once every six months, stably for the

**Table II.** List and characteristics of the heterozygous variants identified in FAF probands.

| Gene | Inheritance | Refseq | hgvs_c | hgvs_p | refSNP | Variant interpretation† | MAF | Phenotype segregation | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *AKAP9* | AD | NM_005751 | c.5246T>C | p.(Ile1749Thr) | rs150016098 | **VUS** | C:0.0009 | Fam-11; | 52 |
| *AKAP9* | AD | NM_005751 | c.11543A>G | p.(Asn3848Ser) | rs773891725 | **Likely benign** | G:0.000008 | Fam-1; | |
| *ANK2* | AD | NM_001148 | c.11231C>A | p.Thr3744Asn | rs121912705 | **VUS** | A:0.0006 | ***Fam-18***; | 37 |
| *ANK2* | AD | NM_001148 | c.11716C>T | p.(Arg3906Trp) | rs121912706 | VUS | T:0.0011 | Fam-6; | 37 |
| *CACNA1C* | AD | NM_001129827 | c.212C>T | p.(Ala71Val) | rs755579963 | VUS | T:0.0001 | ***Fam-2; Fam-3***; | |
| *CACNA1C* | AD | NM_001129827 | c.6040G>A | p.(Val2014Ile) | rs199473660 | Likely benign | A:0.0004 | ***Fam-9***; | 19 |
| *CACNB2* | AD | NM_ 201593 | c.121-1G>T | – | NA | **VUS** | NA | Fam-3; | |
| *CACNA2D1* | | NM_000722 | c.2126G>A | p.Ser709Asn | rs78086631 | **VUS** | T:0.0027 | Fam-9; | 19 |
| *CACNA2D1* | | NM_000722 | c.2264G>C | p.Ser755Thr | rs151327713 | Likely benign | G:0.0008 | Fam-12; | 53 |
| *CTNNA3* | AD | NM_001127384 | c.1133G>A | p.(Arg378His) | rs143682596 | **VUS** | T:0.0009 | Fam-16; | |
| *CTNNA3* | AD | NM_001127384 | c.232C>T | p.(Gln78*) | rs201306690 | **Pathogenic** | A:0.00002 | ***Fam-18***; | |
| *CTNNA3* | AD | NM_001127384 | c.1721T>C | p.(Leu574Pro) | rs375428912 | **VUS** | G:0.000008 | Fam-1; | |
| *JUP* | AR AD | NM_021991 | c.2078A>G | p.(Tyr693Cys) | rs782475413 | **VUS** | C:0.00003 | Fam-13; | 54 |
| *KCNH2* | AD | NM_000238 | c.526C>T | p.Arg176Trp | rs36210422 | VUS | A:0.0003 | Fam-2; Fam-8; | 55 |
| *LMNA* | AR AD | NM_170707 | c.-1C>A | – | rs886043355 | VUS | NA | Fam-18; | |
| *PKP2* | AD | NM_004572 | c.1093A>G | p.Met365Val | rs143900944 | Likely benign | C:0.0004 | Fam-14; | 29 |
| *PKP2* | AD | NM_004572 | c.1114G>C | p.(Ala372Pro) | rs200586695 | **VUS** | G:0.0005 | ***Fam-14***; | 27 |
| *RYR2* | AD | NM_001035 | c.5923A>G | p.(Met1975Val) | rs200318013 | VUS | G:0.00008 | Fam-3; | |
| *SCN10A* | AD | NM_006514 | c.41G>T | p.Arg14Leu | rs141207048 | **VUS** | A:0.0019 | ***Fam-12***; | 24 |
| *SCN5A* | AD | NM_000335 | c.3578G>A | p.Arg1193Gln | rs41261344 | **VUS** | A:0.0062 | ***Fam-17***; | 31 |
| *TTN* | AD AR | NM_133378 | c.3913G>T | p.(Gly1305Trp) | rs199889888 | VUS | G:0.0001 | Fam-5; | 56 |
| *TTN* | AD AR | NM_133378 | c.21394C>T | p.(Pro7132Ser) | rs375209098 | VUS | T:0.0001 | Fam-2; Fam-7; | |
| *TTN* | AD AR | NM_133378 | c.24775G>A | p.(Val8259Ile) | rs202160275 | Likely benign | T:0.0004 | Fam-15; | |
| *TTN* | AD AR | NM_133378 | c.26542C>T | p.(His8848Tyr) | rs72650011 | **Likely benign** | A:0.0041 | Fam-11; | 57 |
| *TTN* | AD AR | NM_133378 | c.47114C>T | p.(Pro15705Leu) | rs201035511 | **VUS** | A:0.0005 | Fam-9; | |
| *TTN* | AD AR | NM_133378 | c.48221T>A | p.(Leu16074Gln) | rs140714512 | Likely benign | T:0.0004 | Fam-9; | |
| *TTN* | AD AR | NM_133378 | c.63596G>A | p.(Arg21199Gln) | rs370516890 | **VUS** | T:0.0001 | Fam-9; | |
| *TTN* | AD AR | NM_133378 | c.64522T>G | p.(Leu21508Val) | rs202098308 | **VUS** | C:0.00006 | Fam-4; | |
| *TTN* | AD AR | NM_133378 | c.76050delT | p.(Leu25351*) | NA | **VUS** | NA | Fam-18; | |
| *TTN* | AD AR | NM_133378 | c.86929C>T | p.(Arg28977Cys) | rs202187398 | VUS | T:0.0002 | Fam-18; | |
| *TTN* | AD AR | NM_133378 | c.90538C>T | p.(Arg30180Cys) | rs72648272 | **Likely benign** | A:0.004 | Fam-11; | |

AD; autosomal dominant; AR, autosomal recessive; MAF, minor allele frequency; VUS, variant of unknown significance. †In bold, variants classified in this study according to the criteria of ACMG Standards and Guidelines that differ from the last ClinVar evaluation; in normal type, variants classified in this study that do not differ from the last ClinVar evaluation. Protein substitutions not in parenthesis were functionally characterized; In **bold** and *italics*, variants segregating in affected family members

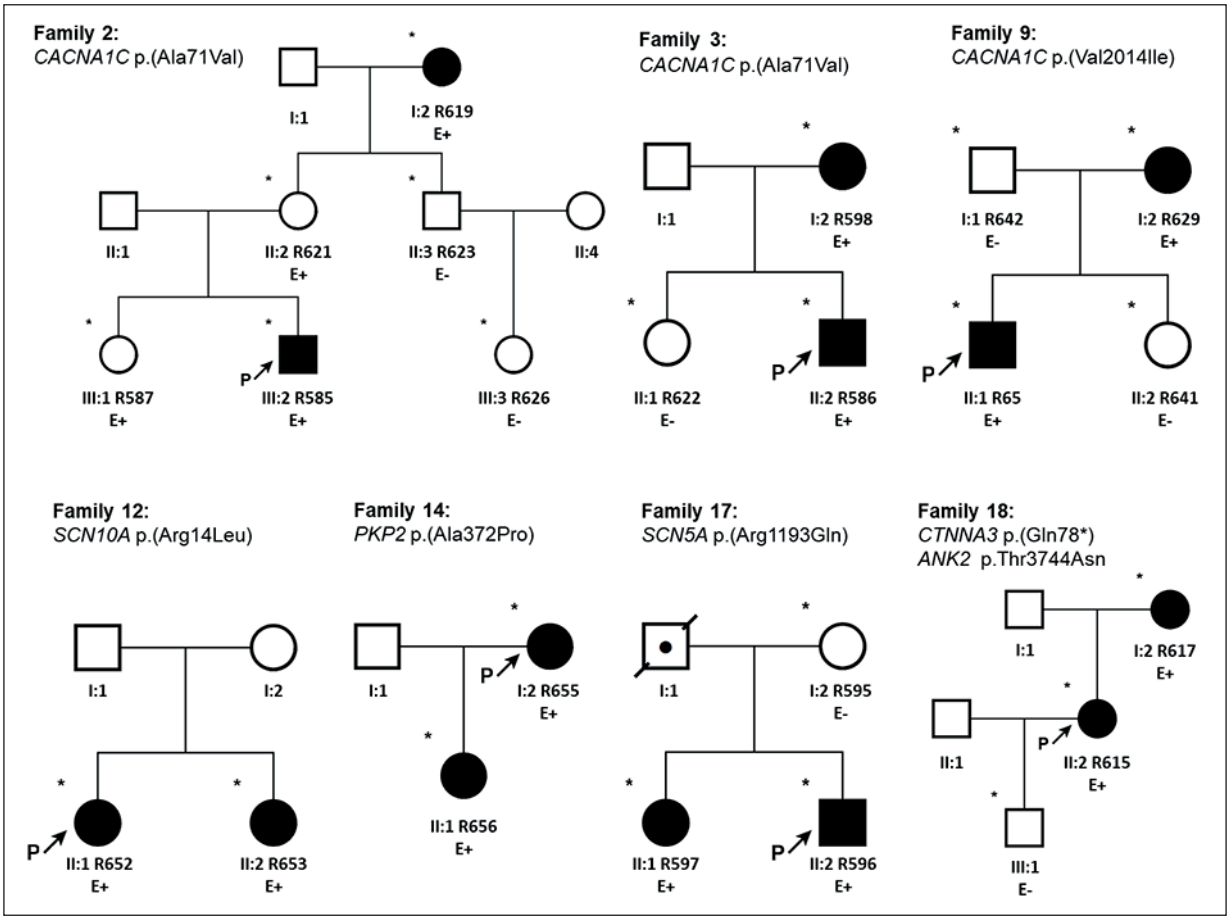


**Figure 1.** Family pedigree illustrating cosegregation of gene mutations and atrial fibrillation phenotypes. P, proband; *Documented clinical evaluation; E+ and E-, positive and negative to genetic test, respectively; ● Obligate carrier.

last 10 years. He feels tired and debilitated. The mother (R598), 67 years of age, has had similar cardiac features since she was 50 (Table III). Since onset, she has suffered AF attacks about once a month. She also complains of headache, irregular heartbeat, shortness of breath and persistent fatigue.

**Family 9:** The 34-year-old male proband (R65) was diagnosed with idiopathic paroxysmal AF with onset at 25 years of age and recurrent palpitations and dizziness as main symptoms. In the family, only the 66-year old mother (R629) has been diagnosed with paroxysmal AF (at age 50 years) (Table III). In the last 5 years, she has suffered AF attacks twice a year. She complains of headache, dizziness and fatigue.

**Family 12:** Only two members were evaluated in this family, the proband (R652), an 80-year-old woman, and her younger sister (R653), age 78 years (Table III). The proband and her sister were diagnosed with lone paroxysmal AF at age 58 and 54 years, respectively. Signs and evolution were quite similar, with one AF episode per year accompanied by debilitation, dizziness and shortness of breath.

**Family 14:** Two members of this family were assessed clinically and genetically: a 78-year-old woman (R655) and her 38-year-old daughter (R656), both diagnosed with lone paroxysmal AF. The proband's daughter had her first AF episode lasting more than one minute at age 26, provoked by transesophageal echocardiography. The mother suffered her first AF episode at age 59 years (Table III). Since then, clinical course and associated symptoms have been similar in the two patients.

**Family 17:** This 30-year-old male proband (R596) is the patient with the earliest onset of all affected subjects in the seven families. His first attack of AF occurred at age 16 years. Attacks have continued with a frequency of one or two per year. The proband's 34-year-old sister (R597) man-

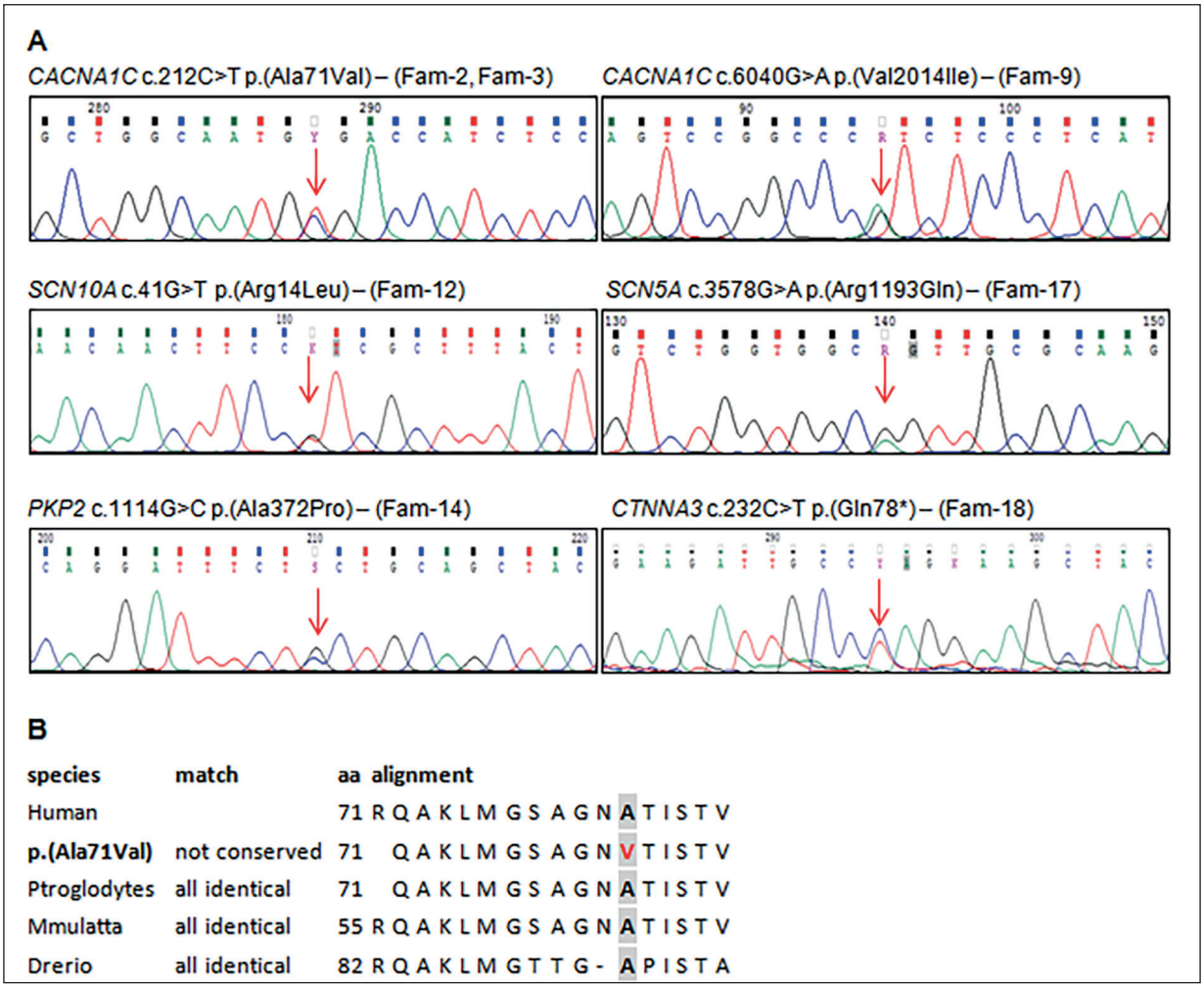


**Figure 2.** ***A,*** Sequence chromatograms of the four variants identified in AF patients. ***B,*** Amino acid (aa) conservation across species of the novel *CACNA1C* variant showing that Cav1.2 Ala71 is highly conserved.

ifested similar disease features with late onset at age 28. Since the first attack, the frequency has been similar to that of her brother and both complain of associated weakness and dizziness (Table III). Their father died of cardiac arrest.

**Family 18:** The 51-year-old female proband (R615) suffered her first episode of AF when she was 35. Since then, she has had episodes once every 6 months, accompanied by debilitation and dizziness. Her 71-year-old mother (R617) showed a similar course and symptoms but with later onset at age 59 years (Table III).

## Discussion

Genetic screening of Russian families having members with AF produced interesting results. Probands from family 2 and family 3 (R619 and R586, respectively) showed the p.(Ala71Val) variant in exon 2 of the *CACNA1C* gene, which encodes an alpha-1 subunit of a voltage-dependent calcium channel or Cav1.2. Genetic variants in specific sites (i.e., exon 8 or 8a) of this gene are known to be associated with Timothy Syndrome, a very rare condition in which LQTS is accompanied by physical and neurological involvement[12], BS[13] and LQTS type 8 without extracardiac abnormalities[14,15]. The amino acid alanine in position 71 is highly conserved through evolution, suggesting that it may be important for protein function (Figure 2B).

The p.(Ala71Val) variant is located near the N-terminus domain of the protein in a highly conserved region of the Cav1.2 protein. Only four variants in exon 2, namely p.Ala28Thr,

**Table III.** . Clinical characteristics of AF families.

| Family ID, sex | Age of onset | Type of AF | AF attack frequency | Echocardiogram | | Electrocardiogram | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | LAD* cm | EF % | HR (beats/min) | P (msec) | PQ (msec) | QRS (msec) | QT (msec) | QTc (msec) |
| **Fam-2** | | | | | | | | | | | |
| R585 (P), M | 18 | PA | 1/2 months | 3.3 | 68 | 65 | 60 | 160 | 80 | 320 | 333 |
| R626, F | | | | | | | | | | | |
| First cousin | – | Healthy | – | 3.3 | 70 | 60 | 60 | 160 | 80 | 320 | 320 |
| R623, M | | | | | | | | | | | |
| Maternal uncle | – | Healthy | – | 3.0 | 66 | 68 | 80 | 160 | 80 | 360 | 383 |
| R621, F | | | | | | | | | | | |
| Mother | – | Healthy | – | **2.6** | 76 | 76 | 60 | 140 | 80 | 360 | 405 |
| R619, F | | | | | | | | | | | |
| Grandmother | 52 | PA | 1/month | **4.5** | 75 | 60 | 100 | 180 | 80 | 360 | 360 |
| R587, F | | | | | | | | | | | |
| Brother | – | Healthy | – | 3.3 | 72 | 66 | 60 | 160 | 80 | 320 | 335 |
| **Fam-3** | | | | | | | | | | | |
| R586 (P), M | 21 | LPA | 1/6 months | 3.7 | 69 | 72 | 80 | 140 | 80 | 360 | 394 |
| R598, F | | | | | | | | | | | |
| Mother | 50 | LPA | 1/month | 3.7 | 70 | 78 | 80 | 160 | 100 | 300 | 342 |
| R622, F | | | | | | | | | | | |
| Sister | – | Healthy | – | 3.6 | 71 | 65 | 80 | 140 | 80 | 320 | 333 |
| **Fam-9** | | | | | | | | | | | |
| R65 (P), M | 25 | IPA | – | – | – | 65 | 80 | 160 | 100 | 380 | 396 |
| R642, M | | | | | | | | | | | |
| Father | – | Healthy | – | – | – | 66 | 80 | 160 | 80 | 380 | 399 |
| R629, F | | | | | | | | | | | |
| Mother | 50 | PA | 1/6 months | 3.6 | 66 | 65 | 80 | 180 | 80 | 360 | 375 |
| R641, F | | | | | | | | | | | |
| Brother | – | Healthy | – | – | – | 70 | 80 | 160 | 80 | 340 | 367 |
| Fam–12 | | | | | | | | | | | |
| R652 (P), F | 58 | LPA | 1 per year | 3.7 | 65 | 57 | 80 | 180 | 80 | 400 | 390 |
| R653, F | | | | | | | | | | | |
| Sister | 54 | LPA | 1 per year | 3.3 | 67 | 82 | 80 | 140 | 100 | 340 | 397 |
| **Fam-14** | | | | | | | | | | | |
| R655 (P), F | 59 | LPA | 1/6 months | 3.6 | 68 | 55 | 80 | 160 | 80 | 400 | 383 |
| R656, F | | | | | | | | | | | |
| Daughter | 26 | LPA | 1/6-8 months | 3.3 | 70 | 65 | 60 | 160 | 80 | 320 | 333 |

*Table continued*

**Table III** *(Continued).* . Clinical characteristics of AF families.

| Family ID, sex | Age of onset | Type of AF | AF attack frequency | Echocardiogram | | Electrocardiogram | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | LAD* cm | EF % | HR (beats/min) | P (msec) | PQ (msec) | QRS (msec) | QT (msec) | QTc (msec) |
| **Fam-17** | | | | | | | | | | | |
| R596 (P), M | 16 | LPA | 1/6–12 months | 3.3 | 78 | 68 | 80 | 160 | 80 | 340 | 362 |
| R597, F | | | | | | | | | | | |
| Sister | 28 | LPA | 1/6-12 months | 3.3 | 75 | 65 | 80 | 160 | 80 | 340 | 354 |
| R595, F | | | | | | | | | | | |
| Mother | – | Healthy | – | 3.3 | 70 | 60 | 80 | 160 | 80 | 320 | 320 |
| **Fam-18** | | | | | | | | | | | |
| R615 (P), F | 35 | LPA | 1/6 months | 3.3 | 68 | 70 | 60 | 180 | 80 | 340 | 367 |
| R617, F | | | | | | | | | | | |
| Mother | 59 | LPA | 1/6 months | 3.3 | 65 | 60 | 80 | 180 | 80 | 360 | 360 |
| R618, M | | | | | | | | | | | |
| Son | – | Healthy | – | 3.2 | 70 | 66 | 80 | 160 | 80 | 320 | 336 |

P: proband; AF: atrial fibrillation; PA: paroxysmal; LPA: lone paroxysmal; IPA: idiopathic paroxysmal; LAD: left atrial diameter; *Normal ranges are 2.7-3.8 cm and 3.0-4.0 cm for women and men, respectively (Bold font indicates abnormal values); EF: ejection fraction; HR: heart rate. QTc: corrected QT interval (Bazett's Formula).

p.(Ala34Val), p.(Gly37Arg) and p.Ala39Val, are described in association with non-syndromic long-QT (LQT)15, sudden cardiac death[16], sudden arrhythmic death syndrome[17] and BS associated with shorter-than-normal QT interval, respectively[13]. Two of them were also characterized functionally: the p.Ala28Thr variant showed an in vitro gain-of-function effect that prolonged action potential duration in line with the associated LQT phenotype15; p.Ala39Val has been described in the literature associated with BS and the authors demonstrated that the observed loss of current was due to a defect in trafficking of mature Cav1.2 channels from the endoplasmic reticulum/Golgi complex to the cell membrane[13].

Our study shows that p.(Ala71Val) segregated with phenotype in family 3, whereas segregation in family 2 suggests that the variant may have incomplete penetrance. Although incomplete penetrance of *CACNA1C* variants has already been described[18], involvement of this variant in onset of the disease in both families can only be postulated from the above observations and therefore the predicted pathogenicity needs to be demonstrated by functional studies.

Genetic testing of family 9 revealed that the proband (R65) has the *CACNA1C*, p.(Val2014Ile) variant. This variant was shown by Burashnikov et al[19] to cause loss of calcium channel current function. Interestingly, Burashnikov et al[19] described the variant in a patient who showed a BS type I ECG after sodium block challenge and had a family history of sudden death of unknown cause at an early age.

Although electrical remodelling led by changes in calcium current channel density has been associated with persistence of AF[20], and although there is increasing evidence suggesting that microRNAs are responsible for decreasing L-type $Ca^{2+}$ current (ICa) through downregulation of CACNA1C mRNA expression[21,22], no genetic variants in *CACNA1C* has ever previously been described in association with lone AF[23].

In our report, three out of seven families showed a genetic variant in the *CACNA1C* gene, and one of these variants is already associated with BS in the literature. A primary role of changes in cell $Ca^{2+}$ loading in the onset of AF, as distinct from the maintenance of AF, remains to be demonstrated. A reason for the original association could be the fact that *CACNA1C* is not usually evaluated in genetic association studies of AF and the results may have been missed.

The variant *SCN10A* p.Arg14Leu found in two affected members of family 12 is a known variant already associated with BS in various independent reports[24-26]. Although the frequency of this variant did not differ between BS patients and controls[25], functional evaluation of the mutant protein has shown that it causes loss-of-function of Nav1.5 current, which can be expected to reduce excitability and lead to development of the arrhythmogenic substrate responsible for inherited cardiac arrhythmia syndromes, including BS and AF[24].

The two affected subjects in family 14 share the variant p.(Ala372Pro) in the *PKP2* gene, a likely disease-causing variant, associated in the literature with ARRVD[27,28]. The variant in proband R655 is in compound heterozygous state with p.(Met365Val), previously described in association with BS in at least two different reports[26,29]. However, R655 did not show a more severe phenotype than her daughter (R656). This finding, in addition to the result of the segregation study, indicates that if there is any association between *PKP2* and AF in this family, it can only be attributed to the variant p.(Ala372Pro).

The p.Arg1193Gln variant in the *SCN5A* gene found in the proband of family 17 (R596) is a variant well-known in the literature due to its great ethnic heterogeneity. The earliest reports from two different research groups linked this variant with LQTS[30] and sudden unexplained nocturnal death syndrome, a typical presentation of BS in individuals from southeast Asia[31]. The two groups performed functional characterization but obtained different results. The functional evaluation performed by Wang et al[30] showed that the mutant channel generates a persistent and non-activating current leading to prolonged cardiac action potential duration. p.Arg1193Gln was therefore described as a "gain-of-function" variant consistent with previously defined variants causing LQT type 3. The functional characterization of p.Arg1193Gln performed by Vatta et al[31] showed that the variant accelerates fast inactivation of the sodium channel, resulting in a reduced sodium current consistent with a "loss-of-function" variant: this explained the BS phenotype. Finally, independent research groups definitively associated this variant with both cardiac conditions, showing that distinct phenotypes depend on the background splice variant used for expression[32,33]. These results suggest caution in interpreting findings of arrhythmia variants

in genetic studies and highlight the contribution of interaction of environment and genetic background with genetic variants[32].

Given the relatively common occurrence, with a certain ethnic specificity (i.e., the variant is common in Asians with a MAF of 8%, rare in whites with a MAF of 0.3% and unseen in Hispanics and blacks)[34], this functional genetic variant was described as a risk factor for cardiac arrhythmias in the general population. The variant segregated with disease phenotype in AF family 17 here described. Moreover, the proband and her sister inherited the variant from their father, obligate carrier of the genetic variant, who died of cardiac arrest before the start of the study (Figure 1). This evidence, together with the above mentioned literature, seems to increase the potential role of this variant in sudden deaths due to heart failure, while at the same time underlining a new association with lone AF.

In family 18, the two affected subjects, the proband R615 and her daughter R617, share two variants with possible influence on the pathogenic phenotype: a truncated variant p.(Gln78*) in *CTNNA3*, a gene associated with ARRVD-13 (OMIM disease 615616), and a substitution p.(Thr3744Asn) in *ANK2*, a gene associated with cardiac arrhythmia or LQTS 4 (OMIM disease 600919). ARRVD is characterized by progressive fibro-fatty replacement of the right ventricle with structural and functional abnormalities of the ventricles, electrocardiographic depolarization/repolarization changes, re-entrant arrhythmias, and sudden death as main clinical features[35]. In proband R615, 12-lead ECG showed no specific signs of ARRVD but cardiac magnetic resonance was not performed.

*CTNNA3* encodes for catenin alpha-3, an 895 amino-acid protein; the variant found in our family introduces a stop codon in position 78 and can be classified as pathogenic on the basis of autosomal dominant transmission of the gene described in ARRVD patients.

*ANK2* encodes for ankyrin-2, a 220 KDa protein involved in the localization and membrane stabilization of ion transporters and channels in cardiomyocytes. It has been shown that mice heterozygous for a null variant in *ANK2*, which disrupts cell organization of various ion pumps and channels, manifest arrhythmia[36,37]. In addition, the present variant has been described as pathogenic in a patient with cardiac arrhythmia[36].

In conclusion, six of the AF families here reported carry variants in genes commonly associated with BS (*CACNA1C*, *PKP2*, *SCN10A* and *SCN5A*). BS is characterized by a right bundle branch block pattern with ST-segment elevation in leads V1 to V3. Interestingly, none of our six probands showed ECG patterns compatible with BS or LQTS (Figure 3), and none had any organic heart disease. It is known from the literature that some drugs can provoke electrocardiographic changes consistent with BS in patients with AF[38]. Drug challenges to clarify the phenotypes were not performed for ethical reasons.

AF and BS share some pathogenic genes, i.e., *SCN1B*, *SCN3B* and *SCN5A* (data from OMIM); however, the association with the BS gene *CACNA1C* described here is new.

Variants in *PKP2*, *ANK2* and *SCN10A* seem somehow involved in AF. In particular, *PKP2* is known to influence atrial volume[39] and to be associated with AF in the setting of ARRVD/cardiomyopathy[40]. *SCN10A* variants seem to be associated with late sodium current and alterations in heart conduction[41] and to modulate risk of AF[42]. *ANK2* variants are associated with LQTS and cardiac arrhythmia. However, the association with FAF in these two cases is new. Involvement of the ARRVD-associated gene, *CTNNA3*, in AF is new and requires further study.

The present study has a number of limitations. First, segregation studies were only performed in families with two affected members. Second, we only selected variants shared by affected members, i.e., we chose to disregard variants described as pathogenic in the literature if they were only found in probands and not in affected relatives. We decided to use the Mendelian inheritance model to simplify interpretation of the results, but we cannot exclude the possibility that phenotype differences between members of the same family could be driven by a common pathogenic variant (shown in this report) that acts in a different genetic setting. There is growing conviction that heritable arrhythmia syndromes are oligogenic or even polygenic diseases[43] and some of the genes evaluated in our study have been demonstrated to behave in this way[18,44]. Likewise in family 18, we cannot exclude the possibility that both *ANK2* and *CTNNA3* are involved in the resulting phenotype. Third, the study was conducted exclusively on Russian patients and it may not be possible to generalize the results to other populations.

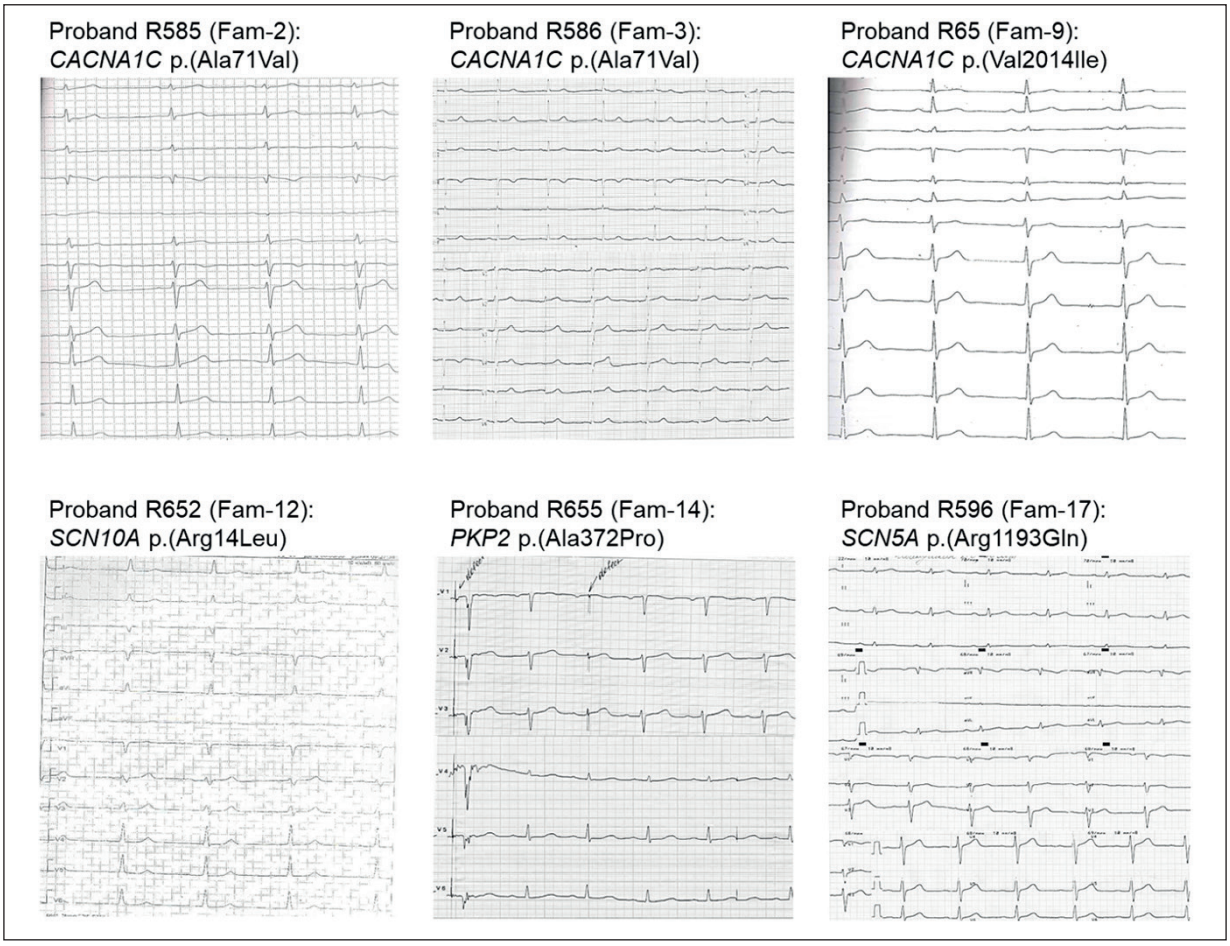


**Figure 3.** Absence of ST-segment elevation in the AF probands.

## Conclusions

To the best of our knowledge, this is the first report of lone AF families with genetic variants and genes known to be associated with other inherited cardiac diseases, although the differences in their phenotypes remain unclear. In light of our results, we suggest that hereditary cardiomyopathies may share a number of genes and variants, and phenotype differences could be explained by the different genetic background in which they act. This is also the first report in Russian families.

Clinically, the result of this study is interesting for identification of subjects at risk of sudden death. This hypothesis needs to be verified in a larger population of subjects with BS and FAF.

In support of the hypothesis, many recent studies suggest that atrial fibrillation is effectively associated with increased risk of cardiovascular pathologies and mortality, especially sudden death and heart failure[45-48]. The mechanisms by which AF increases risk are not completely clear, but we are sure that genetic studies will shed light on them.

Sudden cardiac death is caused by ventricular arrhythmias and Brugada syndrome is a right ventricular disease. In any case, genetically it is not strange that genes associated with ventricular disease be associated with atrial phenotypes and such evidence is not new. Indeed, Francis et al[49] remarked that the arrhythmogenic substrate of Brugada syndrome may not be restricted to the ventricles. Even more importantly, Alhassani et al[50] observed a family with lone AF characterized by a large deletion in *PKP2*, a gene normally associated with arrhythmogenic right ventricular cardiomyopathy. The authors suggest that in certain patients and families, cardiomyopathy gene variants may manifest preferentially with atrial rather than ventricular

phenotypes, and that this could depend on the fact that genes causative for ventricular cardiomyopathy may serve similar functions in the atria, and pathogenic variants in these genes may manifest with isolated atrial phenotypes, potentially secondary to differential penetrance in the atria and ventricles.

It seems clear that besides implementing the normal measures for reducing the risk of thromboembolic stroke, it is also necessary to prevent all these other complications. This can be done through a multidimensional therapeutic programme that considers all the comorbidities that frequently accompany atrial fibrillation[46]. In the near future, genetic data may help in the management of AF by improving the identification of individuals at risk for heart failure and sudden cardiac death.

The success rate (39%) of genetic testing obtained with our approach indicates that AF is genetically heterogeneous and that other genes, not evaluated in this study, may be implied in the pathogenesis. Although a comprehensive gene panel for FAF is still far from completion, since the molecular and pathophysiological foundations are still not fully understood[51], the NGS approach remains the best choice for this kind of study. Familial forms of the disease are not uncommon and it is therefore important to identify individuals at risk for the purpose of prevention or for determining appropriate treatments and planning clinical follow-up.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

### Acknowledgements

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