Commentary: Peptide-Based Targeting of the L-Type Calcium Channel Corrects the Loss-of-Function Phenotype of Two Novel Mutations of the CACNA1 Gene Associated With Brugada Syndrome

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A Commentary on

Peptide-Based Targeting of the L-Type Calcium Channel Corrects the Loss-of-Function Phenotype of Two Novel Mutations of the CACNA1 Gene Associated With Brugada Syndrome by Di Mauro, V., Ceriotti, P., Lodola, F., Salvareoni, N., Modica, J., Bang, M.L., Mazzanti, A., Napolitano, C., Priori, S.G., and Catalucci, D. (2020). Front. Physiol. 11:616819. doi: 10.3389/fphys.2020.616819

We read with great interest a recently published article by Di Mauro et al. (2020) describing for the first time the use of a mimetic peptide (R7W-MP) to restore impaired forward trafficking and reduced half-life of L-type calcium channels (LTCC) caused by mutations in the CACNA1C gene, restoring channel function in vitro. The two novel mutations in the CACNA1C gene (Cavα1.2 T320M and Cavα1.2 Q428E) were found in patients with Brugada syndrome (BrS), one asymptomatic (T320M), and one with a history of cardiac arrest, ICD placement, two episodes of self-terminating polymorphic ventricular tachycardia, and runs of atrial fibrillation (Q428E). The mutations in the CACNA1C gene, encoding for the pore-forming unit (Cavα1.2), studied in HEK293 cells, exhibited reduced protein trafficking to, and half-life in, the membrane, resulting in reduced calcium current.

Variants in more than 26 different genes have been implicated in BrS (Monasky et al., 2020), the most accepted being the SCN5A gene, encoding for the sodium voltage-gated channel alpha subunit 5, or the Nav1.5 protein. While some studies have suggested a role for the CACNA1C gene in BrS (Fukuyama et al., 2014), the causative effect of the CACNA1C gene in BrS has been recently challenged (Hosseini et al., 2018; London, 2019; Wilde et al., 2019), citing the lack of systematic, evidence-based evaluations supporting the causality of this gene. Thus, systematic, evidence-based evaluations are of utmost importance, after several studies suggested an important role for calcium in BrS (Antzelevitch et al., 2007; Cordeiro et al., 2009; Burashnikov et al., 2010; Hoogendijk et al., 2011; Betzenhauser et al., 2015; Monaky et al., 2018).

Calcium plays a pivotal role in cardiac contractility, and the control of intracellular Ca²⁺ cycling depends on the relationships between the various channels and pumps that are involved
Phase 2 and 3 of the action potential correspond to the ST segment and T wave, respectively. These coincide with the rise and fall of intracellular calcium that governs cardiac myocyte contractility (Monasky et al., 2018). Much of the calcium enters the cell via L-type calcium channels, while an additional amount of calcium enters the cell via sodium–calcium exchange (NCX) channels. Calcium that enters the cell through both of these mechanisms triggers release of calcium from the sarco(endo)plasmic reticulum. Alterations in calcium handling could result in mechanical abnormalities, since calcium links the electrical and mechanical functions of the cell. An increase in a risk for arrhythmic events has been observed while patients with BrS were engaging in activities related to parasympathetic stimulation (Monasky et al., 2018), which results in an elevated ST segment, possibly through a reduction in ICa−t (Litovsky and Antzelevitch, 1990; Meregalli et al., 2005; Hoogendijk et al., 2011; Monasky et al., 2018). The reduced heart rate during parasympathetic stimulation results in a decrease in intracellular calcium amplitude (Hiranandani et al., 2006; Varian and Janssen, 2007). CACNA1C mutations could lead to a reduced intracellular concentration of calcium able to bind to troponin C of the myofilaments, thus disrupting excitation-contraction coupling (Monasky et al., 2018), the extent to which is still unclear. In fact, the induction of the BrS pattern has been associated with reduced contractility, particularly in the anterior free wall of the outflow tract, and reduced right ventricular ejection fraction (Pappone et al., 2019, 2020b). Therefore, further investigation of the role of calcium channel genes in BrS is warranted.

Antzelevitch et al. (2007) first described loss-of-function mutations in the LTCC genes CACNB2b, CACNA2D1, and CACNA1C in association with familial sudden cardiac death syndrome, the phenotype combining BrS and shorter-than-normal QT intervals. A role for CACNA2D1 as a contributing factor in cardiac sudden death associated with a short QT interval has been described by significantly decreasing the cell surface protein expression of CaV2.2 (Bourdin et al., 2015). Importantly, in that study, the most significant reduction in CaV2.2 cell surface density was achieved by the combined effect of two genetic variants with little individual impact, highlighting the importance of polymorphisms. In fact, several other studies have highlighted the importance of common polymorphisms as genetic modulators of BrS (Lizotte et al., 2009), explaining the variable expression of the BrS phenotype (Wijeyeratne et al., 2020). Thus, in addition to rare mutations, also polymorphisms in calcium channel genes should be considered in future BrS research.

In their study, Di Mauro et al. (2020) state that CACNA1C mutations are the second most common cause of BrS. However, studies differ, likely due to differences in the gene panels used to screen patients, as well as the size and characteristics of the patient population. For example, in a recent report, variants in the CACNA1C gene were identified in about 7% of BrS patients who tested positive during genetic testing but who did not harbor variants in the SCN5A gene, making CACNA1C the fifth most popular gene screened after SCN5A, AKAP9, SCN10A, and MYBPC3 (Pappone et al., 2020a). AKAP9 encodes for A-kinase anchoring protein 9, a signaling protein that binds to the regulatory subunit of protein kinase A and has been implicated also as a genetic modifier of congenital long-QT syndrome type 1 (De Villiers et al., 2014). SCN10A encodes for the sodium voltage-gated channel alpha subunit 10. MYBPC3 encodes for the myosin-associated protein cardiac myosin-binding protein C, which is involved in the regulation of force production and can be regulated by protein kinase A (Yang et al., 2001). Another study investigating the frequency of variants found in BrS patients also reported a higher frequency in CACNA1C compared to SCN10A, with variants in CACNA1C present in 2.6% of BrS patients overall, and 3.3% of BrS patients negative for variants in SCN5A (Di Resta et al., 2015). However, yet another study specifically looking at mutations in the genes CACNA1C and CACNB2b, encoding the α1- and β2b-subunits of the cardiac L-type calcium channel, respectively, found that 8.5% of the patients had mutations in at least one of these genes, although it is unclear how many harbored mutations in CACNA1C vs. how many harbored mutations in CACNB2b (Antzelevitch et al., 2007). Also, it was unclear if patients harbored mutations in other genes. However, regardless, it is clear by many studies that calcium channel variants have been found across various studies, by various authors, with various patient populations.

BrS is increasingly being recognized as an oligogenic disease (Monasky et al., 2020), with mutations in the SCN5A gene being more useful as a prognostic indicator, rather than a diagnostic one (Ciconte et al., 2020). To date, there is much that remains to be discovered about BrS genetics. Future studies need to identify and test new candidate genes. The genetics of BrS likely varies greatly from family to family, highlighting our need to move toward personalized medicine in BrS. Physiological studies such as the one by Di Mauro et al. (2020) are a good first step toward confirming the pathological effects of particular variants and treating patients with individual variants. However, much work remains before new pharmaceuticals can be developed, tested, and safely used in the clinic.

In conclusion, the study by Di Mauro et al. (2020) provides strong evidence of a possible gene-specific treatment in the future for BrS patients and is the first example of an LTCC-targeting therapeutic molecule that can correct ICa defects through modulation of channel density at the plasma membrane. Although preliminary, this is a promising step toward the development of pharmacological therapies to treat conductance abnormalities of the heart.

**AUTHOR CONTRIBUTIONS**

MM suggested the project and significantly reworked the article and provided guidance. CR wrote the first draft. EM and CP provided useful feedback. CP obtained financial support. All authors contributed to the article and approved the submitted version.

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REFERENCES

Antzelevitch, C., Pollevick, G. D., Cordeiro, J. M., Casis, O., Sanguinetti, M. C., Aizawa, Y., et al. (2007). Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. Circulation 115, 442–449. doi: 10.1161/CIRCULATIONAHA.106.668392

Betzenhauser, M. J., Pitt, G. S., and Antzelevitch, C. (2015). Calcium channel mutations in cardiac arrhythmia syndromes. Curr. Mol. Pharmacol. 8, 133–142. doi: 10.2174/1874467208666150518114857

Bourdin, B., Shakeri, B., Tetreault, M. P., Sauve, R., Lesage, S., and Parent, L. (2015). Functional characterization of CaValpha2delta mutations associated with sudden cardiac death. J. Biol. Chem. 290, 2854–2869. doi: 10.1074/jbc.M114.597930

Burashnikov, E., Pfeiffer, R., Barajas-Martinez, H., Delpon, E., Hu, D., Desai, M., et al. (2010). Mutations in the cardiac L-type calcium channel associated with inherited I-wave syndromes and sudden cardiac death. Heart Rhythm. 7, 1872–1882. doi: 10.1016/j.hrthm.2010.08.026

Ciconte, G., Monasky, M. M., Santinelli, V., Micaglio, E., Vicedomini, G., Anastasia, L., et al. (2020). Brugada syndrome genetics is associated with phenotype severity. Eur. Heart J. 42, 1082–90. doi: 10.1093/eurheartj/ehaa942

Cordeiro, J. M., Marieb, M., Pfeiffer, R., Calleo, K., Burashnikov, E., and Antzelevitch, C. (2009). Accelerated inactivation of the L-type calcium current due to a mutation in CACNB2 underlies Brugada syndrome. J. Mol. Cell Cardiol. 46, 695–703. doi: 10.1016/j.yjmcc.2009.01.014

De Villiers, C. P., Van Der Merwe, L., Crotti, L., Goosen, A., George, A. L. Jr., Schwartz, P. J., et al. (2014). AKAP9 is a genetic modifier of congenital long-QT syndrome type 1. Circ. Cardiov. Genet. 7, 599–606. doi: 10.1161/CIRCGENETICS.113.005580

Di Mauro, V., Ceriotti, P., Lodola, F., Salvarani, N., Modica, J., Bang, M. L., et al. (2020). Peptide-based targeting of the L-type calcium channel corrects the loss-of-function phenotype of two novel mutations of the CACNA1 gene associated with brugada syndrome. Front. Physiol. 11:616819. doi: 10.3389/fphys.2020.616819

Di Resta, C., Pietrelli, A., Sala, D., Della Bella, P., De Bellis, G., Ferrari, M., et al. (2015). High-throughput genetic characterization of a cohort of Brugada syndrome patients. Hum. Mol. Genet. 24, 5828–5835. doi: 10.1093/hmg/ddy302

Eisner, D. A., Caldwell, J. L., Kistamas, K., and Trafford, A. W. (2017). Calcium in brugada syndrome: questions for future research. Front. Physiol. 9:1088. doi: 10.3389/physiol.2018.01088

Fukuyama, M., Ohno, S., Shirayama, T., Itoh, H., and Horie, M. (2014). Nonsense-mediated mRNA decay due to a CACNA1C splicing mutation in a patient with Brugada syndrome. Heart Rhythm. 11, 629–634. doi: 10.1016/j.hrthm.2013.12.011

Hoogendijk, M. G., Potse, M., Saris, S., Vinet, A., and Crotti, L., et al. (2020). SCN5A mutation type and a genetic risk score associate variably with brugada syndrome phenotype in SCN5A families. Circ. Genom. Precis. Med. 13, e002911. doi: 10.1161/CIRCGEN.120.002911

Lizotte, E., Junttila, M. J., Dube, M. P., Hong, K., Benito, B., M., D.E. Z., et al. (2009). Genetic modulation of brugada syndrome by a common polymorphism. J. Cardiovasc. Electrophysiol. 20, 1137–1141. doi: 10.1111/j.1540-8167.2009.01508.x

London, R. (2019). Letter by London regarding article, “reappraisal of reported genes for sudden arrhythmic death: evidence-based evaluation of gene validity for brugada syndrome”. Circulation 139, 1758–1759. doi: 10.1161/CIRCULATIONAHA.118.036889

Meregalli, P. G., Wilde, A. A., and Tan, H. L. (2005). Pathophysiological mechanisms of Brugada syndrome: depolarization disorder, repolarization disorder, or more? Cardiovasc. Res. 67, 367–378. doi: 10.1016/j.cardiores.2005.03.005

Monasky, M. M., Micaglio, E., Ciconte, G., and Pappone, C. (2020). Brugada syndrome: oligogenic or mendelian disease? Int. J. Mol. Sci. 21:31687. doi: 10.3390/ijms21051687

Monasky, M. M., Pappone, C., Piccoli, M., Ghirardi, A., Micaglio, E., and Anastasia, L. (2018). Calcium in brugada syndrome: questions for future research. Front. Physiol. 9:1088. doi: 10.3389/physiol.2018.01088

Pappone, C., Mecarocci, V., Manguo, F., Ciconte, G., Vicedomini, G., Sturla, F., et al. (2019). New electromechanical substrate abnormalities in high-risk patients with Brugada syndrome. Heart Rhythm. 17:637–645. doi: 10.1016/j.hrthm.2019.11.019

Pappone, C., Micaglio, E., Locati, E. T., and Monasky, M. M. (2020a). The omics of channelopathies and cardiomyopathies: what we know and how they are useful. Eur. Heart J. Suppl. 22, L105–L109. doi: 10.1093/eurheartj/suaa146

Pappone, C., Monasky, M. M., Micaglio, E., and Ciconte, G. (2020b). Right ventricular electromechanical abnormalities in Brugada syndrome: is this a channelopathy? Eur. Heart J. Suppl. 22, E101–E104. doi: 10.1093/eurheartj/suaa071

Varian, K. D., and Janssen, P. M. (2007). Frequency-dependent acceleration of relaxation involves decreased myofilament calcium sensitivity. Am. J. Physiol. Heart Circ. Physiol. 292, H2212–2219. doi: 10.1152/ajpheart.00778.2006

Wijeyaratne, Y. D., Tanck, M. W., Mizusawa, Y., Batchvarov, V., Barc, J., Crotti, L., et al. (2020). SCN5A mutation type and a genetic risk score associate variably with brugada syndrome phenotype in SCN5A families. Circ. Genom. Precis. Med. 13, e002911. doi: 10.1161/CIRCGEN.120.002911

Wilde, A.A.M., and Gollob, M.H. (2019). 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 139, 1760–1761. doi: 10.1161/CIRCULATIONAHA.119.039065

Yang, Q., Hewett, T. E., Klevisky, R., Sanbe, A., Wang, X., and Robbins, J. (2001). PKA-dependent phosphorylation of cardiac myosin binding protein C in transgenic mice. Cardiovasc. Res. 51, 80–88. doi: 10.1016/S0008-6363(01)00273-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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