KCNJ2 encodes the pore-forming α-subunit of the Kir2.1 inward-rectifier potassium channel, the main determinant of the current (I_K1), which is responsible for the final repolarization phase of the ventricular action potential, as well as a stable resting membrane potential in the working myocardium. Variants in the KCNJ2 gene have been associated with familial atrial fibrillation (gain of function), short-QT syndrome (gain of function), catecholaminergic polymorphic ventricular tachycardia; loss of function), as well as the Andersen–Tawil syndrome (ATS; loss of function). ATs is a rare hereditary multisystem disorder leading to periodic paralysis, dysmorphic features, and ventricular arrhythmias. Penetration of the disease is extremely variable, and not all patients present with the full triad of symptoms. Regarding the cardiac phenotype, these patients present with frequent polymorphic ventricular contractions and bidirectional ventricular tachycardia. Typically, these arrhythmias are present at rest and disappear during exercise. This is an important differentiation from catecholaminergic polymorphic ventricular tachycardia, which also presents with bidirectional tachycardia, but typically during catecholaminergic stimulation or exercise. ATS patients frequently show prominent U waves in the ECG, and some also show prolongation of the corrected QT interval. Although QT prolongation is not a typical sign, ATS was classified as LQTS-subtype (LQT7). Using a conventional human embryonic kidney cell model, Gélinas et al could show that coexpression of Kir2.1-G52 with Kir2.1-52V resulted in a >80% reduction of I_K1 current density, indicating a dominant-negative effect of the G52V variant, which is probably because of a defect in channel maturation or trafficking. In a parallel approach, they analyzed the usability of commercially available hiPS-CM to study the functional effects of the G52V variant. Human pluripotent stem cells can be derived either from the inner cell mass of blastocysts or from adult somatic cells by manipulation of the epigenetic state of the cells (induced pluripotent stem cells). Both types of cells proliferate indefinitely in the undifferentiated state and can be induced to differentiate into specific terminal cell types, for example, cardiomyocytes (ie, hiPS-CMs), under appropriate culture conditions. hiPS-CM models have already been extensively used for basic research, cardiotoxicity assessment, as well as assessment of patient- or mutation-specific disease mechanisms and treatment. Several studies could already recapitulate cellular disease phenotypes, as well as pharmacological rescue of different congenital channelopathies in hiPS-CM models. Novel techniques for efficient genetical modification (eg, CRISPR-Cas9) further boosts the potential of the hiPS-CM model for future research. However, differentiating between (1) truly monogenic disease causing mutations, (2) variants increasing arrhythmia susceptibility with additional repolarization prolonging insults (eg, drugs that influence repolarizing currents), (3) variants influencing phenotype together with additional variants (allelic heterogeneity, compound variants), and (4) truly innocent bystanders is one of the most challenging tasks in clinical genetic research and needs to be done with great caution. Erroneous misclassification can have serious consequences for the patients and their family members. Ackermann introduced the term genetic purgatory for this situation. Just recently, for example, a consortium

See Article by Gélinas and El Khoury et al

In this issue of Circulation: Cardiovascular Genetics, Gélinas et al describe their approach to identify a putative disease-causing variant in the KCNJ2 gene and support the use of commercially available human pluripotent stem cell–derived cardiomyocytes (hiPS-CMs) in studying the functional effects of this variant. Their index patient presented at the age of 13 years with asymptomatic polymorphic ventricular contractions and frequent episodes of nonsustained ventricular tachycardia, which disappeared during exercise. Although the QT interval was normal, prominent U waves were present. Because the patient was asymptomatic at that time, and there was no sign of structural heart disease in the echocardiogram, she received no treatment until the age of 29 years, when she suffered a syncope attributed to ventricular arrhythmias. She received a 2-chamber implantable cardioverter-defibrillator and treatment with β-blockers.

After negative genetic testing of the 5 most frequently implicated LQTS genes (KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2), whole exome screening was performed. This screening identified a putative causal mutation (Gly52Val) in the KCNJ2 gene.

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of large genetic research groups has questioned many—if not all—to date described KCNE2 variants: instead of being truly monogenic disease causing mutations, they rather seem to be proarrhythmic variants needing additional repolarization prolonging impacts to cause QT prolongation.18

Therefore, for the approaches using hiPS-CM, some challenges and considerations remain for a wider clinical application. First, hiPS-CM exhibit a relatively immature phenotype. While Roux et al nicely demonstrate in their hiPS-CM line the time-dependent expression (at mRNA level) of channel-related genes typically found in differentiated cardiomyocytes, the action potentials in hiPS (as shown) have a morphology that is different from human ventricular cardiomyocytes (eg, less of a typical plateau phase II). This may be explained by not all action potential–forming channels being expressed in these hiPS-CM (at least at the differentiation stage investigated). Also, despite their detectable expression, ion channels in hiPS-CM and those in human-differentiated cardiomyocytes may differ in function, as has been discussed in another hiPS-CM model for LQTS.20 Besides other structural and electrophysiological differences, especially, the Ik1 density of hiPS-CM is almost absent, compared with adult human cardiomyocytes.21 Different groups have already tried to artificially enhance Ik1 density in hiPS-CM, for example, by adenoviral transduction22 or by injection of an in silico Ik123 to study the effects of KCNJ2 mutations. Consistently with these previous studies, Gélinas et al24 could show that there was initially no Ik1 current detected in their hiPS-CM model. Interestingly, and probably the most important finding of this study, after 14 days of electric stimulation from day 7 post-thaw, there was a robust increase of Kir2.1 plasma membrane expression, a typical Ik1 current, as well as a physiologically resting membrane potential in their hiPS-CM model. Thereafter, after transient transfection, they could recapitulate the functional effects of the G52V variant seen in the heterologous expression model in the commercially available hiPS-CM line. Second, the action potentials in the adult differentiated heart is highly regulated, and loss-of-function of an ion channel may be compensated by (eg, posttranscriptional) regulation of other channels to preserve repolarization capacity.24 Functional testing in a hiPS-CM is a step forward (toward integration) from the often used, more reductional approach of heterologous expression in the human embryonic kidney cell model. However, for the reasons given above, translation of putative disease-causing gene variations from experimental findings into the clinic should be done with caution.

Third, genetic variants may reveal their pathogeneity only in the individual context of the patient’s history. Acquired conditions, such as age, systemic comorbidities, and cardiac remodeling, may result in chronic post-transcriptional changes to the gene product (eg, channel) or its cellular compartment that are not recapitulated in hiPS-CM (even if derived from the same patient). As a result, the effects of a putative causative gene expressed in commercially available (healthy) hiPS-CM may be misclassified if it not properly reflects the observed phenotype.

Another aspect is that if hiPS-CMs are taken from a diseased patient to evaluate underlying pathomechanisms, they harbor all genetic variants of the patient, not only the putative disease-causing gene. For example, around 5% of LQTS patients are carriers of >1 LQTS-associated variant,25 which has an important influence on disease expression and severity of the cardiac phenotype. A functional effect seen in these cells attributed to a certain genetic variant might only cause these effects in the specific genetic context of this patient and might cause no or a less severe phenotype in another genetic context. This is of special importance because in most cases, the patients from whom the cells are obtained present with the most severe phenotype. In line with this, Krych et al,26 as well as Jagodzińska et al27 expressed the assumption that the severity of the cardiac phenotype of ATS might also depend on other genetic polymorphisms, such as the K897T polymorphism in the KCNH2 (herG1) gene. Thus, it seems necessary to validate the functional relevance of a putative disease-forming mutation in (eg, commercially available) hiPS-CM with different genetic background.

In summary, with improving methods for stable differentiation toward adult cardiomyocytes commercially available, hiPS-CMs become an increasingly valuable additional tool in our armamentarium to study the genetic mechanisms of channelopathies. Their future role in clinical application also depends on the careful interpretation of experimental results in the integrative context of the patient’s phenotype.

Disclosures
None.

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