© IJHG 2020 Int J Hum Genet, 20(3): 138-146 (2020)
PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330 DOI: 10.31901/24566330.2020/20.03.727

Comparing the SCN5A Gene Sequence and Expression in Three Different Brugada Syndrome Profiles of Patients: New Insights for Genotype-Phenotype Correlation

Hajer Foddha[1,2], Houria Daimi[1], Abdelhak Foddha[3], Nadia Leban[1], Rahma Touhami[1,2], Habib Gamra[3], Ali Saad[4], Jemni Ben Chibani[1] and Amel Haj Khelil[1,2]

[1]Biochemistry and Molecular Biology Laboratory, Department of Clinical Biology, Faculty of Pharmacy, University of Monastir, 5000, Tunisia
[2]Department of Molecular and Cellular Biology and Biotechnology, Higher Institute of Biotechnology, University of Monastir, 5000, Tunisia
[3]Cardiology A Service, Fattouma Bourguiba Hospital, Monastir, 5000, Tunisia
[4]Cytogenetics, Molecular Genetics and Reproduction Biology Service, Farhat Hached Hospital, Sousse, 4000, Tunisia

KEYWORDS Brugada Syndrome. Electrocardiogram. Genotype. Phenotype. Gene Expression

ABSTRACT Brugada Syndrome (BrS) is a rare inherited cardiac arrhythmia. In this paper, the researchers aim to identify the molecular defect in three unrelated patients with different clinical BrS profiles. Therefore, the researchers analysed, by real time PCR, the expression amount of SCN5A gene, which is the most described in BrS, in the three selected patients. Thereafter, the researchers sequenced the total exome and the untranslated regions of SCN5A. The results showed a variable decrease in SCN5A expression and no deleterious mutation, but 11 exonic and 3' UTR variants. The number of these variants is correlated to the gene expression and the phenotype severity in each patient. These results confirm the implication of SCN5A, and the researchers hypothesise that in the absence of causal mutation, the presence of one or numerous variants in the SCN5A gene could be involved in the disease. This finding may provide new insights in genotype-phenotype correlation and personalised diagnosis.

INTRODUCTION

The Brugada Syndrome (BrS) is a rare inherited channelopathy described for the first time by the Brugada brothers (Brugada and Brugada 1992). Its prevalence ranges from 1 to 5 out of 10,000 in Europe (Hermida et al. 2000; Sinner et al. 2009). In Tunisia, the disease is still poorly known, but it could be responsible for four to twelve percent of all sudden deaths (Tlili et al. 2012). The typical electrocardiogram (ECG) pattern consists of an ST segment elevation in the right pericardial leads and a high risk of episodes of ventricular fibrillation, causing syncope or sudden cardiac death (SCD) (Wilde et al. 2002).

Positive diagnosis is based on an algorithm developed by Berne and Brugada (2012) in relation to ECG type occurring spontaneously or after a provocative test with the administration of Class 1 antiarrhythmic drugs, such as flecainide or ajmaline, family history of SCD, and documented ventricular arrhythmias.

Familial inheritance has traditionally been described as Mendelian, with autosomal dominant, incomplete penetrance and predominantly affected males (Antzelevitch et al. 2005). More than 22 genes have been linked to BrS, most of them code for membrane channels in cardiomyocytes and are involved in cardiac contraction. Among these, SCN5A, SCN10A and GPD1L are responsible for sodium current (Gourraud et al. 2016).

The SCN5A gene, containing 28 exons (80 Kb), encodes the α-subunit of the cardiac Na+ channel Nav1.5 (Wang et al. 1996). Mutations in this gene (more than 300) cause Nav1.5 loss of function, leading to an aberrant action potential

Address for correspondence:
Pr. Amel Haj Khelil
Department of Molecular and
Cellular Biology and Biotechnology,
University of Monastir,
Higher Institute of Biotechnology,
BP 74, Street Tahar Haddad, 5000, Tunisia
Telephone: (00216) 73465405;
Fax: (00216) 73465404
E-mail: hajkhelil@yahoo.fr

(Kapplinger et al. 2010; Berne and Brugada 2012; Gourraud et al. 2016; Juang and Horie 2016; Wu et al. 2017). Only thirty-seven to forty-seven percent of identified variants are classified as pathogenic or likely pathogenic for BrS (Denham et al. 2019; Kroncke et al. 2018).

The diagnosis of BrS may be difficult due to ECG dynamism, the incomplete penetrance of the disease, the age-dependent symptoms and the asymptomatic state of mutation carriers (Wilde et al. 2010).

Objectives

In this paper, the researchers aim to test the implication of the SCN5A gene and identify the molecular defects that may be involved in three different BrS profiles in order to reinforce the clinical diagnosis and establish a genotype-phenotype correlation for risk stratification.

MATERIAL AND METHODS

Patients

This study includes three unrelated Tunisian patients (P1, P2 and P3) enrolled in the cardiology service at Monastir Fattouma Bourguiba Hospital and clinically diagnosed with different BrS profiles (tolerated, medium and severe). The diagnosis was essentially based on the ECG pattern. The patients were asked about personal and family history of disease signs. All common causes of acquired BrS or phenocopy were eliminated, according to (Juang and Lin 2017) protocol. A written informed consent for participation in the study was signed by all patients. The work was approved by the Medical Ethical Committee of Farhat Hached Hospital, Sousse, Tunisia.

Methods

Clinical Features

All relevant data (age, symptoms, family history of SCD, and ventricular fibrillation induction) were collected. An ECG was performed on each patient. Intravenous administration of Flecainide, which is a Na+ channel blocking drug (Meregalli et al. 2006), was performed to confirm diagnosis.

Genetic Analysis

SCN5A Gene Expression Quantification

Reverse Transcription (RT) was performed on the RNA of all patients and healthy controls by Qiagen QuantiTect® kit. Real time quantitative PCR was performed five times for each RT product using a BIORAD C1000 Thermal Cycler using SCN5A primers (forward: 5'GGAGTACGCCGACAAGATGT3'; reverse: 5'CCTGGACGCCTGTACAGTTT3'). Beta Actin was used as reference gene. Analysis of relative gene expression data was performed by $2^{-\Delta\Delta CT}$ method, as previously described (Livak and Schmittgen 2001).

SCN5A Gene Sequencing

All 28 SCN5A exons and 5' and 3' untranslated regions were directly sequenced using PCR-based Sanger sequencing (Sistemas Genómicos). Sequences were aligned against consensus sequences using NCBI Blastn program.

RESULTS

Phenotypic Analysis

The clinical characteristics of the three patients are summarised in Table 1. Patient 1 (P1) was a 23-year old man with no significant pathological history. He consulted the cardiology department suffering from atypical chest pain evolving since one year without associated signs (in particular no syncope) and without history of sudden death in the family. His ECG, presented in Figure 1A, was of type 1 (J point elevation, coved type ST and inverted T wave)

Table 1: Clinical characteristics of BrS patients

	Patient 1	*Patient 2*	*Patient 3*
Age (Years)	23	33	39
Symptoms	Asymptomatic	Syncope	Collapse
Family history of sudden death	No	No	Yes
Family history of BrS	No	No	No
ECG	Type 1 drug challenge	Type 2 spontaneous	Type 1 spontaneous
Defibrillator	No	Yes	Yes

BrS: Brugada syndrome, ECG: electrocardiogram.

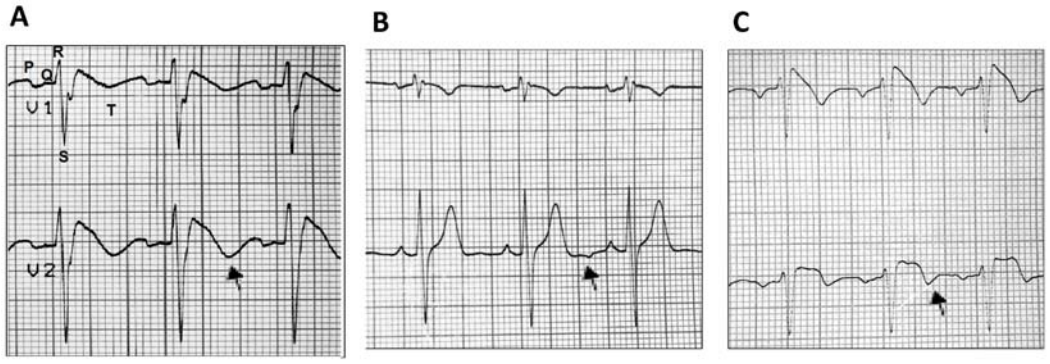

Fig. 1. ECG patterns of the three patients. A: Patient 1 after flecainid test; B: Patient 2; C: patient 3. Arrows indicate the inverted T wave that follows ST elevation. Cardiac revolution intervals (PQRST) are indicated in A

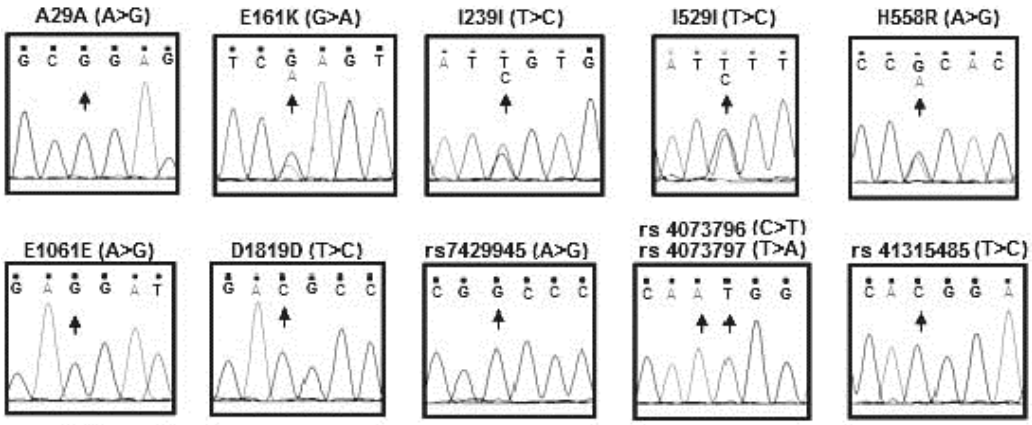

Fig. 2. Sequence of the eleven SCN5A variants identified in patient P1, P2 and P3. The arrows indicate the mismatch position

after Flecainide test. However, the physical and biological examinations were normal. He, therefore, did not need an implantable defibrillator. Patient 2 (P2) was a 33-year old man with no notable pathological history. He arrived at the emergency department suffering from a frontal traumatism following a syncope that occurred during medium physical effort. He did not report any previous similar symptoms or family history of sudden death. Physical and biological examinations were within normal range. His ECG, presented in Figure 1B, showed an incomplete right bundle branch aspect consistent with a type 2 BrS (>2mm of saddleback shaped ST elevation). He was treated with a single-chamber defibrillator. Patient 3 (P3) was a 39-year old man who consulted the emergency department due to a brief collapse associated with chest pain. He reported several episodes of collapse in the previous months. Family history showed the sudden death of a brother at the age of 36. The ECG was of typical type 1 BrS pattern, as shown in Figure 1C. A defibrillator was implanted in him due to ventricular fibrillation.

Molecular Analysis

SCN5A Quantification

The results showed a significant decrease in SCN5A expression compared with healthy control for all three patients. The decrease is posi-

tively correlated to the severity of the clinical profile. This result confirms the implication of the SCN5A gene in each BrS patient profile. The mean values normalized to Beta Actin and standard deviations for five tests are presented in Table 2.

Table 2: Real time PCR results showing the decrease in SCN5A gene expression (normalized to Beta Actin gene). C: healthy control; P1, P2 and P3: patients. The mean value and standard deviation are calculated from five quantification tests

Sample	*Normalized units (Mean value)*	*Standard deviation*
C	1	
P1	0.21730	0.001301
P2	0.09250	0.005359
P3	0.00031	0.000028

SCN5A Sequencing

The screening of the entire coding sequence of SCN5A revealed no causative mutation. However, a total of 11 single nucleotide polymorphisms were detected, that is, 3 exonic in patient 1 (A29A, E161K, E1061E), 3 exonic in patient 2 (A29A, H558R, E1061E) and 9 in patient 3 distributed as follows: 5 exonic (A29A, I239I, I529I, E1061E, D1819D) and 4 in the 3' UTR (rs7429945, rs4073797, rs4073796 and rs41315485), as shown in Table 3 and Figure 2.

All of these variants, with different minor allele frequencies (MAF) as shown in Table 4, have already been described as SCN5A variants associated with BrS.

SIFT and Polyphene prediction software packages showed one pathogenic variant (E161K), while the others were benign or likely benign, as presented in Table 4.

DISCUSSION

The diagnosis of BrS is based on an ECG pattern typically distinguished by a coved type ST segment. However, the ST segment elevation in BrS is very dynamic and can be modified by several factors, such as fever, diet, autonomic activity, alcohol and cocaine toxicity (Benito et al. 2009). These dynamic ECG manifestations added to the incomplete penetrance of BrS mutations cause difficulties in establishing clinical diagnosis (Wilde et al. 2010). Molecular analyses have led to new findings in establishing a genotype-phenotype correlation. In fact, mutations in SCN5A exhibit more pronounced electrophysiological defects in BrS and more severe prognosis in Asian and Caucasian populations (Chen et al. 2020). In a recent study, an evaluation of 21 genes that were previously indicated to have a correlation with BrS demonstrated only SCN5A as a definitive causative gene. All other genes were classified as contributors of limited evidence (Hosseini et al. 2018).

In this context, the researchers chose SCN5A as a candidate gene for molecular exploration in order to identify potential defects that may be implicated in the three patients BrS onset. These patients were selected on the basis of clinical

Table 3: Genetic SCN5A variants identified in the three patients (P1, P2, and P3)

Region in SCN5A gene	*Variant*	*Genotype*		
		P1	*P2*	*P3*
Exon 2	c.87 A>G (A29A)	***G/G***	***G/G***	***G/G***
Exon 4	c.481 G>A (E161K)	***A***/G	G/G	G/G
Exon 7	c.717 C>T (I239I)	C/C	C/C	C/***T***
Exon 12	c.1587 T>C (I529I)	T/T	T/T	T/***C***
	c.1673 A>G (H558R)	A/A	A/***G***	A/A
Exon 17	c.3183 A>G (E1061E)	***G/G***	***G/G***	***G/G***
Exon 28	c.5454 T>C (D1819D)	T/T	T/T	***C/C***
3'UTR	c.6365 A>G	A/A	A/A	***G/G***
	c.7205 C>T	C/C	C/C	***T/T***
	c.7204 T>A	T/T	T/T	***A/A***
	c.7779 T>C	T/T	T/T	***C/C***
	Total changes	**3**	**3**	**9**

Mutated alleles are shown in bold and *italics*

Int J Hum Genet, 20(3): 138-146 (2020)

Table 4: Clinical significance of the variants identified in the three patients

Variants (Positionon Chr. 3)	*Nucleotide change*	*Alleles*	*MAF (associated allele)*	*Consequence type*	*Syndromes associated*	*Clinical significance*
rs6599230(38633221)	Exon 2c.87 A→G	T/C	0.218 (T)	Synonymous (A29A)	* Cardiovascular phenotype	Benign Likely benign
rs199473062(38622401)	Exon 4c.481 G>A	C/G/T	<0.001 (T)	Missens/splice region (E161K)	BrS	Deleterious Associated (Smits et al, 2005)
rs41285129(38609951)	Exon 7c.717 C→T	G/A	<0.001 (A)	Synonymous (I239I)	BrS Cardiocascular phenotype	Benign Likely benign
rs45624133(38604015)	Exon 12c.1587 T→C	A/G	0.002 (G)	Synonymous (I529I)	BrS Cardiocascular phenotype	Benign
rs1805124(38603929)	Exon 12c.1673 A→G	T/C	0.230 (C)	Missens mutation (H558R)	*Cardiovascular phenotype Progressive familial heart block type 1A	BenignLikely benign
rs7430407(38580976)	Exon 17c.3183 A→G	A/GT/C/G	0.08 (T)	Synonymous (E1061E)	*Cardiovascular phenotype	Benign Likely benign
rs1805126(38550915)	Exon 28c.5454 T→C	A/G	0.492 (G)	Synonymous (D1819D)	*Cardiovascular phenotype Electro-cardio-graphic conduction measures	Benign Likely benign
rs7429945(38550198)	3'UTRc.6365 A>G	T/C	0.49 (C)		*	Likely benign
rs4073796(38549358)	3'UTRc.7205 C>T	G/A	0.49 (A)		*	Likely benign
rs4073797(38549359)	3'UTRc.7204 T>A	A/C/T	0.49 (T)		*	Likely benign
rs41315485(38548784)	3'UTRc.7779 T>C	A/G	0.180 (G)		*	Likely benign

MAF : minor allele frequency ; *: phenotypes including BrS, Dilated Cardiomyopathy, Long QT Syndrome, Paroxysmal familial ventricular fibrillation, Progressive familial heart block, Romano-Ward Syndrome, and Sick Sinus Syndrome.

exploration. The phenotypes are shown in Table 1. They were moderate in P1 (no physical perturbations, only chest pain without syncope, no history of SCD and no defibrillator), less moderate in P2 (no physical perturbations, syncope and implantable defibrillator, but no history of SCD) and severe in P3 (no physical perturbations but chest pain, several episodes of collapse, defibrillator and SCD in one brother). This increasing severity may be due to age (23, 33 and 39 years in P1, P2 and P3, respectively) or genotype defects.

In the molecular analyses, real time quantification showed a variable decrease in SCN5A expression in the three patients (4.6 fold in P1, 10 fold in P2 and more than 3000 times in P3). First, these results confirm the implication of SCN5A in the disease with potential loss-of-function mutations, and second, they indicate a positive correlation between SCN5A expression and phenotype severity. In fact, it was stated that loss-of-function mutations in SCN5A lead to lower gene expression levels or production of defective Nav1.5 proteins and cause BrS, an electrical disease with no or minor structural changes in the heart (Wilde and Amin 2018), which is the case with the patients explored in this work (BrS related ECG, no heart structural perturbation and lower SCN5A expression levels).

Sequencing analysis of the SCN5A gene in the three patients revealed no mutations but the presence of 11 polymorphisms, including 2 non-synonymous variants: E161K (MAF <0.001), predicted to be deleterious or possibly damaging (Smits et al. 2005), and H558R (MAF = 0.230), predicted to be likely benign or probably damaging (Magnani et al. 2014). The five other exonic variants are synonymous, and therefore, taken individually, are benign or likely benign. The four variants found in the 3' UTR are also predicted to be likely benign. However, according to NCBI ClinVar database, all of the variants detected were reported in BrS, conduction disease or cardiovascular phenotype with variable clinical significance, as shown in Table 4. In addition, synonymous and non-synonymous nucleotide changes in SCN5A were reported to be associated with SCD in a previous case control study (Stecker et al. 2006).

Interestingly, in this work, the researchers observed different distribution of variants in the three patients.

In P1, E161K, A29A and E1061E are simultaneously present. These variants could explain the moderate phenotype observed in P1. In fact, previous functional tests revealed that the E161K missense mutation in segment 2 of domain I in Nav 1.5 protein produces notably decreased sodium current (Smits et al. 2005). In addition, *in-silico* predictions of A29A variant in secondary mRNA structure showed that in the presence of this variant, there was loss of four stems and loops in the mRNA secondary structure compared with the wild type. This variation may alter the binding of splicing proteins, thus affecting the splice mechanism. Equally, *in silico* analyses for E1061E revealed that this variant leads to complete transformation of the mRNA, thus affecting the binding sites of five enhancer and/or silencer spliceosome proteins (Qureshi et al. 2015). These genomic data are in concordance with the SCN5A quantification results, which revealed a 4.6 fold decrease in SCN5A transcripts in this patient.

In P2, three variants were detected: A29A, E1061E and H558R. The A29A and E1061E variations revealed in this patient might alter the splice mechanism in addition to what has been described for patient 1. Also, H558R variation was revealed in the heterozygous state in P2. Even though this common variation does not alter the wild-type channel functions by itself (Paulussen et al. 2004), it could have detrimental effects if associated with the other two variants. These genomic findings could be correlated to P2 phenotype, which is less moderate than P1, and to the tenfold decrease transcript expression in SCN5A.

In P3, a total of nine variants were described (five exonic and four in 3'UTR), as shown in Table 3. A29A and E1061E might alter the splicing function, leading to a decrease in transcript production. Synonymous I239I, I529I and D1819D probably do not have, taken alone, any clinical significance, even though D1819D was previously reported to be associated with cardiac atrioventricular and ventricular conduction (Magnani et al. 2014). For the 3'UTR in which rs7429945, rs4073796, rs4073797, and rs41315485 are revealed, *in silico* analysis using miRNA TargetScanHuman software showed a set of miRNAs having binding sites on this region of the SCN5A gene, such as mir-217, mir-200bc and mir-

219. In autosomal dominant diseases (case of BrS), single nucleotide polymorphisms (SNPs) in the 3' UTR may create a binding site for miRNAs that recruits a selective destructive complex, providing the cellular and molecular basis for mRNA cleavage (Fazi and Nervi 2008), thereby reducing the clinical expression of the protein. In addition, a previous work demonstrated that rs4073796 and rs4073797 create a new binding site for miR-1270, which can, if overexpressed, modulate the SCN5A transcripts, and thus contributes to the genetic bases of BrS (Daimi et al. 2015). These genomic findings showing multiple variants on exonic and 3' UTR might explain the drastic gene expression decrease (more than three-thousand-times) and the severe phenotype in P3.

The identification of genetic variants in the patients explored gives extra evidences about the complexity of the disease and suggests that the occurrence and prognosis of BrS is, as previously described, most likely controlled by a combination of multiple genetic variants, rather than a single one (Daimi et al. 2019; Qureshi et al. 2015). Indeed, the simultaneous presence on the same haplotype of various alleles associated with the disease increases the risk of its occurrence and the severity of the phenotype. This relative risk can be calculated assuming a multiplicative model, which is relevant in case of multiple genetic variants (Chatterjee et al. 2016).

In this paper, all variants found were previously reported to be associated with BrS in different populations, but their effects had not been tested by functional analyses. Bioinformatics software gives a slight insight into their single but not combined effect. Based on the results of this study, the researchers hypothesise that the combination of several polymorphisms described individually without any effect or with a minor influence could decrease the expression of the underlying gene. The number of variants found could be directly proportional to the decrease in gene expression, resulting in a more severe phenotype. This hypothesis should be further investigated.

CONCLUSION

In this paper, the researchers selected three unrelated patients with three different BrS profiles. The researchers aimed, first, to confirm the clinical diagnosis, which could be difficult due to ECG dynamism and incomplete disease penetrance, and, second, to identify their molecular defects in order to establish a genotype-phenotype correlation. The researchers chose the well-described SCN5A gene as the candidate for exploration. The gene expression decrease observed and its correlation with the three patients' phenotype severity confirms the implication of SCN5A gene with a loss of function effect as previously described in BrS. Sequencing results showed no deleterious mutation, but seven synonymous and non-synonymous exonic variants and four 3'UTR SNPs. The researchers observed that the decrease in SCN5A expression is directly proportional to the number of variants in each patient. Based on these results and the bibliographic data, the researchers hypothesise that in the absence of causal mutation, multiple but not a single alteration in SCN5A might be responsible for BrS onset and that the number of defects is positively correlated to the phenotype severity. A larger dataset and/or functional explorations may confirm this hypothesis, which gives new insights for better genetic counselling in BrS diagnosis and risk stratification.

RECOMMENDATIONS

For genetic counselling of a patient for whom the influence of SCN5A gene is suspected, the researchers recommend that cardiologists analyse the expression of this gene before exploring its sequence in search of mutations because these may not exist in the region analysed or may be replaced by one or more benign or likely benign variants.

ABBREVIATIONS

BrS: Brugada Syndrome
ECG: Electrocardiogram
Kb: Kilobase
P1, 2, 3: Patients 1, 2, 3
RT: Reverse Transcription
SCD: Sudden cardiac death
SCN5A: Sodium voltage-gated channel alpha subunit 5
SNP: Single nucleotide polymorphism
UTR: Untranslated region.

ACKNOWLEDGEMENT

This work was funded by the Ministry of Higher Education and Scientific Research (Ex-Research Unit: Biology and Molecular Anthropology Applied to Development and Health, Faculty of Pharmacy, University of Monastir, Tunisia). The authors would like to thank Dr. Amelia Aranega Gimene and Dr Diego Franco Jame from the Department of Experimental Biology, Jaén University, Spain, for their precious help in DNA sequencing. The authors would also like to thank the patients and their families for agreeing to participate in this study.

REFERENCES

Antzelevitch C, Brugada P, Borggrefe M et al. 2005. Brugada syndrome: Report of the second consensus conference. *Heart Rhythm*, 2: 429-440.

Benito B, Brugada J, Brugada R et al. 2009. Brugada Syndrome. *Rev Esp Cardiol*, 62: 1297-1315.

Berne P, Brugada J 2012. Brugada syndrome. *Circ J*, 76: 1563-1571.

Brugada P, 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.

Chatterjee N, Shi J, García-Closas M 2016. Developing and evaluating polygenic risk prediction models for stratified disease prevention. *Nat Rev Genet*, 17(7): 392-406.

Chen C, Tan Z, Zhu W et al. 2020. Brugada syndrome with SCN5A mutations exhibits more pronounced electrophysiological defects and more severe prognosis: A meta-analysis. *Clin Genet*, 97(1): 198-208.

Daimi H, Haj Khelil A, Neji A et al. 2019. Role of SCN5A coding and non coding sequences in Brugada syndrome onset: What's behind the scene? *Biomed J*, 42(4): 252-260.

Daimi H, Lozano-Velasco E, Haj Khelil A et al. 2015. Regulation of SCN5A by microRNAs: miR-219 modulates SCN5A transcript expression and the effects of flecainide intoxication in mice. *Heart Rhythm*, 12: 1333-1342.

Denham NC, Pearman CM, Ding WY et al. 2019. Systematic re-evaluation of SCN5A variants associated with Brugada syndrome. *J Cardiovasc Electrophysiol*, 30(1): 118-127.

Fazi F, Nervi C 2008. MicroRNA: Basic mechanisms and transcriptional regulatory networks for cell fate determination. *Cardiovascular Research*, 79: 553-561.

Gourraud JB, Barc J, Thollet A et al. 2016. The Brugada Syndrome: A rare Arrhythmia Disorder with complex inheritance. *Front Cardiovasc Med*, 3: 9.

Hermida JS, Lemoine JL, Bou Aoun F et al. 2000. Prevalence of the Brugada syndrome in an apparently healthy population. *Eur Heart J*, 86: 91-94.

Hosseini SM, Kim R, Udupa S et al. 2018. Reappraisal of reported genes for sudden arrhythmic death. *Circulation*, 18;138(12): 1195-1205.

Juang JJ, Horie M 2016. Genetics of Brugada syndrome. *J Arrhythm*, 32(5): 418-425.

Juang JJ, Lin JL 2017. Brugada syndrome: Merely an ion channelopathy, a structural heart disease, or mixed? *Heart Rhythm*, 14: 590-591.

Kapplinger JD, Tester DJ, Alders M 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.

Kroncke BM, Glazer AM, Smith DK et al. 2018. SCN5A (NaV1.5) variant functional perturbation and clinical presentation: Variants of a certain significance. *Circ Genom Precis Med*, 11(5): e002095.

Livak KJ, Schmittgen TD 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods*, 254: 402-408.

Magnani JW, Brody JA, Prins BP et al. 2014. Sequencing of SCN5A identifies rare and common variants associated with cardiac conduction: Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium. *Circ Cardiovasc Genet*, 7: 365-373.

Meregalli PG, Ruijter JM, Hofman N, Bezzina et al. 2006. Diagnostic value of flecainide testing in unmasking SCN5A-related Brugada syndrome. *J Cardiovasc Electrophysiol*, 17: 857–864.

Paulussen AD, Gilissen RA, Armstrong M et al. 2004. Genetic variations of KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2 in drug-induced long QT syndrome patients. *J Mol Med*, 82: 182-188.

Qureshi SF, Ali A, John P et al. 2015. Mutational analysis of SCN5A gene in long QT syndrome. *Meta Gene*, 6: 26-35.

Sinner MF, Pfeufer A, Perz S et al. 2009. Spontaneous Brugada electrocardiogram patterns are rare in the German general population: results from the KORA study. *Europace*, 11: 1338-1344.

Smits JPP, Koopmann TT, Wilders R et al. 2005. A mutation in the human cardiac sodium channel (E161K) contributes to sick sinus syndrome, conduction disease and Brugada syndrome in two families. *J Mol Cell Cardiol*, 38: 969-981.

Stecker EC, Sono M, Wallace E et al. 2006. Allelic variants of SCN5A and risk of sudden cardiac arrest in patients with coronary artery disease. *Heart Rhythm*, 3: 697-700.

Tlili R, Ben Ameur Y, Sdiri W et al. 2012. La mort subite d'origine rythmique chez le sportif: Revue de la Littérature. *La Tunisie Medicale*, 90: 345-350.

Wang Q, Li Z, Shen J, Keating MT 1996. Genomic organization of the human SCN5A gene encoding the cardiac sodium channel. *Genomics*, 15;34(1): 9-16.

Wilde AA, Antzelevitch C, Borggrefe M et al. 2002 Proposed diagnostic criteria for the Brugada syn-

drome: consensus report. *Circulation*, 106: 2514-2519.
Wilde AA, Postema PG, Di Diego et al. 2010. The pathophysiological mechanism underlying Brugada syndrome: Depolarization versus repolarization. *J Mol Cell Cardiol*, 49: 543-553.
Wilde AM, Amin AS 2018. Clinical spectrum of SCN5A mutations long QT Syndrome, Brugada Syndrome, and Cardiomyopathy. *JACC: Clinical Electrophysiology*, 4(5): 569-579.
Wu Y, Ai M, Bardeesi AS et al. 2017. Brugada syndrome: A fatal disease with complex genetic etiologies – still a long way to go. *Forensic Sciences Research*, 2: 115-125.

Paper received for publication in May, 2019
Paper accepted for publication in August, 2020