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RESEARCH NOTE

A high number of 'natural' mitochondrial DNA polymorphisms in a symptomatic Brugada syndrome type 1 patient

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Abstract. Brugada syndrome (BrS) is a rare genetic arrhythmic disorder with a complex model of transmission. At least 20 different genes have been identified as BrS-causal or susceptibility genes. Of these, *SCN5A* is the most frequently mutated. Coregulation of different mutations or genetic variants, including mitochondrial DNA (mtDNA), may contribute to the clinical phenotype of the disease. In the present study, we analysed the mitochondrial genome of a symptomatic BrS type 1 patient to investigate a possible mitochondrial involvement recently found in the arrhytmogenic diseases. No pathogenic mutation was identified; however, a high number of single-nucleotide polymorphisms were found (n=21) and some of them were already been reported in molecular autopsy case for sudden death. The results reported here further support our hypothesis on the potential role of mtDNA polymorphisms in mitochondrial dysfunction, which may represent a risk factor for arrhythmogenic disease.

Keywords. ventricular arrhythmia; Brugada syndrome; mitochondrial DNA; single-nucleotide polymorphism.

Introduction

Brugada syndrome (BrS) is a hereditary cardiac disease characterized by electrocardiographic alterations and malignant ventricular arrhythmias, which result in syncope and/or sudden cardiac death (SCD) (Brugada and Brugada 1992). The typical patient profile for BrS is a middle-aged man, presenting an elevation of the ST segment in precordial derivations, who sometimes has a family history of cardiac events and suddenly collapses due to degenerative polymorphic ventricular tachycardia (PVT). At present, the defibrillator implant (ICD) is the only effective strategy for the prevention of SCD in patients with symptoms or a family history of SCD, although it has several drawbacks.

The genetic studies carried out to date define BrS as a genetic disease transmitted according to an autosomal dominant pattern with incomplete penetrance. Genetic mutations in the *SCN5A* gene coding for the α subunit of the Na^+ channel have been found in 25–30% of BrS patients. Specifically, more than 300 different mutations, responsible for a functional loss of the canal have been identified in this gene (Kapplinger *et al.* 2010). However, a negative screening result for the *SCN5A* gene does not exclude BrS, which can no longer be considered a monogenic disease. In fact, mutations have been described in at least 20 other genes, each responsible for a very low percentage of Brugada cases or BrS-susceptibility genes (Le Scouarnec *et al.* 2015). Moreover, coregulation of different mutations or genetic variants (Roden 2004; Bezzina *et al.* 2013), including mitochondrial DNA (mtDNA) (Stocchi *et al.* 2016), may contribute to the clinical phenotype. The present investigation, which examines the entire mt genome of a type 1 symptomatic BrS patient, is based on a previous study concerning the possible role of mtDNA alterations in BrS (Stocchi *et al.* 2016).

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Material and methods

Case

This study was approved by the Human Ethics Committee of the University of Urbino Carlo Bo and was conducted according to the ethical guidelines and principles of the International Declaration of Helsinki. The proband, a 52 years old male with clear marked ST upsloping in V1 and V2, and no symptoms or family history, was enrolled by the Comprehensive Cardiology Care Unit of the Institute for Treatment and Research (IRCCS), 'Casa Sollievo della Sofferenza' Hospital, San Giovanni Rotondo (Italy). He provided consent to share his clinical history, electrocardiogram (ECG) and DNA analyses. He was not examined by programmed ventricular stimulation (PVS). According to current guidelines, he would be classified as a middle risk patient (Priori and Napolitano 2018); however, he presented a number of other particular features. Taken individually, these features were not necessarily associated with a bad outcome, but their sheer number was cause for concern. The patient showed a strong ST upsloping, uncommon in the Brugada population, and a series of minor well-documented signs : F wave-QRS fragmentation (Priori *et al.* 2012, 2013; Priori and Napolitano 2018), dynamic ST changes (Priori *et al.* 2013; Priori and Napolitano 2018) and ST abnormalities in inferior and lateral leads (Priori *et al.* 2013; Priori and Napolitano 2018). His ECGs were highly unusual, suggesting a complex membrane disturbance with a possible overlapping of common channellopathies and conduction disturbances (BrS, J-wave syndrome and right bundle branch block) that may share a common ionic mechanism. Even in the absence of symptoms, he received a conventional single-chamber ICD programmed as shock only. After one year, the ICD discharged on true nocturnal ventricular fibrillation, confirming the electrical instability highlighted by a series of strange atypical ECGs in a middle risk patient (male sex, ECG pattern type 1). The death of the patient allowed us to define him as symptomatic type 1 with a severe phenotype. The genetic test on the *SCN5A* gene yielded negative results.

DNA extraction

Total DNA was isolated from peripheral blood using the QIAamp DNA blood Mini kit (Qiagen, Hilden, Germany) and the concentration was determined using a Nanodrop spectrophotometer (ND-1000 Spectrophotometer, ThermoScientific, Wilmington, USA).

The mtDNA genome analysis

The mtDNA amplification and sequencing were performed using the primer pair and amplification protocol described by Stocchi *et al.* (2016). As already reported, specificity for mtDNA target sequences of designed primers was tested on DNA from osteosarcoma 143B.TK2 Rho0 cells completely lacking mtDNA. The sequences were compared with the revised Cambridge Reference Sequence (NC_012920)) in the Mitomap database (https://www.mitomap.org) using SeqScape v. 2.5V, Sequencing Analysis 5.2 (Applied Biosystems, Foster City, USA) and DNA Baser v. 3.5 (Heracle BioSoft S.R.L.) software programs. PolyPhen-2 and PredictSNP were used to analyse the functional impact of nonsynonymous genetic variation on protein and SNP effect.

Results and discussion

In the present study, we sequenced the whole mt genome of a type 1 symptomatic BrS patient with a severe phenotype and an ECG polymorphic whose features are shown in figure 1. The aim of the investigation was to find an association between the patient's mtDNA profile and his BrS clinical phenotype in light of the fact that mitochondrial mutations have recently been found in cardiomyopathies and in an increasing number of other human diseases (Alston *et al.* 2017). Mutations in the mitochondrial genome or mitochondrial-related nuclear genes lead to mitochondrial dysfunction with consequent insufficient energy production. There is a high demand for the energy generated in mitochondria in the central nervous system, kidneys, liver, endocrine system, skeletal and cardiac muscles. Cardiac muscle is one of the tissues with a high energy demand and it uses almost adenosine triphosphate produced in mitochondria for contraction and ion transport. Mitochondrial cardiomyopathies can range in severity from asymptomatic status to severe manifestations, including heart failure, arrhythmia and SCD (El-Hattab and Scaglia 2016). Hypertrophic cardiomyopathy is the most frequent cardiac manifestation in mitochondrial diseases (El-Hattab and Scaglia 2016), but mitochondrial cardiomyopathies are also found in dilated (Tang *et al.* 2010), restrictive, left ventricular noncompaction, and histiocytoid cardiomyopathies (Finsterer and Kothari 2014). Little is known regarding the involvement of mtDNA in inherited arrhythmogenic diseases (Khatami *et al.* 2010, 2014; Stocchi *et al.* 2016; Tafti *et al.* 2018), including BrS.

In the present study, the analysis of whole mt genome did not detect novel pathogenic mutations; however, 21 variants, all in homoplasmic form, were found. Of these, 11 variants fall into mRNA genes (three nonsynonymous and eight synonymous); two into rRNA genes, eight into noncoding regions, of which seven fall into d-loop. All the variants have already been described and are present in normal populations.

Among the nonsynonymous SNPs, variant G8616 T falls into ATP6 and leads to the substitution of leucine by phenilalanine at amino acid position 30. As shown in table 1, this SNP has a low GenBank frequency, and when

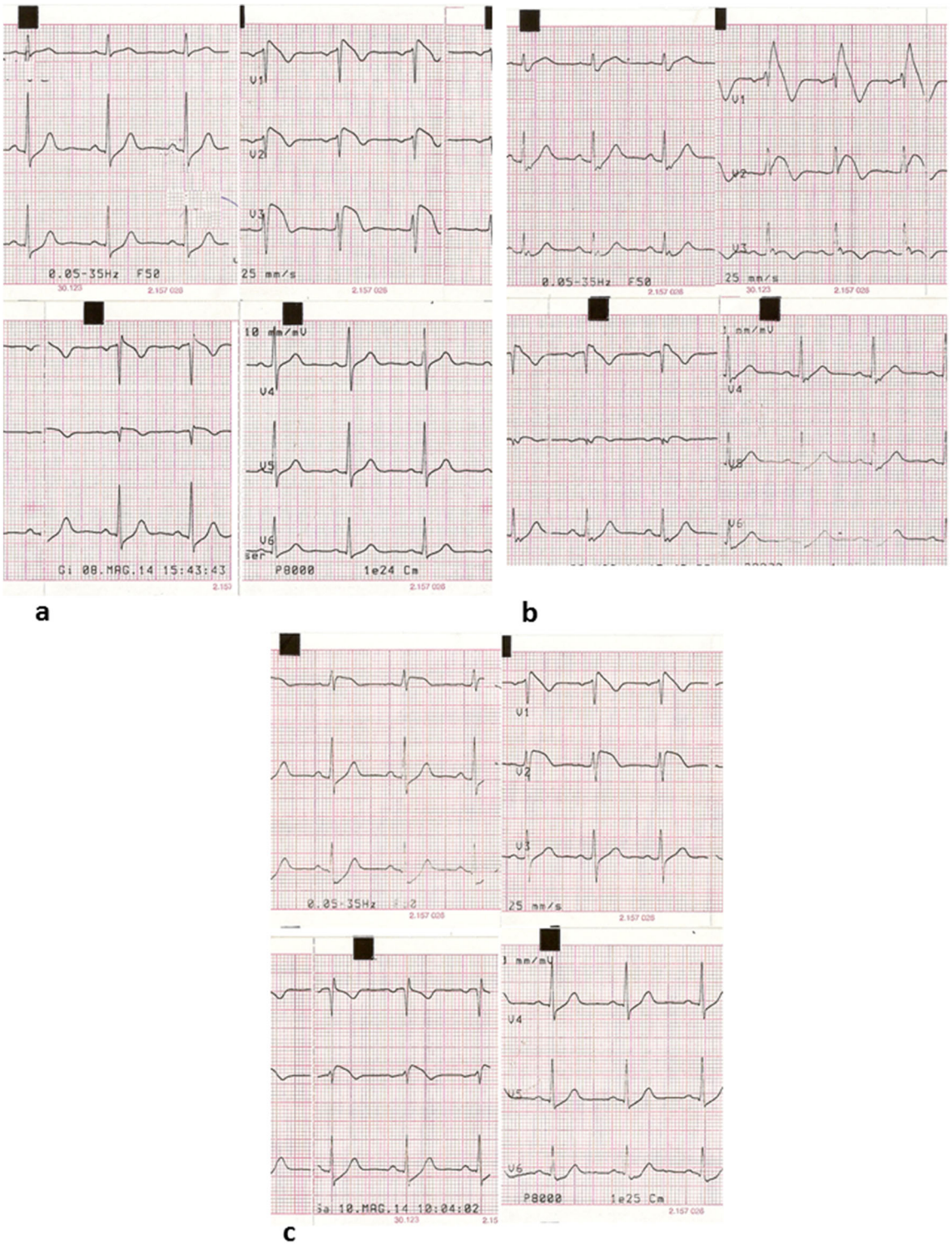

Figure 1. The patient's electrocardiographic pattern on three different consecutive days: (a) first day; (b) second day; (c) third day. Remarkable features of the ECG: typical ST upsloping type I in right precordial leads (coved type); ST upsloping in lateral leads; ST downsloping in inferior leads; Intermittent right bundle branch block and extreme variability of ECG; fragmented QRS as described by Priori in PRELUDE study (Priori *et al.* 2012).

bioinformatics programs (PolyPhen-2 and PredictSNP) were applied, it was found to be 'probably damaging' with a 'deleterius' SNP prediction.

Among the variants identified in the subject under study A2706G, T3197C, A11467G, G11719A, T13617C, C14766T, C16270T, T16311C have already been observed

Table 1. Identification and localization of mitochondrial SNPs updated on March 2020, https://www.mitomap.org.

mtDNA variants	State	Localization	Variant type	GenBank frequence
A2706G	Homoplasmic	rRNA	rRNA	39658
T3197C	Homoplasmic	rRNA	rRNA	1982
A5656G	Homoplasmic	tRNA	NC4	619
A7768G	Homoplasmic	COX2	Syn	952
A8577G	Homoplasmic	ATP8-ATP6	Syn	60
G8616T	Homoplasmic	ATP6	Non syn L-F	162
G9477A	Homoplasmic	COX3	Non syn V-I	1965
A11467G	Homoplasmic	ND4	Syn	6234
G11719A	Homoplasmic	ND4	Syn	38921
G13194A	Homoplasmic	ND5	Syn	227
T13617C	Homoplasmic	ND5	Syn	1935
T14182C	Homoplasmic	ND5	Syn	1284
C14766T	Homoplasmic	cytB	Non syn T-I	38624
T15191C	Homoplasmic	cytB	Syn	74
C16174T	Homoplasmic	d-loop HV1	Noncoding	503
T16189C	Homoplasmic	d-loop HV1	Noncoding	32097
C16270T	Homoplasmic	d-loop HV1	Noncoding	4665
T16311C	Homoplasmic	d-loop HV1	Noncoding	23087
A73G	Homoplasmic	d-loop HV2	Noncoding	75319
C150T	Homoplasmic	d-loop HV2	Noncoding	13784
C516T	Homoplasmic	d-loop HV3	Noncoding	44

separately in different cases of sudden infant death syndrome (Divne *et al.* 2003; Opdal and Rognum 2004). Further, A2706G, G11719A, C14766T and A73G have already been found together in six spontaneous previously analysed type 1 BrS patients (Stocchi *et al.* 2016). In the same work performed by our research group (Stocchi *et al.* 2016), we found that symptomatic type 1 Brugada patients with the most severe phenotype showed a highly polymorphic mtDNA (>20) and the specific mtDNA SNP combination: T4216C, A11251G, C15452A and T16126C. The BrS patient examined in the present investigation did not show this allelic combination; however, the proband did show a highly polymorphic mtDNA (21 variants) with the major distribution in the coding region rather than in the d-loop, which is usually the most variable region. The high number of mtDNA variants found could therefore be indicative of a severe clinical state. A high number of mitochondrial genome variations was also found in Persian patients with long QT syndrome (LQTS), another arrhythmogenic disorder. Further, in the same study, it was reported that the number of variations in LQTS patients with syncope was higher than it was in patients who did not present syncope (Khatami *et al.* 2010). Indeed, mitochondrial gene polymorphisms frequently occur and can lead to mitochondrial dysfunction, whose impact can be observed in various human diseases (Lott 2013). The link between mitochondrial dysfunction, conduction block and arrhythmic risk may be explained by mitochondrial ROS overproduction, which regulates the cardiac sodium channel directly or indirectly, reducing current (I_{Na}) in cardiomyocytes.

The results presented here, corroborate with the hypothesis formulated in our previous paper (Stocchi *et al.* 2016), that mitochondrial genetics may contribute to complex diseases. Moreover, our findings support recent investigations on mt genome polymorphisms aimed to associate non-pathogenic mtDNA variability with phenotypic features such as the mitochondrial function (Marcuello *et al.* 2009) and cardiovascular function, and pathology (Bray and Ballinger 2017).

In conclusion, our findings support the consensus in the scientific community regarding the complexity of BrS, a disease in which the cosegregation of different mutations or genetic variants, including mtDNA, can contribute to the clinical phenotype. The identification of additional genetic markers, both nuclear and mitochondrial, which may be associated with different clinical phenotypes of BrS, could provide support to the clinical and electrophysiological approach. Risk stratification of this syndrome, which is especially important for asymptomatic patients could thus be improved. Further, the results presented here highlight the role that 'natural' polymorphisms play in the clinical phenotype of cardiomyopathies.

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References

Alston C. L., Rocha M. C., Lax N. Z., Turnbull D. M. and Taylor R.W. 2017 The genetics and pathology of mitochondrial disease. *J. Pathol*. **241**, 236–250.

Bezzina C. R., Barc J., Mizusawa Y., Remme C. A., Gourraud J.-B., Simonet F. *et al*. 2013 Common variants at SCN5A-SCN10A and HEY2 are associated with Brugada syndrome, a rare disease with high risk of sudden cardiac death. *Nat. Genet.* **45**, 1044–1049.

Bray A. W. and Ballinger S. W. 2017 Mitochondrial DNA mutations and cardiovascular disease. *Curr. Opin. Cardiol.* https://doi.org/10.1097/hco.0000000000000383.

Brugada P. and Brugada J. 1992 Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. *J. Am. Coll. Cardiol*. **20**, 1391–1396.

Divne A. M., Rasten-Almqvist P., Rajs J., Gyllensten U. and Allen M. 2003 Analysis of the mitochondrial genome in sudden infant death syndrome. *Acta Paediatr*. **92**, 386–388.

El-Hattab A.W. and Scaglia F. 2016 Mitochondrial Cardiomyopathies. *Front. Cardiovasc. Med.* **3**, 25.

Finsterer J. and Kothari S. 2014 Cardiac manifestations of primary mitochondrial disorders. *Int. J. Cardiol.* **177**, 754–763.

Kapplinger J. D., Tester D. J., Alders M., Benito B., Berthet M., Brugada J. *et al.* 2010 An international compendium of mutations in the SCN5A-encoded cardiac sodium channel in patients referred for Brugada syndrome genetic testing. *Heart Rhythm*. **7**, 33–46.

Khatami F., Mehdi Heidari M. and Houshmand M. 2014 The mitochondrial DNA mutations associated with cardiac arrhythmia investigated in an LQTS family. *Iran. J. Basic Med. Sci.* **17**, 656–661.

Khatami M., Houshmand M., Sadeghizadeh M., Eftekharzadeh M., Heidari M. M., Saber S. *et al*. 2010 Accumulation of mitochondrial genome variations in Persian LQTS patients: a possible risk factor? *Cardiovasc. Pathol.* **19**, e21–e27.

Le Scouarnec S., Karakachoff M., Gourraud J.-B., Lindenbaum P., Bonnaud S., Portero V. *et al*. 2015 Testing the burden of rare variation in arrhythmia-susceptibility genes provides new insights into molecular diagnosis for Brugada syndrome. *Hum. Mol. Genet.* **24**, 2757–2763.

Lott M. T. 2013 mtDNA variation and analysis using mitomap and mitomaster. *Curr. Protoc. Bioinformatics* **44**, 1–26.

Marcuello A., Martinez-Redondo D., Dahmani Y., Casajus J. A., Ruiz-Pesini E., Montoya J. *et al.* 2009 Human mitochondrial variants influence on oxygen consumption. *Mitochondrion* **9**, 27–30.

Opdal S. H. and Rognum T. O. 2004 The sudden infant death syndrome gene: does it exist? *Pediatrics* **114**, e506–e512.

Priori S. G. and Napolitano C. 2018 J-wave syndromes: electrocardiographic and clinical aspects. *Card. Electrophysiol. Clin.* **10**, 355–369.

Priori S. G., Gasparini M., Napolitano C., Della Bella P., Ottonelli A. G., Sassone B. *et al*. 2012 Risk stratification in Brugada syndrome: results of the PRELUDE (PRogrammed ELectrical stimUlation preDictive valuE) registry. *J. Am. Coll. Cardiol.* **59**, 37–45.

Priori S. G., Wilde A. A., Horie M., Cho Y., Behr E. R., Berul C. *et al*. 2013 HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC in June 2013. *Heart Rhythm*. **10**, 1932–1963.

Roden D. M. 2004 Human genomics and its impact on arrhythmias. *Trends Cardiovasc. Med.* **14**, 112–116.

Stocchi L., Polidori E., Potenza L., Rocchi M. B. L., Calcabrini C., Busacca P. *et al*. 2016 Mutational analysis of mitochondrial DNA in Brugada syndrome. *Cardiovasc. Pathol.* **25**, 47–54.

Tafti M. F., Khatami M., Rezaei S., Heidari M. M. and Hadadzadeh M. 2018 Novel and heteroplasmic mutations in mitochondrial tRNA genes in Brugada syndrome. *Cardiol. J.* **25**, 113–119.

Tang S., Batra A., Zhang Y., Ebenroth E. S. and Huang T. 2010 Left ventricular noncompaction is associated with mutations in the mitochondrial genome. *Mitochondrion* **10**, 350–357.

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