Case Report

Novel SCN5A p.V1429M Variant Segregation in a Family with Brugada Syndrome

Michelle M. Monasky 1†, Emanuele Micaglio 1†, Giuseppe Ciconte 1, Valeria Borrelli 1, Luigi Giannelli 1, Gabriele Vicedomini 1, Andrea Ghiroldi 2, Luigi Anastasia 23, Emanuela T. Locati 1, Sara Benedetti 4, Chiara Di Resta 45, Giorgio Casari 45 and Carlo Pappone 1,5,*

1 Arrhythmology Department, IRCCS Policlinico San Donato, San Donato Milanese, 20097 Milan, Italy; michelle.monasky@grupposandonato.it (M.M.M.); emanuele.micaglio@grupposandonato.it (E.M.); g.ciconte@gmail.com (G.C.); valiborrelli91@gmail.com (V.B.); giannelli.luigi@gmail.com (L.G.); gabriele.videdomini@grupposandonato.it (G.V.); EmanuelaTeresina.Locati@grupposandonato.it (E.T.L.)
2 Stem Cells for Tissue Engineering Laboratory, IRCCS Policlinico San Donato, San Donato Milanese, 20097 Milan, Italy; andrea.ghiroldi@gmail.com (A.G.); luigi.anastasia@unimi.it (L.A.)
3 Department of Biomedical Sciences for Health, University of Milan, 20122 Milan, Italy
4 Clinical Genomics—SMEL, IRCCS San Raffaele Hospital, 20132 Milan, Italy; benedetti.sara@hsr.it (S.B.); diresta.chiara@hsr.it (C.D.R); casari.giorgio@unisr.it (G.C.)
5 Vita-Salute San Raffaele University, 20132 Milan, Italy
* Correspondence: carlo.pappone@af-ablation.org; Tel./Fax: +390252774260/4306
† These authors contributed equally to this work.

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Abstract: Brugada syndrome (BrS) is diagnosed by the presence of an elevated ST-segment and can result in sudden cardiac death. The most commonly found mutated gene is SCN5A, which some argue is the only gene that has been definitively confirmed to cause BrS, while the potential causative effect of other genes is still under debate. While the issue of BrS genetics is currently a hot topic, current knowledge is not able to result in molecular confirmation of over half of BrS cases. Therefore, it is difficult to develop research models with wide potential. Instead, the clinical genetics first need to be better understood. In this study, we provide crucial human data on the novel heterozygous variant NM_198056.2:c.4285G>A (p.Val1429Met) in the SCN5A gene, and demonstrate its segregation with BrS, suggesting a pathogenic effect. These results provide the first disease association with this variant and are crucial clinical data to communicate to basic scientists, who could perform functional studies to better understand the molecular effects of this clinically-relevant variant in BrS.

Keywords: Brugada syndrome; sudden cardiac death; genetic testing; mutation; variant; SCN5A; sodium channel; arrhythmia; channelopathy; human; family

1. Background

There has been an ever-increasing interest in Brugada Syndrome (BrS) ever since its first description almost three decades ago, due to its ability to cause ventricular tachycardia/fibrillation (VT/VF) and sudden cardiac death (SCD) in young and otherwise healthy individuals [1]. Accordingly, BrS genetics has gained widespread popularity and is currently a hot topic, as not even half of BrS cases can be molecularly confirmed [2], and all but the involvement of the SCN5A gene is fiercely debated [3–5]. However, even the roles of several specific variants within the SCN5A gene are disputed, with many listed as variants of unknown significance, and many are thought to result
in pathologies other than BrS, such as atrial standstill, atrial fibrillation, left ventricular non-compaction, dilated cardiomyopathy, Long QT syndrome, idiopathic ventricular fibrillation, and heart block [6,7]. Some SCN5A variants found in patients undergoing routine genetic testing have never been described before in the literature, or are listed in popular genetic databases, such as Varsome [8], as benign or likely benign.

Novel mutations in the SCN5A gene and their likely causative role in BrS have been of recent interest [9–15], as well as new candidate genes [13,16,17]. Most studies have reported autosomal dominant inheritance with incomplete penetrance [18–20], with a few suggesting a recessive or X linked inheritance [21,22] and a possible involvement of mitochondrial mutations [23]. Variants in the SCN5A gene associated with BrS result in a loss of function of the voltage-gated sodium channel subunit (Na\textsubscript{v}1.5) [17,24,25].

It is necessary to better understand clinical genetics to increase the power of the diagnostic capability based upon genetics alone. Genetic testing is a far easier test to perform clinically than other diagnostic tests, such as an ajmaline challenge, which requires the patient to travel to a highly-specialized facility because of the high risks of the procedure [26]. Travel restrictions or the cost of an invasive procedure could impair the ability to diagnose this potentially fatal disease, which often results in sudden death in otherwise asymptomatic and seemingly otherwise healthy individuals. Genetic testing, on the other hand, can be performed in more remote areas, either with a blood or saliva sample, without serious risks related to the procedure.

In this study, the variant NM_198056.2:c.4285G>A (p.Val1429Met) in the SCN5A gene is characterized for the first time, generally and in a family with BrS, providing crucial human data that are the first step in advancing diagnostic capabilities.

2. Case Presentation

Written informed consent of human subjects included in this case series report was obtained for their participation in the study and for publication. The procedures employed were reviewed and approved by the local ethics committee. The study was conducted in accordance with the Declaration of Helsinki, and written informed consent of human subjects was obtained for their participation in the study and for publication. The procedures employed were reviewed and approved by the local Ethics Committee (approver number: M-EC-006/A, rev. 1 March 2013).

The proband is a 38-year-old female with a personal history of syncope. Both her father (at 46 years) and maternal grandfather (at 70 years) died suddenly (Figure 1). Her maternal uncle experienced an aborted cardiac arrest at the age of 36 years old, and was diagnosed with BrS due to the presence of the type 1 BrS ECG pattern identified elsewhere after the diagnostic workup for aborted cardiac arrest. Thus, the proband underwent an ajmaline challenge at our facility, which resulted positive (Figure 2). She then underwent an electrophysiology study (EPS), and was inducible for ventricular tachycardia/fibrillation (VT/VF) (Figure 2). An ICD was implanted.
Figure 1. Family pedigree. Proband identified with arrow. Square: male; Circle: female; Shaded: clinically affected by Brugada syndrome; Star: molecularly confirmed SCN5A variant; Triangle: molecularly confirmed CACNB2 variant; Triangle with slash: negative for CACNB2 variant; y = years old at diagnosis.

Figure 2. Electrocardiogram at baseline, after ajmaline administration, and ventricular tachycardia/ventricular fibrillation inducibility during electrophysiological study for proband.

Genetic testing of several genes described in BrS research literature (ABCC9, AKAP9, CACNA1C, CACNA2D1, CACNB2, DSG2, GPD1L, HCN4, KCN2D, KCND2, KCNE3, KCNE5, KCNH2, KCNJ8, PKP2, RANGRF/MOG1, SCN1B, SCN2B, SCN3B, SCN5A, SCN10A, SEMA3A, TRPM4) by Next Generation Sequencing and confirmed by Sanger sequencing revealed the novel heterozygous variants NM_198056.2:c.4285G>A (p.Val1429Met) in the SCN5A gene (LOVD: https://databases.lovd.nl/shared/variants/0000673713#00018523) (Figure 3) and NM_201596.2:c.1880G>A (p.Arg627His) in the CACNB2 gene (LOVD: https://databases.lovd.nl/shared/variants/0000673714#00024101).
The proband’s 59-year-old mother is asymptomatic. Due to her family history, she underwent an ajmaline challenge at our facility, which resulted positive (Figure 4). She was not found to be inducible for VT/VF at EPS. Genetic testing by Sanger sequencing was positive for the familial variant NM_198056.2:c.4285G>A (p.Val1429Met) in the SCN5A gene but negative for the familial variant NM_201596.2:c.1880G>A (p.Arg627His) in the CACNB2 gene.

The proband’s maternal uncle experienced an aborted cardiac arrest and he came to our facility only for genetic testing, revealing by Sanger sequencing the presence of the familial variant NM_198056.2:c.4285G>A (p.Val1429Met) in the SCN5A gene in him as well. However, he was negative for the familial variant NM_201596.2:c.1880G>A (p.Arg627His) in the CACNB2 gene.
2.2. In Silico Predictions

The novel c.4285G>A variant in the SCN5A gene was classified as likely pathogenic according to ACMG criteria and Varsome database accessed on 13 July 2020 [8,27]:

- PM1 Moderate: Hot-spot of length 61 base-pairs has seven non-VUS coding variants (seven pathogenic and zero benign), pathogenicity = 100.0%, qualifies as hot-spot.
- PM2 Moderate: Variant not found in gnomAD exomes (good gnomAD exomes coverage = 51.3). Variant not found in gnomAD genomes (good gnomAD genomes coverage = 33.1).
- PP2 Supporting: 272 out of 342 non-VUS heterozygous missense variants in gene SCN5A are pathogenic = 79.5% which is more than threshold of 51.0%, and 426 out of 1915 clinically reported variants in gene SCN5A are pathogenic = 22.2% which is more than threshold of 12.0%.
- PP3 Supporting: Pathogenic computational verdict based on 11 pathogenic predictions from DANN, DEOGEN2, EIGEN, FATHMM-MKL, M-CAP, MVP, MutationAssessor, MutationTaster, PrimateAI, REVEL and SIFT vs. no benign predictions.

Table 1 demonstrates the mutational hot-spot in the region harboring the novel SCN5A variant described.

Table 1. Demonstration of mutational hot-spot in the region harboring the novel c.4285G>A SCN5A variant.

| Chromosome | Position | Reference Sequence | Altered Sequence | Mutation Effect |
|------------|----------|-------------------|------------------|-----------------|
| 3          | 38598716 | A                 | G                | c.4137 + 6T > C  |
| 3          | 38598720 | None              | C                | c.4137 + 1dupG   |
| 3          | 38598725 | C                 | A                | c.4134G > T     |
| 3          | 38598726 | CT                | GA               | c.4132, 4133delAGinsTCLike pathogenic |
| 3          | 38598736 | C                 | T                | c.4285G > A     |
| 3          | 38598738 | G                 | A                | c.4121C > T     |
| 3          | 38598759 | C                 | T                | c.4100G > A     |
| 3          | 38598762 | C                 | A                | c.4097G > T     |
| 3          | 38598763 | C                 | G                | c.4096G > C     |

The novel c.1880G>A (p.Arg627His) variant in the CACNB2 gene was classified as likely benign according to ACMG criteria and Varsome database accessed on 13 July 2020 [8,27]:

- BS2 Strong: Observed in healthy adults: gnomAD exomes allele count = 8 is greater than the five threshold for dominant gene CACNB2 (good gnomAD exomes coverage = 69.5).
- BP1 Supporting: seven out of eight non-VUS missense variants in gene CACNB2 are benign = 87.5% which is more than threshold of 51.0%, and 79 out of 267 clinically reported variants in gene CACNB2 are benign = 29.6% which is more than threshold of 24.0%.

3. Discussion

In the present study, we report for the first time the variant NM_198056.2:c.4285G>A (p.Val1429Met) in the SCN5A gene, both generally and in BrS. The family segregation analysis and the in silico predictions support the hypothesis of a pathogenic effect of this variant and provide the first step towards understanding the pathophysiology in these patients and improving diagnostic capabilities.

The clinical presentations of the family members presented are severe, ranging from cardiac arrest to spontaneous type 1 pattern, syncope, inducibility for VT/VF during EPS, and a family history of sudden death. Clearly, aborted cardiac arrest is the most severe presentation of the disease possible. However, the presence of both syncope and a spontaneous type 1 ECG pattern have also been associated with a poor prognosis (6%–19% of people experiencing an arrhythmic event within 24–39 months during the follow-up period) [16,28]. Inducibility for VT/VF during EPS is also indicative of a poor prognosis [29,30]. Therefore, the clinical phenotypes indicate the presence of a severe disease, potentially requiring ICD implantation to prevent life-threatening arrhythmias.

The SCN5A gene encodes for the alpha subunit of the NaV1.5 protein. Disease-causing variants found in the SCN5A gene responsible for BrS result in a loss of function of the NaV1.5 protein and
reduced sodium transport due to any of a variety of mechanisms, including reduced expression [31–33], non-functional channels [34], and changes in gating properties [35,36].

The c.4285G>A variant in the SCN5A gene is currently listed as likely pathogenic [8] and has several factors supporting the possible pathogenicity of this variant, including its genic expression and apparent low frequency. In fact, to date, the heterozygous c.4285G>A variant has never been found in the GnomAD database, consistent with the hypothesis of having a very low frequency in the general population. Supporting the hypothesis of a pathogenic role of this variant, the interspecies conservation of the p.Val1429 residue is shown in Figure 3B, according to Uniprot. Several other variants in this gene are known to be either pathogenic or likely pathogenic. In particular, Table 1 shows at least seven of such heterozygous mutations localized very close to our variant. Therefore, the region from position 38598716 to 38598763 might be a hot-spot for pathogenic variants. Moreover, several computational studies and many bioinformatic tools predict this heterozygous variant to be pathogenic, while no computational studies or bioinformatic tools predict the variant to be benign.

The NM_201596.2:c.1880G>A (p.Arg627His) in the CACNB2 gene was additionally found in the proband, but not in her mother, who exhibits the SCN5A familial variant and BrS. The proband’s father died suddenly at the age of 46 years old. Therefore, it is likely that the proband inherited the CACNB2 variant from her father (as opposed to being a de novo variant). Some variants in the CACNB2 gene are currently disputed as possibly causative for BrS. However, considering also the presence of the SCN5A variant in the proband in the current study, the family segregation information for the CACNB2 variant is inconclusive.

4. Concluding Remarks

The novel heterozygous variant NM_198056.2:c.4285G>A (p.Val1429Met) in the SCN5A gene segregates with BrS in the family presented, suggesting a pathogenic effect of this variant. These crucial human data are the first step in understanding the pathology of BrS for patients with this variant and set the stage for both functional studies to better understand the molecular pathways involved, and eventually better diagnostic capabilities, based upon very minimally invasive and safe genetic tests.

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Reference

1. Kusumoto, F.M.; Bailey, K.R.; Chaouki, A.S.; Deshmukh, A.J.; Gautam, S.; Kim, R.J.; Kramer, D.B.; Lambarakos, L.K.; Nasser, N.H.; Sorajja, D. Systematic Review for the 2017 AHA/ACC/HRS Guideline for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death. Circulation 2018, 138, doi:10.1161/CIR.0000000000005550.

2. Kapplinger, J.D.; Tester, D.J.; Alders, M.; Benito, B.; Berthet, M.; Brugada, J.; Brugada, P.; Fressart, V.; Guercicchio, A.; Harris-Kerr, C.; et al. An international compendium of mutations in the SCN5A-encoded cardiac sodium channel in patients referred for Brugada syndrome genetic testing. Hear. Rhythm. 2010, 7, 33–46, doi:10.1016/j.hrthm.2009.09.069.
21. Hosseini, S.M.; Kim, R.; Udupa, S.; Costain, G.; Jobling, R.; Liston, E.; Jamal, S.M.; Szybowska, M.; Morel, C.F.; Bowdin, S.; et al. Reappraisal of Reported Genes for Sudden Arrhythmic Death. *Circulation* **2018**, *138*, 1195–1205, doi:10.1161/circulationaha.118.035070.

20. London, B. Letter by London Regarding Article, “Reappraisal of Reported Genes for Sudden Arrhythmic Death: Evidence-Based Evaluation of Gene Validity for Brugada Syndrome.” *Circulation* **2019**, *139*, 1758–1759, doi:10.1161/circulationaha.118.036889.

19. Wilde, A.A.; Gollob, M.H. Response by Wilde and Gollob to Letter Regarding Article, “Reappraisal of Reported Genes for Sudden Arrhythmic Death: Evidence-Based Evaluation of Gene Validity for Brugada Syndrome.” *Circulation* **2019**, *139*, 1760–1761, doi:10.1161/circulationaha.119.039065.

18. Gosselin-Badaroudine, P.; Moreau, A.; Chahine, M., Nav 1.5 mutations linked to dilated cardiomyopathy phenotypes: Is the gating pore current the missing link? *Channels (Austin)* **2014**, 8, 90-94.

17. Zaklyazminskaya, E.; Dzemeshkevich, S.D. The role of mutations in the SCN5A gene in cardiomyopathies. *Biochim. Biophys. Acta (BBA) - Biomembr.* **2016**, *1863*, 1799–1805, doi:10.1016/j.bbamcr.2016.02.014.

16. VarSome: The Human Genomics Community. https://varsome.com/ (Accessed on: July 13, 2020).

15. Micaglio, E.; Monasky, M.M.; Ciconte, G.; Vicedomini, G.; Conti, M.; Mecarocci, V.; Giannelli, L.; Giordano, F.; Pollina, A.; Saviano, M.; et al. SCN5A Nonsense Mutation and NF1 Frameshift Mutation in a Family With Brugada Syndrome and Neurofibromatosis. *Front. Genet.* **2019**, *10*, doi:10.3389/fgene.2019.00050.

14. Yeates, L.; Ingles, J.; Gray, B.; Singarayar, S.; Sy, R.W.; Sensmarius, C.; Bagnall, R.D.; Couns, G.D.G. A balanced translocation disrupting SCN5A in a family with Brugada syndrome and sudden cardiac death. *Hear. Rhythm. 2019*, 16, 231–238, doi:10.1016/j.hrthm.2018.08.027.

13. Yagihiara, N.; Watanabe, H.; Barnett, P.; Duboscq-Bidot, L.; Thomas, A.C.; Yang, P.; Ohno, S.; Hasegawa, K.; Kuwano, R.; Chatel, S.; et al. Variants in the SCN5A Promoter Associated With Various Arrhythmia Phenotypes. *J. Am. Hear. Assoc.* **2016**, *5*, doi:10.1161/jaha.116.003644.

12. Micaglio, E.; Monasky, M.M.; Ciconte, G.; Vicedomini, G.; Conti, M.; Mecarocci, V.; Giannelli, L.; Giordano, F.; Pollina, A.; Saviano, M.; et al. Novel SCN5A Frameshift Mutation in Brugada Syndrome Associated With Complex Arrhythmic Phenotype. *Front. Genet.* **2019**, *10*, 547, doi:10.3389/fgene.2019.00057.

11. Monasky, M.M.; Micaglio, E.; Vicedomini, G.; Locati, E.T.; Ciconte, G.; Giannelli, L.; Giordano, F.; Crisà, S.; Vecchi, M.; Borrelli, V.; et al. Comparable clinical characteristics in Brugada syndrome patients harboring SCN5A or novel SCN10A variants. *EP Europace* **2019**, 21, 1550–1558, doi:10.1093/epace/ezu186.

10. Micaglio, E.; Monasky, M.M.; Resta, N.; Bagnulo, R.; Ciconte, G.; Giannelli, L.; Locati, E.T.; Vicedomini, G.; Borrelli, V.; Ghiroldi, A.; et al. Novel SCN5A p.W697X Nonsense Mutation Segregation in a Family with Brugada Syndrome. *Int. J. Mol. Sci.* **2019**, *20*, 4920, doi:10.3390/ijms20194920.

9. Monasky, M.M.; Micaglio, E.; Giachino, D.; Ciconte, G.; Giannelli, L.; Locati, E.T.; Ramondini, E.; Cotugno, R.; Vicedomini, G.; Borrelli, V.; et al. Genotype–Phenotype Correlation in a Family with Brugada Syndrome Harboring the Novel p.Gln371* Nonsense Variant in the SCN5A Gene. *Int. J. Mol. Sci.* **2019**, *20*, 5522, doi:10.3390/ijms20225522.

8. Brugada, J.; Campuzano, O.; Arbelo, E.; Sarquella-Brugada, G.; Brugada, R. Present Status of Brugada Syndrome. *J. Am. Coll. Cardiol.* **2018**, *72*, 1046–1059, doi:10.1016/j.jacc.2018.06.037.

7. Di Resta, C.; Pietrelli, A.; Sala, S.; Bordoni, R.; Benedetti, S.; Della Bella, P.; De Bellis, G.; Ferrari, M. High-throughput genetic characterization of a cohort of Brugada syndrome patients. *Hum. Mol. Genet.* **2015**, *24*, 5828–5833, doi:10.1093/hmg/ddv302.

6. Nademanee, K.; Veerakul, G.; Chandanamattha, P.; Chaothawee, L.; Ariyachaipanich, A.; Jirasirirajakorn, K.; Likitanasombat, K.; Bhuripanyo, K.; Ngarmukos, T. Prevention of Ventricular Fibrillation Episodes in Brugada Syndrome by Catheter Ablation Over the Anterior Right Ventricular Outflow Tract Epicardium. *Circulation* **2011**, 123, 1270–1279, doi:10.1161/circulationaha.110.972612.

5. Lieve, K.V.; Wilde, A.A. Inherited ion channel diseases: a brief review. *EP Europace* **2015**, 17, i1–i16, doi:10.1093/epace/euv105.

4. Chen, Q.; Kirsch, G.E.; Zhang, D.; Brugada, P.; Brugada, P.; Brugada, P.; Potenza, D.; Moya, A.; Borggrefe, M.; Breithardt, G.; et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature* **1998**, *392*, 293–296, doi:10.1038/32675.

3. Janin, A.; Bessière, F.; Georgescu, T.; Chanavat, V.; Chevalier, P.; Millat, G. TRPM4 mutations to cause autosomal recessive and not autosomal dominant Brugada type 1 syndrome. *Eur. J. Med. Genet.* **2019**, *62*, 103527, doi:10.1016/j.ejmg.2018.08.008.
22. Ohno, S.; Zankov, D.P.; Ding, W.-G.; Itoh, H.; Makiyama, T.; Doi, T.; Shizuta, S.; Hattori, T.; Miyamoto, A.; Naiki, N.; et al. KCNE5 Variants Are Novel Modulators of Brugada Syndrome and Idiopathic Ventricular Fibrillation. *J. Arrhythmia* 2011, 27, Y1AB-5, doi:10.4020/jhrs.27.yiab_5.

23. Tafti, M.F.; Khatami, M.; Rezaei, S.; Heidari, M.M.; Hadadzadeh, M. Novel and heteroplasmatic mutations in mitochondrial tRNA genes in Brugada syndrome. *Cardiol. J.* 2018, 25, 113–119, doi:10.5603/cj.a2017.0104.

24. Curcio, A.; Santarpia, G.; Indolfi, C. The Brugada Syndrome — From Gene to Therapy —. *Circ. J.* 2017, 81, 290–297, doi:10.1253/circj.cj-16-0971.

25. Sieira, J.; Dendramis, G.; Brugada, P. Pathogenesis and management of Brugada syndrome. *Nat. Rev. Cardiol.* 2016, 13, 744–756, doi:10.1038/nrcardio.2016.143.

26. Ciconte, G.; Monasky, M.M.; Vicedomini, G.; Borrelli, V.; Giannelli, L.; Pappone, C. Unusual response to ajmaline test in Brugada syndrome patient leads to extracorporeal membrane oxygenator support. *Europace* 2019, 21, 1574, doi:10.1093/europace/euz139.

27. Richards, S.; on behalf of the ACMG Laboratory Quality Assurance Committee; Aziz, N.; Bale, S.; Bick, D.; Das, S.; Gastier-Foster, J.; Grody, W.W.; Hegde, M.; Lyon, E.; et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* 2015, 17, 405–423, doi:10.1038/gim.2015.30.

28. Priori, S.G.; Gasparini, M.; Napolitano, C.; Della Bella, P.; Ottonelli, A.G.; Sassone, B.; Giordano, U.; Pappone, C.; Marsigli, G.; Rossetti, G.; et al. Risk Stratification in Brugada Syndrome. *J. Am. Coll. Cardiol.* 2012, 59, 37–45, doi:10.1016/j.jacc.2011.08.064.

29. Sroubek, J.; Probst, V.; Mazzanti, A.; Delise, P.; Hevia, J.C.; Ohkubo, K.; Zorzi, A.; Champagne, J.; Kostopoulou, A.; Yin, X.; et al. Programmed Ventricular Stimulation for Risk Stratification in the Brugada Syndrome: A Pooled Analysis. *Circulation* 2016, 133, 622–30, doi:10.1161/CIRCULATIONAHA.115.017885.

30. Antzelevitch, C.; Yan, G.-X.; Ackerman, M.J.; Borggrefe, M.; Corrado, D.; Guo, J.; Gussak, I.; Hasdemir, C.; Horie, M.; Huikuri, H.; et al. J-Wave syndromes expert consensus conference report: Emerging concepts and gaps in knowledge. *EP Europace* 2017, 19, 665–694, doi:10.1093/europace/euw235.

31. Valdivia, C.R.; Tester, D.J.; A Rok, B.; Porter, C.-B.J.; Munger, T.M.; Jahangir, A.; Makielski, J.C.; Ackerman, M.J. A trafficking defective, Brugada syndrome-causing SCN5A mutation rescued by drugs. *Cardiovasc. Res.* 2004, 62, 53–62, doi:10.1016/j.cardio.2004.01.022.

32. Herfst, L. Na+ channel mutation leading to loss of function and non-progressive cardiac conduction defects. *J. Mol. Cell. Cardiol.* 2003, 35, 549–557, doi:10.1016/s0022-2828(03)00078-6.

33. Tfelt-Hansen, J.; Jespersen, T.; Hofman-Bang, J.; Rasmussen, H.B.; Cedergreen, P.; Skovby, F.; Abriel, H.; Svendsen, J.H.; Olesen, S.-P.; Christiansen, M.; et al. Ventricular tachycardia in a Brugada syndrome patient caused by a novel deletion in SCN5A. *Can. J. Cardiol.* 2009, 25, 156–160, doi:10.1016/s0828-282x(09)70043-1.

34. Kyndt, F.; Probst, V.; Potet, F.; Demolombe, S.; Chevallier, J.-C.; Baró, I.; Moisan, J.-P.; Boisseau, P.; Schott, J.-J.; Escande, D.; et al. Novel SCN5A mutation leading either to isolated cardiac conduction defect or Brugada syndrome in a large French family. *Circulation* 2001, 104, 3081–3086, doi:10.1161/hc5001.100834.

35. Bezzina, C.; Veldkamp, M.W.; Berg, M.P.V.D.; Postma, A.V.; Rook, M.B.; Viersma, J.-W.; Van Langen, I.M.; Tan-Sindhumata, G.; Bink-Boelkens, M.T.E.; Van Der Hout, A.H.; et al. A single Na(+) channel mutation causing both long-QT and Brugada syndromes. *Circ. Res.* 1999, 85, 1206–1213, doi:10.1161/01.res.85.12.1206.

36. Dumaine, R.; Towbin, J.A.; Brugada, P.; Vatta, M.; Nesterenko, D.V.; Nesterenko, V.V.; Brugada, J.; Brugada, R.; Antzelevitch, C. Ionic mechanisms responsible for the electrocardiographic phenotype of the Brugada syndrome are temperature dependent. *Circ. Res.* 1999, 85, 803–809, doi:10.1161/01.res.85.9.803.

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