Down the rabbit hole: deciphering the short QT syndrome

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This editorial refers to ‘Transgenic short-QT syndrome 1 rabbits mimic the human disease phenotype with QT/ action potential duration shortening in the atria and ventricles and increased ventricular tachycardia/ventricular fibrillation inducibility’, by K.E. Odening et al., doi:10.1093/eurheartj/ehy761.

The short QT syndrome (SQTS) is a relatively new player in the field of inherited arrhythmia syndromes. The syndrome was first described in the early 2000s, with affected individuals presenting with atrial fibrillation (AF), a short QT interval, and increased risk for sudden cardiac death (SCD).1 While clinical presentation was found to be heterogeneous, common features among SQTS patients include poor rate adaptation of the QT interval during exercise, shortened atrial and ventricular refractory periods, and increased susceptibility to atrial and ventricular arrhythmias.1–3 SQTS type 1 (SQT1) is associated with mutations in the human ether-a-go-go-related gene (HERG; also named KCNH2) underlying the a-subunit of the rapid component of the delayed rectifier potassium current (IKr). The first identified SQT1 mutation, HERG-N588K, was shown in heterologous expression systems to cause a mixed phenotype with, on the one hand, IKr ‘loss-of-function’ alterations and, on the other hand, an increased IKr density during depolarizing pulses and ventricular action potential (AP) waveforms.4–6 In sum, the overall effect of the mutation is thus a ‘gain of function’ of IKr, which in computer simulation studies was indeed predicted to accelerate repolarization, shorten refractory periods, and enhance susceptibility to re-entrant arrhythmias in atrial and ventricular preparations.7,8

While highly informative, studies employing expression systems and computer simulations do not fully recapitulate the cardiomyocyte and whole heart environment. This was partly overcome by the recent generation of human induced pluripotent stem cell-derived cardiomyocytes (hiPCS-CMs) from a HERG-N588K mutation carrier, which confirmed the mutation-induced increased IKr, and AP shortening.9,10 Two-dimensional sheets of N588K hiPSC-CMs further recapitulated the impaired rate adaptation of AP duration and increased susceptibility for arrhythmias.10 Despite these observations, modulators of SQTS disease severity and expressivity are as yet unknown, and arrhythmia risk prediction is elusive. Hence, studies linking electrophysiological alterations at the level of the cardiomyocyte, the whole heart, and the intact animal are required in order to gain essential mechanistic insight. A pharmacologically induced SQTS model was previously successfully employed,11 yet is fundamentally different from the situation in patients who carry the genetic defect from birth. Zebrafish offer the possibility to study genetic alterations in a short period of time, but essential anatomical and functional differences may limit transferability of the results to humans. Mouse models are most often used for genetic studies; however, their repolarizing currents are very different from those of humans, limiting their use for studying genetic alterations in, for example, potassium channels. In addition, the ‘gain-of-function’ effect of N588K was found to be absent during Purkinje AP waveforms which typically have a fast repolarization phase-1 and low plateau amplitude.5 Hence, mice carrying the N588K mutation are likely to have only a mild phenotype (if any), since the murine AP morphology is more Purkinje like. Clearly, generation of a different animal model is required to overcome these limitations.

In their study in the current issue of European Heart Journal, Odening and colleagues present the generation and first assessment in vivo and ex vivo features to human mutation carriers, including short QT and T wave alterations, reduced QT adaptation to heart rate, shortened atrial and ventricular refractory periods, and increased atrial and ventricular arrhythmia susceptibility.12 Quinidine, which was shown to correct the QT interval and effectively suppress ventricular arrhythmias in SQT1 patients,4,13 reversed AP and QT shortening in N588K rabbits, substantiating its anti-arrhythmic potential. Cellular measurements demonstrated that N588K rabbits reproduce the augmented IKr amplitude and AP...
shortening previously seen in cell expression systems, computer simulations, and hiPSC-CMs. The study thus provides conclusive evidence that the HERG-N588K mutation is causally linked to QT interval shortening and increased tissue arrhythmia susceptibility in SQT1 (Take home figure). As with all experimental models, however, the use of transgenic rabbits also carries disadvantages and limitations. Due to their relatively long breeding cycle, generation of mutant offspring is slow and costly. As a consequence, group sizes are sometimes small, which in the case of the N588K rabbits probably prevented assessment of spontaneous arrhythmia incidence. More importantly, in contrast to SQT1 patients who carry one N588K allele and one wild-type allele, N588K rabbits have an overexpression of human mutant channels on top of their endogenous HERG channels, with potential consequences for channel stoichiometry. Since a control line of rabbits overexpressing human wild-type HERG was not studied, it remains unknown to what extent (some of) the observed alterations are due to the mutation or secondary to the overexpression model. This may be of particular relevance when exploring in more detail (long-term) remodelling and its impact on arrhythmogenesis (see below).

Apart from confirming the causal role of Ikr ‘gain of function’ in SQT1, what mechanistic insight has been obtained so far from N588K rabbits? The co-occurrence of SQTS and AF can be readily explained by the reduced AP duration and the resulting stability of wavelets in the atria. In relation to ventricular arrhythmogenesis, transmural dispersion in repolarization has been shown to be significantly higher in SQTS patients than in normal controls. In line with this, both transmural dispersion in repolarization and Tpeak–Tend intervals were reported to be prolonged in dogs with pharmacologically induced SQT1. Moreover, intact tissue computer simulations demonstrated that decreased pseudo QT intervals, increased T wave amplitude, and increased tissue vulnerability for unidirectional conduction block and formation of re-entrant excitation waves only occurred if a heterogeneous transmural Ikr density was incorporated into the model. While transmural repolarization patterns were not assessed in N588K rabbit hearts, the monophasic AP data indicate an increased regional dispersion in repolarization. This could be in line with the observation that Tpeak–Tend, which is increased in SQT1 patients and rabbits, may in fact reflect repolarization dispersion in the entire heart. While this suggests a potential role for enhanced regional heterogeneity in both SQT1-related T wave alterations and arrhythmogenicity, these observations require further in-depth analysis of regional vs. transmural repolarization patterns in N588K rabbits.

Another finding of potential relevance for arrhythmogenesis is the observation of electromechanical remodelling in N588K rabbit hearts. Apart from changes in Ikr, the authors also report a decrease in inward rectifier potassium current (Ikr) and L-type calcium current (ICa,L), and an increased slow delayed rectifier potassium current (IKs). These findings are in contrast to previous observations in hiPSC-CMs, which could (in part) be explained by species- and model-specific differences. To date, evidence is increasing that a particular type of ion channel may have synergistic effects on other ion channels (see Potero et al., and primary references cited therein). For example, wild-type HERG expression may affect Iks function, and this is even more pronounced with HERG mutations. While the ICa,L decrease and Ikr increase may contribute to the shorter QT and action potentials, the Ikr decrease may partially reverse the SQT1

**Take home figure** Transgenic rabbits with N588K-HERG are a valuable model in short QT type 1 syndrome studies.
phenotype. However, it must be noted that the observed $i_{K1}$ decrease and change in $i_{K1}$ reversal potential were not associated with alterations in resting membrane potential, making potential effects on AP repolarization less likely. The authors also report increased mechanical dispersion and alterations in diastolic relaxation in NS88K rabbits (in the absence of cardiac fibrosis), demonstrating the complexities of ion channel dysfunction on the whole-heart level. Such mechanical remodelling could impact on arrhythmogenesis, and one may even speculate that progressive remodelling with age may change the arrhythmogenic substrate and/or mechanism. The $i_{Ca,L}$ reduction was indeed most pronounced in older rabbits, suggesting progressive ionic remodelling, but age-dependent effects on SQT1 phenotype, $i_{K1}$, $i_{Kr}$, and $i_{ks}$ were not evaluated. Without assessment of calcium homeostasis, the relevance of the observed changes in $i_{Ca,L}$, but also the cause of mechanical (dys)function in NS88K rabbits remains unclear. Moreover, it is as yet unknown to what extent the apparent electrical and mechanical remodelling is due to the NS88K mutation, or merely consequent to the use of the overexpression model. Hence, further research is required to explore (age-dependent) remodelling, ideally using a rabbit model generated with the use of CRISPR/Cas9 technology to overcome the potential limitation of the currently used overexpression model.

In conclusion, the newly developed HERG-NS88K rabbit model nicely recapitulates the clinical phenotype of SQT1 patients and provides a comprehensive explanation for the observed repolarization shortening and increased arrhythmia susceptibility. Future studies employing this model may further decipher complex disease mechanisms and arrhythmia triggers in SQT1 (Take home figure). In addition, the model will facilitate identification of factors modifying SQT1 disease severity and expressivity, including age, gender, environmental factors, and co-morbidities.

Funding
C.A.R. is supported by an Innovative Research Incentives Scheme Vidi grant (91714371) from the Netherlands Organisation for Health Research and Development (ZonMW) and by the Netherlands Cardiovascular Research Initiative, an initiative supported by the Dutch Heart Foundation (CVON2015-12 e-DETECT).

Conflict of interest: none declared.

References