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EDITORIAL COMMENT

CRISPRed Cardiomyocytes to Decrypt Variants of Uncertain Significance*

Jean-Sébastien Hulot, MD, PhD, Eva Vermersch, PhD, Charlène Jouve, BSc

Clinical genetic testing is the standard of care for many diseases, including inherited cardiovascular conditions and the advent of high-throughput next-generation sequencing is leading to ever-increasing reports of known and novel genetic variants in tested patients. As a consequence, this progressive shift in genetic testing comes with key challenges in sequence interpretation. Different standards, guidelines, and online tools (1) have been developed for the interpretation of sequence variants and are typically using genetic, knowledge-based, and computational criteria to help classifying variants. The standard terminology describes variants as pathogenic/likely pathogenic versus benign/likely benign, but there is a challenging category where the significance of identified variants remains uncertain (so-called variants of uncertain significance [VUS] for variants of uncertain/unknown significance) (2). The discovery of multiple VUS for an individual patient has now become a routine part of clinical practice. This problem is particularly prevalent for cardiovascular genetic disorders with strong allelic heterogeneity and/or a preponderance of "private" mutations. A typical example is the long QT syndrome (LQTS), a rare congenital disorder, with an estimated prevalence of 1 in 2,000 individuals, that is characterized by a delayed repolarization of the heart and a consequent high risk of sudden death from cardiac arrhythmias. To date, 15 genes have been identified and are associated with different types of LQTS. Despite significant advances in the management of LQTS based on an improved understanding of implicated genes and underlying ion currents, the care of almost one-third of LQTS patients is often challenged by unyielding clinical genetic testing due to identification of VUS (3). The Expert Consensus Statement (4) on management of inherited arrhythmias requires the presence of an unequivocally pathogenic mutation in LQTS susceptibility genes as a Class I criterion for LQTS diagnosis.

During this last decade, 2 technologies have emerged as promising tools for the prediction of VUS pathogenicity. The increasingly refined capacity to differentiate human induced pluripotent stem cells (hiPSCs) into disease-relevant cell types, such as cardiomyocytes (iPSC-CMs), has offered new opportunities to model cardiomyopathies and to test effects of drugs (5). Of importance, at least 5 LQTS types have been reproduced using patient-specific iPSCs (6). In contrast, the rise of genome editing technologies based on programmable nucleases have greatly improved our ability to make precise modifications in the genomes of eukaryotic cells, including hiPSCs (5). Specifically, programmable nucleases enable precise genome editing by creating DNA double-strand breaks at specific genomic loci. In the presence of a repair template, the DNA lesion will be repaired through homology-directed repair. Consequently, the DNA sequence can be changed at a targeted position, thus allowing mutation correction or insertion. This latter point is of importance because the correction of a VUS in hiPSCs will generate a corrected cell line with an isogenic genetic background for comparison. Reciprocally, the same approach can be performed to introduce the studied VUS in a healthy control hiPSC line and analyze its pathogenicity in a neutral genetic background.

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In this issue of the *Journal*, Garg et al. (7) provide the first report of integrating hiPSC-CMs and CRISPR genome editing technologies as tools to examine the pathogenicity of VUS in LQTS. The patient is a 39-year-old man with a diagnosis of symptomatic

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From INSERM, UMR_S 970, Paris Cardiovascular Research Center (PARCC), Paris, France. The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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LQTS but borderline QT prolongation or symptoms in the relatives. The genetic testing found a p.Thr983Ile (c.2948 C>T) variant in the *KCNH2* gene, a rare variant classified as a VUS based on available data (1,7). Using the CRISPR/Cas9 genome editing approach, the homozygous variant T983I was introduced in a healthy hiPSC control line and then corrected in the patient hiPSC line, followed by examination of the electrophysiological properties of genome-edited lines. The introduction of the VUS led to characteristic LQTS phenotype with significantly prolonged action potential duration and generation of baseline arrhythmias, such as early afterdepolarizations. Reciprocally, the correction of the VUS in the patient cell line normalized the LQTS phenotype, confirming a causative role of the underlying *KCNH2* VUS in the patient. Single cell voltage-clamp recordings further confirmed that the VUS is associated with a significant decrease in iKr tail current density and biochemical investigations showed that the underlying mechanism of the variant pathogenicity is reduced total hERG protein and deficiency of mature channel trafficking to the cell surface.

This study illustrates how iPSC and genome editing technologies provide unique opportunities to elucidate the contribution of genetics to cardiovascular diseases, by allowing the unprecedented and fast creation of appropriate cellular models of pathological processes. Previous studies have shown the ability of iPSC to model LQTS caused by pathogenic variants with major effects (6). This study further demonstrates that hiPSC can also be an appropriate platform to detect a less severe phenotype, as anticipated with VUS. Recent studies have similarly shown that iPSC can recapitulate phenotypes associated with a predisposing genetic background, such as drug-induced long QT (8). In comparison with previous LQTS model studies, the T983I variant was considered as driving a moderate arrhythmogenicity, but a direct comparison with other *KCNH2* pathogenic variants was not performed. Whether the reported strategy could not only serve to predict the pathogenicity of a given VUS but also to develop a comparative catalogue of VUS would represent an important future step. Phenotypic investigations of different VUS inserted in a control hiPSC (i.e., a neutral genetic background) using large-scale genome editing technology could indeed provide useful comparative data and be used as a first guidance for risk prediction.

Finally, this study represents an important new step in the field of precision medicine. The incorporation of results into the clinical management of patients was not an endpoint of this study, but it is obvious that methods to predict a priori whether a given VUS predisposes an individual to a life-threatening risk are critically needed to better guide anti-arrhythmic therapies in these patients. Inherited cardiac arrhythmias and chanelopathies represent an ideal area to test and develop these methods as it would have important consequences on appropriate clinical management (i.e., indications of implantable cardioverter-defibrillator or long-term pharmacological therapies). In addition, this cellular platform can serve to predict drug response at the individual level. The response to high-risk torsadogenic drugs (as defined by a Comprehensive in Vitro Proarrhythmia Assay [9]) first help predicting the arrhythmogenic risk. The cellular platform can also be used to test innovative drugs, as was tested in this study with a recently discovered hERG channel activator that normalized action potential duration in hiPSC-CM carrying the VUS (7). Similarly, it was recently shown on iPSC-CMs derived from LQT2 patients not protected by β-blockers that lumacaftor, a drug already in clinical use for cystic fibrosis, can be a novel therapeutic option to rescue pathological phenotype (10). And this is just the beginning of the "CRISPRed" revolution!

ADDRESS FOR CORRESPONDENCE: Dr. Jean-Sébastien Hulot, Paris Cardiovascular Research Center (PARCC), 56 Rue Leblanc 75015 Paris, France. E-mail: jean-sebastien.hulot@aphp.fr. Twitter: @parcc_inserm.

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