Lack of association between mitochondrial DNA haplogroups J and T and clinical manifestation in Russian patients with Brugada syndrome

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Received December 3, 2019; Accepted April 16, 2020

DOI: 10.3892/br.2020.1324

Abstract. Brugada syndrome (BrS) is an inherited disorder characterized by specific ST segment elevation in the right precordial leads, pseudo right bundle branch block, and a high risk of sudden cardiac death due to ventricular tachycardia. It was initially described as a monogenic disorder with an autosomal dominant mode of inheritance. It is hypothesized that modifying genetic factors, in addition to disease-causing mutations, may significantly contribute to the clinical symptoms and the risk of sudden cardiac death. These modifying factors can include mitochondrial DNA (mtDNA) variants. In particular, combination of mtDNA m.T4216C, m.A11251G, m.C15452A and m.T16126C variants (defining haplogroups T and J), is considered to be a factor that promotes manifestation of BrS manifestation, with no pro-arrhythmic effects. The aim of the present study was to confirm the reported association of BrS with MtDNA variants in a cohort of Russian patients. mtDNA haplogroups were genotyped in 47 Russian BrS probands and the prevalence of common mtDNA haplogroups was compared with the general population in European part of Russia. The distribution and prevalence of all but the J mtDNA haplogroups were comparable in BrS probands and the general Russian population. The mitochondrial J haplogroup was not found in the BrS cohort. In conclusion, it was shown that the mtDNA polymorphism, m.T4216C (haplogroups J and T) does not contribute significantly to the clinical manifestation of BrS in Russian patients.

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Key words: Brugada syndrome, SCN5A, inherited channelopathies, mitochondrial DNA, sudden cardiac death, genetic modifier

Introduction

Brugada syndrome (BrS) is an inherited arrhythmia disorder that was first described in 1992 (1). BrS is characterized by ST-segment elevation in the right precordial leads V1-V2 ≥2 mm, atypical right bundle branch block, and a high risk of sudden cardiac death (SCD) due to polymorphic ventricular tachycardia (2). In the majority of cases, the disease manifests around the age of 35-45 years, but life-threatening rhythm disturbances may occur at almost any age (3). The prevalence of BrS is ~10:100,000 in all ethnic groups including Russians (4).

To date, only cardioverter-defibrillator implantation has proven effectiveness in reducing the risk of SCD in BrS patients (5-7). However, the selection of patients for surgical treatment is difficult, as the prediction of clinical manifestation and SCD risk assessment remain controversial and need further clarification.

Mutations in at least 23 genes are known to be responsible for BrS (4). Mutations in the *SCN5A* gene are observed in 15-30% of patients (8), whereas other known genetic factors account for 5-10% of all cases. It is generally accepted that BrS is a monogenic autosomal dominant disorder (1,9,10). However, it has been suggested that the inheritance patterns of BrS do not always fit into classic Mendelian genetics. It is likely that other genetic and environmental factors are associated with the manifestation of BrS (10,11).

There have been two studies highlighting the possible role of mitochondrial DNA (mtDNA) polymorphisms in the clinical symptoms of BrS. In the first study, three likely unique pathogenic heteroplasmic mutations were identified in Iranian patients with BrS (12). The second study, performed in Italian BrS patients, showed that mtDNA haplogroups T and J were found in all symptomatic BrS patients with a spontaneous type 1 ECG pattern (13). The authors proposed that mtDNA haplogroups T and J represent important modifying factors for manifestation of BrS.

The aim of the present study was to assess the findings of Stocchi *et al* (13), which showed an association between BrS and mtDNA haplogroups T and J, and specifically combinations of mtDNA m.T4216C, m.A11251G, m.C15452A and m.T16126C variants, in a Russian cohort of patients with BrS.

Materials and methods

Cohort characteristics. A total of 47 index patients (40 males, 7 females) diagnosed with BrS were included in the present study (median age, 32 years; range, 4-63 years). The male-to-female ratio was 6:1. A total of 26 patients (55%) had a lone Brugada ECG pattern as an accidental finding, and 21 patients were symptomatic, with palpitation/syncope/cardiac arrest and a BrS pattern in their ECG that was accompanied by concomitant arrhythmias.

Clinical investigation. The present study was performed in accordance with the principles of the Declaration of Helsinki and the local Ethics Committee of Petrovsky National Research Centre of Surgery (Moscow, Russia). Written informed consent was obtained from all individual participants included in the study. Data obtained from each individual in the study included personal and familial medical history, general examination, 12-lead resting ECG, 24-h Holter monitoring, transthoracic echocardiogram and a pharmacological challenge test with class Ic anti-arrhythmic drugs. The diagnosis of BrS was established based on the currently recommended diagnostic criteria (14).

Genetic screening. Genetic screening of the target mini-panel genes (*SCN5A, SCN1B, SCN2B, SCN3B* and *SCN4B*), encoding the α- and β-subunits of the $Na_v1.5$ sodium channel, was performed using semi-conductive sequencing on the IonTorrent PGM® instrument (Thermo Fisher Scientific, Inc.). Confirmation of all variants was performed using Next-Generation Sequencing, resequencing of the regions with low coverage and cascade familial screening was performed by bidirectional capillary Sanger Sequencing. Sequencing data were analyzed using the Torrent Suite software v5.0.5 (Thermo Fisher Scientific, Inc.), Ion Reporter annotation software v5.12 (Thermo Fisher Scientific, Inc.) and the Integrative Genomic Viewer visualization tool v2.8.2 (Broad Institute of MIT). Interpretation of clinically relevant findings was performed using population databases (ExAC, gnomAD, 1000 Genomes), variant effect predictors (SIFT v1.1.3, PolyPhen2 v2, MutationTaster, Provean v1.1.3) and splicing analysis tools (UMD HSF v3.1; NetGene2). Following interpretation, variants were classified according to American College of Medical Genetics consensus recommendations (2015) (15). Variants classified as 'pathogenic' and 'likely pathogenic' (Class V and Class IV) were included in the final report.

The following reagents were used for hot-start PCR: HS Taq Turbo buffer, dNTPs (10 mM), Mg^{2+} (50 mM), H_2O (MQ), HS-Taq polymerase and the corresponding primers for each exon. All the reagents used were purchased from Evrogen. Hot-start PCR was performed using a Veriti™ 96-Well thermal cycler. The thermocycling conditions used were: preheating at 95˚C for 8 min; followed by 40 cycles of denaturation at 95˚C for 30 sec, annealing at 60˚C for 30 sec and elongation at 72˚C for 40 sec; and a final elongation step at 72˚C for 1 min.

MtDNA haplogroups were assigned based on the first hypervariable segment (HVS1) haplotype data obtained by Sanger Sequencing. A section of the mtDNA D-loop was PCR-amplified using primers L15997 (5'-CTCCACCATTAG CACCCAAAGC-3') and H408 (5'-CTGTTAAAAGTGCAT ACCGCCA-3'). The PCR products were then sequenced on an ABI 3730 DNA Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.) using the primers L15997 and H16526 (5'-AACGTGTGGGCTATTTAGGC-3').

Sanger Sequencing of the PCR products was performed with BigDye™ Terminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific), according to the manufacturer protocol. Primers L15997 and H16526 (5'-AACGTGTGGGCTATT TAGGC-3') were used as sequencing primers.

The sequences were aligned to a revised human mtDNA reference sequence (rCRS) (16). Each individual haplotype was represented as a list of substitutions compared with the rCRS. Mitochondrial haplogroup affiliations were determined using marker HVS1 substitutions, according to the human mtDNA phylogeny available online (17).

Statistical analysis. The frequencies of the haplogroups in healthy Russian individuals were based on data from (17). Haplogroup and genotype frequencies were compared using a χ^2 test with Yates correction or a Fisher's exact test (when the number in any group was <5). Two-sided 95% confidence intervals for the frequency values were calculated using the Wilson score method. Statistical analysis was performed using Statistica v13.2 (Dell). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Genetic study. Genetic screening revealed 7 mutations in the *SCN5A* gene in 7 probands (14.8%). No disease-causing mutations were found in the *SCN1B-4B* genes, and there were no significant differences found in the clinical manifestation of BrS between patients with different mutations, such as rate of syncope, number of SCD cases, age of manifestation, spectrum of ventricular and supra-ventricular arrhythmias, or ICD implantation between *SCN5A*-positive and *SCN5A*-negative probands (data not shown). The frequency of the p.H558R allele in the *SCN5A* gene was 14.8% in the entire cohort and its frequency did not differ between symptomatic and asymptomatic patients.

Mitochondrial haplotyping. HVS1 in the mtDNA D-loop is known to be the most variable mtDNA region (Fig. 1) (18). In the 47 patients with BrS, 37 different HVS1 haplotypes were identified (Table I). The majority of these haplotypes were only found in 1 sample each, except the 'CRS' haplotype (corresponding to the reference sequence), which was found in 7 individuals, and haplotypes 4, 12 and 22. The mtDNA haplogroups were then assigned for each individual, using characteristic substitutions in the HVS1 and substitutions in positions 72 and 73 (data not shown). In total, 11 different mtDNA haplogroups were identified, five of which (HV, R, X, N1a and F) were only found in one individual each. The estimated frequencies of the haplogroups are presented in Table II, along with 95% confidence intervals. The frequencies of the haplogroup were compared between patients and healthy Russian individuals based on data from (17). For only one haplogroup was a significant difference in the frequency observed, haplogroup J, which was absent in patients (0%), but was present at a frequency of 9.7% in the healthy individuals (Fisher's exact test, P=0.032).

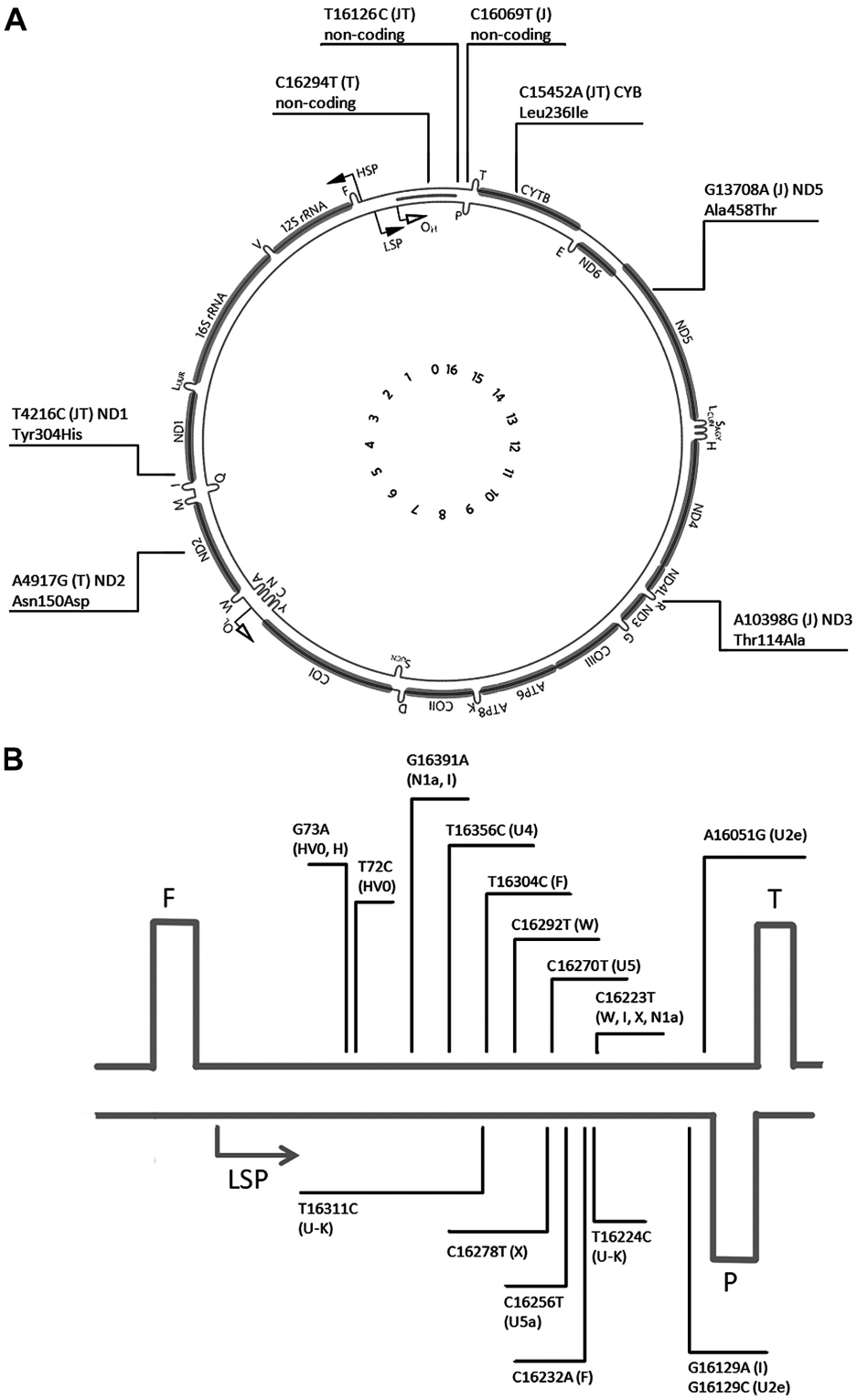

Figure 1. Schematic presentation of mtDNA showing substitutions that define haplogroups. (A) Schematic presentation of mtDNA (obtained from mitowheel.org, with permission). Substitutions in the D-loop typical for haplogroups J and T are shown, as well as substitutions in the coding region of mtDNA which result in amino acid substitutions in these haplogroups. (B) Enlarged region of the D-loop, with substitutions which allow assigning primary mtDNA haplogroups using the first hypervariable segment sequence data. mtDNA, mitochondrial DNA; LSP, light strand promoter.

Discussion

Brugada Syndrome is characterized by considerable genetic and clinical heterogeneity, with incomplete penetrance of the disease-causing mutations. Generally, it is very difficult to assess the risk of sudden cardiac death in patients, even if the causal mutation has been identified (6). Therefore, identification of biomarkers which will facilitate prediction of the clinical manifestation in patients with BrS is required. Common genetic variants in other genes including mitochondrial genes, may be very promising 'modifying' targets. MtDNA encodes 13 subunits of the respiratory chain

Table I. Mitochondrial DNA first hypervariable segment haplotypes in the patients with Brugada syndrome.

Number	Variations comparing to reference mtDNA sequence	Haplogroup	Number of individuals
1	rCRS	H	7
2	C16176T	H	1
3	C16221T	H	1
4	A16293G	H	2
5	C16354T	H	1
6	A16051G, A16162G, C16266T	H	1
7	A16080G, T16189C, T16356C	H	1
8	T16092C, T16140C, A16265G, A16293G, T16311C	H	1
9	A16162G, C16187T	H	1
10	C16188G	H	1
11	A16051G, C16169T	H	1
12	T16298C	HV0	3
13	G16129A, T16172C, C16223T, T16311C, G16319A, G16391A	I	1
14	G16129A, T16172C, C16223T, T16311C, G16391A	I	1
15	T16126C, A16163G, C16186T, T16189C, C16294T	T (T1a)	1
16	T16126C, A16163G, C16186T, T16189C, A16293G, C16294T	T (T1a)	1
17	T16126C, A16163G, C16186T, T16189C, C16294T	T (T1a)	1
18	T16126C, C16294T, C16296T	T (T2)	1
19	T16126C, C16294T, C16296T, T16304C	T (T2b)	1
20	T16126C, C16294T, T16304C	T (T2b)	1
21	A16051G, G16129C, T16189C, T16362C	U (U2e)	1
22	C16134T, T16356C	U (U4)	2
23	A16265G, T16356C, T16362C	U (U4)	1
24	T16136C, T16189C, C16192T, C16256T, C16270T	U (U5a)	1
25	C16192T, C16256T, C16270T, C16291T, A16399G	U (U5a)	1
26	C16256T, C16270T, A16399G	U (U5a)	1
27	T16189C, C16270T	U (U5b)	1
28	C16192T, C16270T	U (U5b)	1
29	T16224C, T16311C	U (K)	1
30	T16224C, T16311C, G16319A	U (K)	1
31	G16145A, C16223T, C16292T	W	1
32	C16192T, C16223T, C16292T, T16311C, T16325C	W	1
33	T16189C, C16223T, G16255A, C16278T	X	1
34	C16193T	R	1
35	C16168T, C16192T, A16220C, T16304C	HV	1
36	C16223T, G16391A	N1a	1
37	T16189C, C16232A, T16249C, T16304C, T16311C, C16360T	F	1

rCRS, human mtDNA reference sequence.

complex, which are crucial for oxidative phosphorylation (19). Several mitochondrial diseases are characterized predominantly by cardiac involvement including progressive rhythm and conduction disturbances. The best known example is Kerns-Sayre syndrome, which is caused by a large mtDNA deletion, and manifests as a progressive AV block and a high risk of SCD (20). Arrhythmias and SCD may also occur in patients with mitochondrial encephalopathy, lactic acidosis and stroke-like episodes syndrome, which is most frequently caused by the point mtDNA mutation, m.A2343G (21). There have been several studies showing that common mtDNA polymorphisms may also be modifying factors in cardiovascular disease phenotypes.

Studies have suggested that mitochondrial dysfunction (decreased ATP production and increased reactive oxygen species production) may have an arrhythmogenic effect on the myocardium (22,23). Decreasing the ATP/ADT ratio results in the opening of potassium channels, which causes retardation of impulse conduction (24). Furthermore, oxidative stress influences the excitability of cardiomyocytes, partially due to decreased sodium and potassium entry into cells (25,26). Thus, mitochondrial dysfunction may reproduce the effects

Table II. Prevalence of the mtDNA haplogroups in Russian BrS patients and in general population from the European region of Russia.

	BrS patients, n=47		Controls, n=953		
mtDNA haplogroup	n	Percentage (CI)	n	Percentage (CI)	P-value
H	18	38.3 (25.8-52.6)	386	40.5 (37.4-43.7)	0.882
U (incl. K)	11	23.4 (13.6-37.2)	240	25.2 (22.5-28.0)	0.919
T	6	12.8 (5.6-25.2)	85	8.9 (7.3-10.9)	0.525
J	0	0 (0-7.6)	92	9.7 (7.9-11.7)	0.032[a]
I	2	4.3 (1.5-18.1)	25	2.6 (1.8-3.8)	0.633
W	2	4.3 (1.5-18.1)	19	2.0 (1.3-3.1)	0.259
HV0	3	6.4 (2.2-17.2)	29	3.0 (2.1-4.3)	0.396
Other	5	10.6 (4.6-22.6)	77	8.1 (6.5-10.0)	0.581

[a]P<0.05. mtDNA, mitochondrial DNA; BrS, Brugada syndrome; CI, confidence interval.

of disease-causing mutations in BrS, and variations in mitochondrial genome may be promising candidates for facilitating diagnosis of BrS.

Human mtDNA is highly polymorphic. As mtDNA has no meiotic recombination, consequent mutations constitute non-disturbed haplotypes. Related haplotypes are organized into haplogroups. Each haplogroup is designated by a letter code and has its own set of nucleotide substitutions in comparison with the reference (or ancestor) sequence, including amino acid changes and variants in mitochondrial rRNA and tRNA genes. It is hypothesized that these haplotypes may have some minor relevance to respiratory chain function (19).

The first study to show the association between mtDNA polymorphisms and specific BrS manifestations was performed by Stocchi *et al* (13) in Italian patients. It was found that all symptomatic and half of the asymptomatic patients with a spontaneous type 1 ECG had mtDNA belonging to either J or T haplogroups (13). These two mtDNA haplogroups constitute the phylogenetic cluster JT, defined by the combination of T4216C, A11251G, C15452A and T16126C substitutions, with T4216C resulting in amino acid substitution, Tyr304His, in the ND1 subunit and C15452A resulting in another amino acid substitution, Leu236Ile, in the Cyt b subunit. In addition, each of the haplogroups has other polymorphisms which result in amino acid substitutions in different complexes of the mitochondrial respiratory chain. It is hypothesized that these missense single nucleotide polymorphisms together result in reduced oxidative phosphorylation efficiency, thereby explaining the association with severe forms of BrS. Stocchi *et al* (13) found a high frequency (56%) of the JT cluster in a group of 40 patients, compared with the frequency in the normal Italian population (20%) (13).

In the present study, mtDNA polymorphisms in Russian patients with BrS were studied and it was shown that while the frequency of the JT haplogroup cluster in the common Russian population did not differ from its frequency in Italians (~20%), in Russian patients, haplogroup J was virtually absent, whereas haplogroup T was observed at almost the same frequency in patients as it was in the normal population. For the haplogroup J, the difference was statistically significant. According to the results, haplogroup J may exhibit a more protective effect than a pathogenic effect on the development of BrS.

Haplotype definition in Stocchi *et al* (13) was based on analysis of the entire mitochondrial genome with assumption that all mtDNA haplogroups are the branches of the common phylogenetic tree and can be unequivocally defined by characteristic combinations of nucleotide substitutions, as a result of the non-recombinant manner of mtDNA inheritance. Stocchi *et al* (13) discussed the combination of four polymorphisms (T4216C, A11251G, C15452A and T16126C) as a factor promoting manifestation of Brugada syndrome, and showed that combination of T4216C and T16126C defined the JT cluster on the mtDNA tree. Two other substitutions were also present at the root of the JT cluster; the mtDNA phylogeny is available online (phylotree.org/tree/JT.htm). Hence, this combination unambiguously identifies the JT cluster only, and a higher frequency of this single nucleotide variant (SNV) combination means a higher frequency of the entire JT cluster. The presence of another 11 variants separates the JT haplogroup from the rCRS, which belongs to the haplogroup H2a2 (not to the ancestral root). The J haplogroup carries 6 more genetic variations, and the T haplogroup contains an additional 10 characteristic SNVs, which makes for a total of >20 variants difference (21 for J haplogroup and 25 for the T haplogroup). This difference reflects the phylogenetic distance of the aforementioned haplogroups from the reference sequence, but does not correspond to any specific clinical significance. As most of the mtDNA variants can be imputed from the haplogroup identification, whole mtDNA sequencing is redundant for the purpose of the present study.

One possible explanation for the discrepancy between the present study and Stocchi *et al* (13) may be that the 'rough' classification of individual haplotypes of mtDNA into major haplogroups does not reflect geographical differences in mtDNA polymorphisms. Major mitochondrial haplogroups consist of several sub-haplogroups defined by additional variants, which can be region-specific (27). These specific variants may contribute to the contradictory results. For example, in the present study, 6 haplotypes belonging to haplogroup T

were represented by three individuals with T1a, one individual with the T2 root haplotype and two with T2b, whereas Italian patients only had representatives of sub-haplogroup T2c (13). Similarly, different J sub-haplogroups may prevail in Italian and Russian populations. Thus, associations between mtDNA polymorphisms and disease manifestation may be population specific.

The small size of the clinical group in the initial study (40 patients with BrS) may introduce a bias into the initial results. Thus, the preliminary data from Stocchi *et al* (13) required a replication study in a larger number of patients and in a different ethnic group.

The striking contradiction of the results obtained in the two independent groups of patients with different ethnic backgrounds suggests that there is no reliable basis for using mtDNA haplogroups in medical practice for risk stratification of patients with BrS.

The primary goal of the present study was to assess the role of common combinations of SNVs (such as haplogroups) in the manifestation of BrS. Whilst certain individuals may carry recently occurred unique genetic variants, which may impart some effect on the clinical course of the disease, the role of these rare and unique mtDNA variants requires additional methodology and thus should be the subject of a separate study.

The results of the present study did not confirm the results of a previously reported association of mtDNA haplogroups J and T (polymorphism m.T4216C) with BrS or with the more severe form of the disease (13). In contrast, haplogroup J was absent in the patients in the present study, but was present in the normal population at a frequency of 9.7%. Thus, it seems unlikely that mitochondrial haplogroup J and T contribute to the clinical manifestation of BrS in Russian patients. However, clinically relevant biomarkers of manifestation and prognosis of BrS (electrocardiographical, biochemical and genetic) are required. Large-scale genome sequencing may provide novel insights and result in the identification of genes responsible for modification of clinical phenotypes in patients with BrS.

Acknowledgements

Not applicable.

Funding

The present study was funded by the Program for Basic Research of the Russian Academy of Sciences with additional partial support from RFBR (grant no. 19-04-01322).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

MG conceived and designed the study, performed the analysis, as well as drafted and revised the manuscript. VM performed the PCR and genotyping, and prepared the illustrations. VR drafted and revised the manuscript, as well as analyzed the data. AS performed the genotyping of BrS patients, analyzed the data and drafted the manuscript. EZ collected the patient data, performed genetic counseling and ECG analysis, as well as wrote and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was performed in accordance with the 1964 Helsinki declaration. The head of local Ethics Committee of Petrovsky National Research Centre of Surgery (Moscow, Russia) signed a permit to conduct research on Brugada syndrome on 27/09/2019. Written informed consent was obtained from all individual participants included in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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