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TRANSLATIONAL SCIENCE
Arrhythmias

KCNQ1 suppression-replacement gene therapy in transgenic rabbits with type 1 long QT syndrome

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Abstract

Background and Aims Type 1 long QT syndrome (LQT1) is caused by pathogenic variants in the *KCNQ1*-encoded $K_v7.1$ potassium channels, which pathologically prolong ventricular action potential duration (APD). Herein, the pathologic phenotype in transgenic LQT1 rabbits is rescued using a novel *KCNQ1* suppression-replacement (SupRep) gene therapy.

Methods *KCNQ1*-SupRep gene therapy was developed by combining into a single construct a *KCNQ1* shRNA (suppression) and an shRNA-immune *KCNQ1* cDNA (replacement), packaged into adeno-associated virus serotype 9, and delivered *in vivo* via an intra-aortic root injection (1E10 vg/kg). To ascertain the efficacy of SupRep, 12-lead electrocardiograms were assessed in adult LQT1 and wild-type (WT) rabbits and patch-clamp experiments were performed on isolated ventricular cardiomyocytes.

Results *KCNQ1*-SupRep treatment of LQT1 rabbits resulted in significant shortening of the pathologically prolonged QT index (QTi) towards WT levels. Ventricular cardiomyocytes isolated from treated LQT1 rabbits demonstrated pronounced shortening of APD compared to LQT1 controls, leading to levels similar to WT (LQT1-UT vs. LQT1-SupRep, $P < .0001$, LQT1-SupRep vs. WT, $P = \text{ns}$). Under β-adrenergic stimulation with isoproterenol, SupRep-treated rabbits demonstrated a WT-like physiological QTi and APD_{90} behaviour.

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Conclusions This study provides the first animal-model, proof-of-concept gene therapy for correction of LQT1. In LQT1 rabbits, treatment with *KCNQ1*-SupRep gene therapy normalized the clinical QTi and cellular APD_{90} to near WT levels both at baseline and after isoproterenol. If similar QT/APD correction can be achieved with intravenous administration of *KCNQ1*-SupRep gene therapy in LQT1 rabbits, these encouraging data should compel continued development of this gene therapy for patients with LQT1.

Structured Graphical Abstract

Key Question

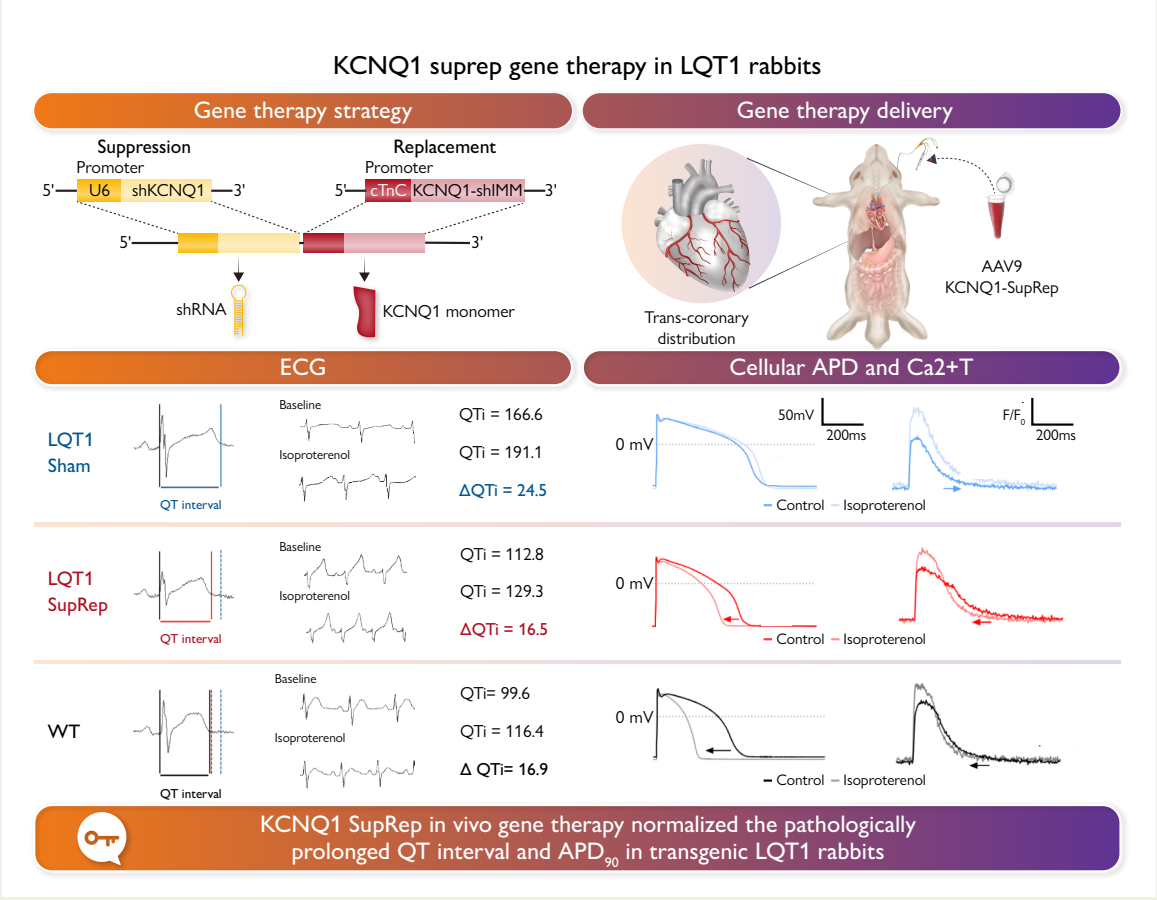
Can AAV9-mediated KCNQ1 suppression-replacement (SupRep) gene therapy restore physiological KCNQ1 function and rescue the diseased phenotype in transgenic LQT1 rabbits?

Key Finding

Treatment of LQT1 rabbits with AAV9-KCNQ1-SupRep gene therapy, delivered via targeted intra-aortic root injections, resulted in significant shortening of the pathologically prolonged QTi and cellular APD_{90} towards those observed in wild type (WT) rabbits. SupRep treated LQT1 rabbits demonstrated a physiological behavior under β-adrenergic stimulation.

Take Home Message

In vivo KCNQ1-SupRep gene therapy rescues the physiological QTi and APD_{90} to those observed in WT rabbits both at baseline and after provocation with isoproterenol. This translational study might impact future LQT1 treatment.



KCNQ1 suppression-replacement (SupRep) gene therapy was developed by combining into a single construct a *KCNQ1* shRNA (suppression) and an shRNA-immune *KCNQ1* cDNA (replacement). This hybrid SupRep gene therapy was packaged into adeno-associated virus serotype 9 (AAV9) and delivered *in vivo* via intra-aortic root injections (1E10 vg/kg). To ascertain the efficacy of SupRep, 12-lead electrocardiograms (ECGs) were assessed in adult type 1 long QT syndrome (LQT1) and wild-type (WT) rabbits. Patch-clamp and calcium transient experiments were performed on isolated ventricular cardiomyocytes (VCMs), both at baseline and under β-adrenergic stimulation with isoproterenol. In LQT1 transgenic rabbits, treatment with *KCNQ1*-SupRep resulted in significant shortening of the pathologically prolonged QT interval, QT index, and cellular action potential

duration (APD) and Ca^{2+}T towards WT levels. Furthermore, unlike sham-treated LQT1 rabbits, SupRep-treated LQT1 rabbits demonstrated a physiological behaviour under β-adrenergic stimulation.

Keywords AAV9 • Gene therapy • KCNQ1 • Long QT syndrome • Transgenic LQT1 rabbits

Translational perspective

This study represents the first ever gene therapy in an animal model for the treatment of type 1 long QT syndrome (LQT1). *KCNQ1* suppression-replacement gene therapy rescues the pathologically prolonged QT index and cellular action potential duration in transgenic LQT1 rabbits both at baseline and under β-adrenergic stimulation with isoproterenol. This novel gene therapy will be applicable to all patients with LQT1 regardless of which variant they possess because it targets the whole *KCNQ1* gene rather than specific variants. This proof-of-concept study encourages continued development of this gene therapy towards a first-in-human clinical trial.

Introduction

Congenital long QT syndrome (LQTS) is an arrhythmogenic heart disease that is characterized by a prolonged QT interval on a 12-lead electrocardiogram (ECG). Patients with LQTS are at an increased risk for syncope, seizure, or sudden cardiac death (SCD) stemming from LQT1-triggered episodes of torsades de pointes (TdP). The prevalence of LQTS is ∼1 in 2000 individuals and when untreated, the highest risk subset of LQTS patients have an estimated 10-year mortality of 50%.[1,2]

Long QT syndrome is caused by autosomal dominant pathogenic variants in genes encoding various cardiac ion channels that lead to prolongation of cardiac repolarization (QT duration on ECG). The most prevalent form is type 1 LQTS (LQT1) which is caused by pathogenic loss-of-function variants in the *KCNQ1*-encoded I_{Ks} (K_v7.1) potassium channel, causing a reduction or complete loss of cardiac I_{Ks}.[3] The K_v7.1 channel is comprised of four *KCNQ1*-encoded α-subunits which tetramerize during channel assembly. This tetramerization is critical for LQT1 pathobiology since incorporation into the tetramer of even a single subunit translated from the disease-causative allele can interfere with the normal subunits, rendering the channel dysfunctional. To date, over 600 unique LQT1-causative variants in *KCNQ1* have been reported. The majority are missense variants which most commonly exhibit a dominant-negative effect on the normal allele.[4–10]

Current symptom-directed therapeutic strategies aimed at reducing arrhythmia triggering events include lifestyle modifications such as avoidance of competitive sports and certain QT-prolonging medications, use of β-adrenergic receptor blockers, and left cardiac sympathetic denervation which surgically reduces localized norepinephrine release to mitigate pro-arrhythmic sympathetic triggers.[11] Furthermore, SCD may be aborted by an implantable cardioverter defibrillator which terminates arrhythmias once they have started.[12] However, current treatment strategies have many side-effects which impact the patients' quality of life and do not address the underlying molecular cause (i.e. dysfunction of ion channels) of LQTS.[1,12–14] Therefore, there remains a crucial unmet need for novel, more efficacious, mechanism-driven therapies to treat congenital LQTS.

To overcome the limitations of current therapies, we discovered a novel hybrid suppression-and-replacement (SupRep) gene therapy for the treatment of LQTS types 1 and 2.[15,16] Specifically, for LQT1, a dual component SupRep gene therapy was created by combining into a single construct a custom-designed *KCNQ1* shRNA (shKCNQ1) that targets human *KCNQ1* and a shRNA-immune *KCNQ1* cDNA (KCNQ1-shIMM). The suppression arm of this gene therapy, shKCNQ1, binds a portion of human *KCNQ1* devoid of any known LQT1-causative variants, thus allowing this strategy to be applicable for virtually every patient with LQT1 regardless of the disease-causing variant. Previously, we demonstrated the efficacy of *KCNQ1*-SupRep gene therapy in shortening the pathologically prolonged action potential duration (APD) in patient-specific, induced pluripotent stem cell-derived cardiomyocyte (iPSC-CM) models of LQT1.[15]

Herein, we demonstrate the efficacy of *in vivo KCNQ1*-SupRep gene therapy in alleviating the pathogenic phenotype in adult transgenic LQT1 (KCNQ1-Y315S) rabbits.[17] These transgenic rabbits are particularly well-suited for examining the impact of gene therapy at the whole animal, cardiac, and cellular levels[17] as rabbits share pronounced similarities with humans in terms of the repolarizing potassium channels and action potential characteristics in general,[18] and as LQT1 rabbits mimic the human LQT1 phenotype on all levels.[17,19–21]

Methods

Animal studies

Adult transgenic LQT1 and wild-type (WT) New Zealand White rabbits (3–4 kg) of both sexes were used. LQT1 rabbits overexpress the human dominant-negative pore mutation KCNQ1-Y315S under the control of the rabbit β-myosin heavy chain promoter and mimic the human phenotype with loss of I_{Ks} and prolonged APD and QT duration.[17] All animal experiments were performed in compliance with European Union (EU) legislation (directive 2010/63/EU) and the Swiss Animal Welfare Ordinance, after approval by the Cantonal Veterinary Office and the Animal Welfare Officer (Kanton Bern, approval number BE55–21).

Generation of the AAV9-KCNQ1-SupRep gene therapy construct

The KCNQ1-SupRep gene therapy construct was developed as described previously.[15] *Figure 1* provides a schematic representation of the KCNQ1-SupRep gene therapy construct design. This SupRep gene therapy was created by combining into a single construct a custom-designed KCNQ1-shRNA (suppression) and a shRNA-immune (shIMM) KCNQ1-cDNA (replacement) which was packaged into AAV9.

Briefly, to generate the KCNQ1-SupRep vector, shRNAs were custom designed and assessed using ClinVar and the Genome Aggregation Database (gnomAD) and were devoid of common genetic polymorphisms and known pathogenic/likely pathogenic variants in *KCNQ1* that could impact the knockdown efficiency. However, given that the human and rabbit *KCNQ1* do not share 100% nucleotide identity, we were unable to find a stretch of 25–29

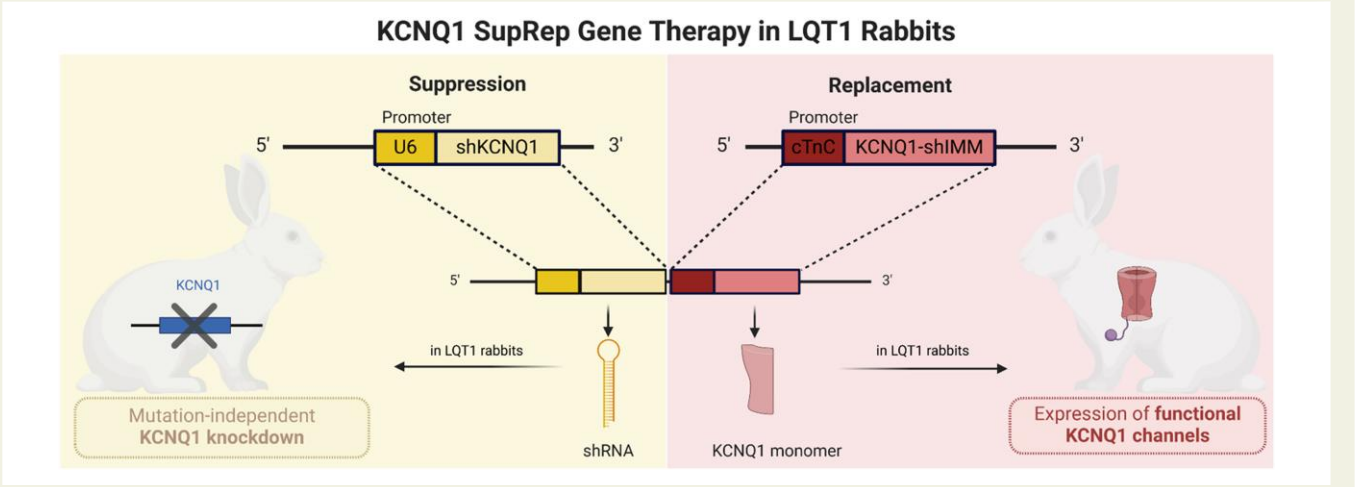


Figure 1 KCNQ1 suppression-and-replacement gene therapy in LQT1 rabbits. *KCNQ1*-SupRep gene therapy consists of two therapeutic components expressed from a single viral vector. The suppression component (depicted in yellow in the left panel) utilizes a U6 promoter element driven KCNQ1 shRNA (shKCNQ1) to silence the expression of *KCNQ1* transcripts. This approach targets the gene itself rather than specific variants. Simultaneously, the replacement component (in red in the right panel) of *KCNQ1*-SupRep restores the lost transcripts by expressing a 'shRNA-immune' (shIMM) version of the KCNQ1 cDNA, featuring synonymous variants at each wobble base within the shRNA target site. Importantly, this does not alter the WT amino acid sequence but prevents shRNA binding, making KCNQ1-shIMM resistant to knockdown. When expressed in tandem, suppression-replacement results in the simultaneous suppression of *KCNQ1* and the replacement with KCNQ1-shIMM. The outcome is an increase in functional *KCNQ1*-encoded $K_v7.1$ potassium channels, rescuing the LQT1 phenotype in transgenic rabbits arising from KCNQ1 loss-of-function. Figure created using Biorender.com

nucleotides within this region with 100% identity to the rabbit *KCNQ1*. As such, we proceeded with the intentionally designed and chosen shRNA—henceforth referred to as shKCNQ1—(5′-GTTCAAGCTGGACAAAGA CAATGGGGTGA-3′) despite its nucleotide mismatches (see Supplementary data online, *Figure S1*). To create the replacement 'shKCNQ1-immune' cDNA copy of *KCNQ1*, called KCNQ1-shIMM, 10 synonymous nucleotide variants were introduced into the wobble base of each codon within the shKCNQ1 target sequence (nucleotides c.1266–1294) to create the final shIMM sequence (5′-ATTTAAACTCGATAAGGATAACGGCGTCA-3′). Expression of the KCNQ1-shIMM cDNA was driven by cardiac troponin C (cTnC) promoter[22] to achieve cardiac specificity. The KCNQ1-shIMM cDNA enables production of a WT amino acid sequence which is impervious to knockdown by the shRNA. The generated KCNQ1-SupRep plasmid was packaged into adeno-associated virus serotype 9 (AAV9), referred to as AAV9-KCNQ1-SupRep.

Testing the efficacy of AAV9-KCNQ1-SupRep gene therapy in transgenic LQT1 rabbits

AAV9-KCNQ1-SupRep was delivered *in vivo* via an intra-aortic root injection (1E10 vg/kg) during balloon occlusion of the proximal aorta through a Swan–Ganz catheter. Twelve-lead ECGs were assessed in adult transgenic LQT1 and WT rabbits to ascertain the efficacy of SupRep on the rabbit's heart rate-corrected QT index (QTi) before and 2–3 weeks after therapy at baseline and during isoproterenol (ISO) challenge. In rabbits, a linear QT/RR relation is noted at physiological heart rates. To ascertain the effect of SupRep on the rabbit's QT duration, we calculated the heart rate-corrected QT index (%, QTi, $\frac{QT_{measured} \times 100}{QT_{expected}}$ with $QT_{expected}$ calculated as $QT_{exp} = 86 + 0.22 * RR$) as described previously.[17] Patch clamp and IonOptix was performed on isolated ventricular cardiomyocytes (VCMs) to evaluate the efficacy of SupRep on the VCM's APD at 90% repolarization (APD_{90}) and calcium transient duration at baseline and after ISO.

β-Adrenergic stimulation with isoproterenol

Clinically, in patients with LQT1, activation of the sympathetic nervous system during exercise and/or stress can trigger arrhythmias.[23] β-Adrenergic stimulation, mediated by PKA phosphorylation of $K_v7.1$, enhances repolarizing current I_{Ks} and shortens the APD.[24] In LQT1, however, this sympathetic activation of I_{Ks} is impaired, leading to a paradoxical prolongation (or lack of shortening) of the APD/QT during catecholamine infusion or exercise test, which is pathognomonic in patients with LQT1.[25] Because this phenomenon is observed in association with LQT1, as a pilot experiment, we investigated the effect of ISO at the whole animal (using one representative animal per group) and cellular levels. The comprehensive methods section is available in the Supplement.[15,17,26–33]

Statistical analyses

Statistical analyses were performed with GraphPad Prism (GraphPad Software, USA). Significance levels of normally distributed data were calculated using *t*-tests or one-way Analysis of variance (ANOVA) with *post hoc* Tukey's or Dunnett's multiple comparisons where applicable and as indicated in the figure legends. For non-normally distributed data, Mann–Whitney was used. A P value $< .05$ was considered statistically significant.

Results

In vitro KCNQ1-SupRep treatment shortens the pathologically prolonged cardiac action potential duration in isolated transgenic LQT1 rabbit ventricular cardiomyocytes

To determine if AAV9-KCNQ1-SupRep can shorten the pathologically prolonged APD_{90} in rabbit VCMs, as a first step, VCMs were isolated from Langendorff-perfused LQT1 and WT rabbit hearts and transfected with either the AAV9-KCNQ1-SupRep plasmid or a non-targeting scrambled (sham) plasmid (*Figure 2A*). Whole cell patch clamp technique was used to measure the APD at 1 Hz stimulation at physiological temperature after 24 h of culturing (see Supplementary data online, *Figure S2*).

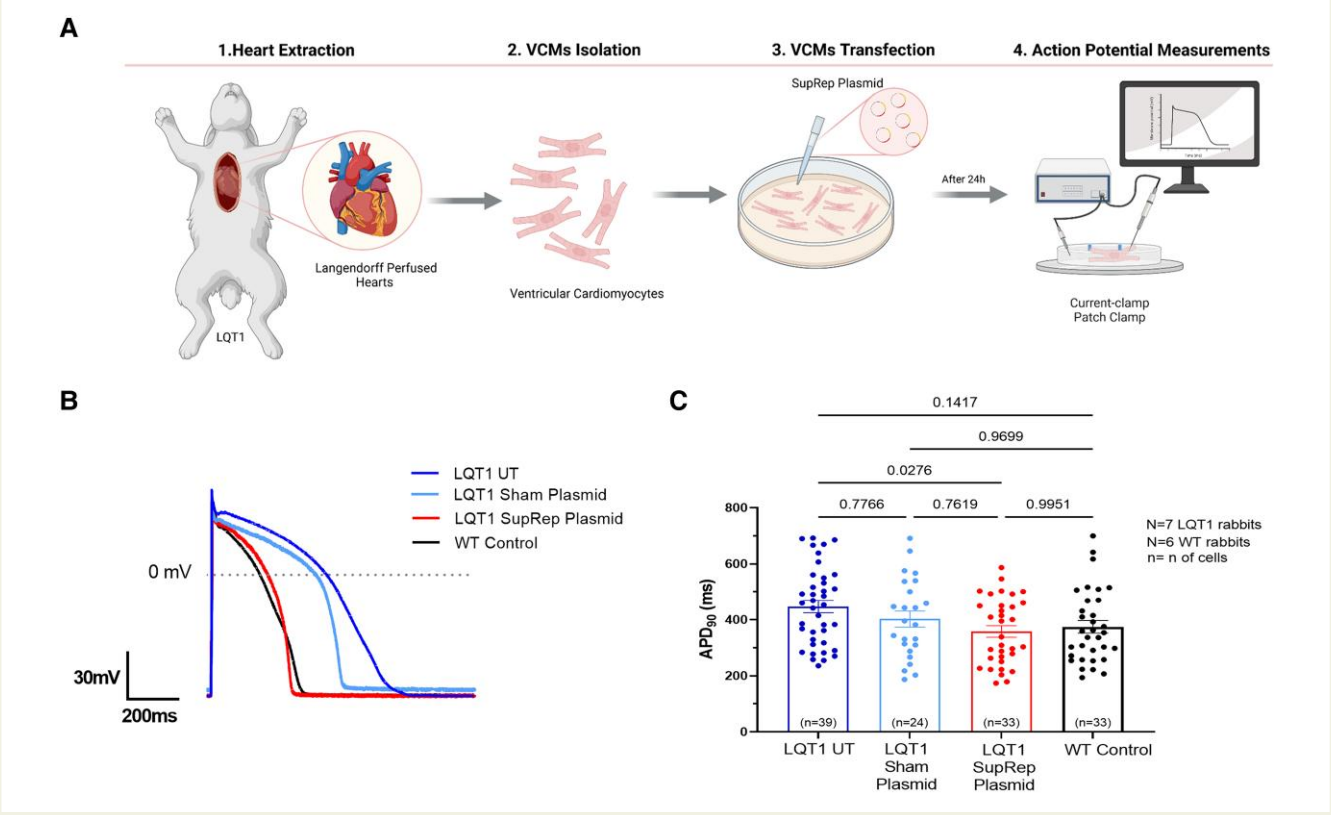


Figure 2 Evaluation of *in vitro* KCNQ1 SupRep efficacy. (*A*) Schematic illustration of ventricular cardiomyocyte (VCM) isolation process. Rabbit hearts were extracted, perfused on a Langendorff system, and ventricular CMs were isolated using enzymatic digestion. Following isolation, cells were either untreated or transfected with SupRep or sham (non-targeting scramble RNA) plasmids. Twenty-four hours following transfection, action potential durations at 90% repolarization (APD_{90}) were measured via whole cell patch clamp. Figure created using Biorender.com. (*B*) Representative whole cell patch clamp action potential recordings in UT-LQT1 (blue), sham-treated LQT1 (light blue), SupRep-treated LQT1 (red), and UT-WT cultured VCMs (black). (*C*) Whole cell patch-clamp APD_{90} measurements at 1 Hz stimulation were performed at 37°C in the various treatment groups. Results are expressed as mean ± standard error of the mean (SEM). One-way ANOVA with *post hoc* Tukey's multiple comparison was performed for statistical analysis. $P < .05$ was considered significant

As expected, untreated (UT)-LQT1 cultured VCMs had significantly longer APD_{90} (ms ± SEM) (447 ± 22 ms) than UT-WT cultured VCMs (375 ± 23 ms, $P = .026$) similar to what was observed in freshly isolated VCMs (see Supplementary data online, *Figure S1B*). Compared to the UT-LQT1 cultured VCMs, there was no significant difference in APD_{90} of the sham-plasmid treated LQT1 cultured VCMs (447 ± 22 ms vs. 403 ± 29 ms, $P = .77$; *Figure 2B and C*). Akin to our previous disease-in-the-dish data with patient-specific iPSC-CMs,[15] treatment with KCNQ1-SupRep plasmid significantly shortened APD_{90} of the LQT1 rabbits' cultured VCMs from 447 ± 22 ms to 358 ± 21 ms (UT-LQT1 vs. SupRep-treated LQT1, $P = .02$). Notably, after treatment with AAV9-KCNQ1-SupRep plasmid, there was no difference in the APD_{90} of the SupRep-treated LQT1 cultured VCMs (358 ± 21 ms) and UT-WT cultured VCMs (375 ± 23 ms, $P = .99$), indicating a normalization of the APD_{90}.

Successful targeted AAV9-KCNQ1-SupRep delivery into the aortic root

A total of 21 LQT1 rabbits and 4 WT rabbits underwent the targeted intra-aortic delivery of AAV9-KCNQ1-SupRep or Sham-NaCl (vehicle control), as described in the online Supplement and depicted in *Figure 3*. While perioperatively, during anaesthesia induction, one untreated LQT1 rabbit experienced TdP and ventricular fibrillation which was successfully resuscitated with external defibrillation, no arrhythmias were observed in any of the rabbits during the aortic balloon occlusion used for construct delivery, during the subsequent recovery period, or in the 2–3 weeks following the surgical procedure. The haemodynamic characteristics of the rabbits during the procedure are summarized in Supplementary data online, *Table S1*.

In vivo AAV9-KCNQ1-SupRep gene therapy shortens the pathologically prolonged QT index in treated transgenic LQT1 rabbits

To determine the efficacy of *in vivo* AAV9-KCNQ1-SupRep gene therapy on the QTi of transgenic LQT1 rabbits, 1E10 vg/kg of AAV9-KCNQ1-SupRep gene therapy was administered via an intra-aortic root injection (*Figure 3*). Electrocardiograms were obtained to measure the QT, RR, and the corrected QTi of these rabbits 2–3 days prior to AAV9-KCNQ1-SupRep injection and two and/or three weeks post-treatment with AAV9-KCNQ1-SupRep gene therapy.

At baseline, the QTi (%) of the UT-LQT1 rabbits (122 ± 3%) was significantly longer compared to the WT rabbits (QTi = 105 ± 2%;

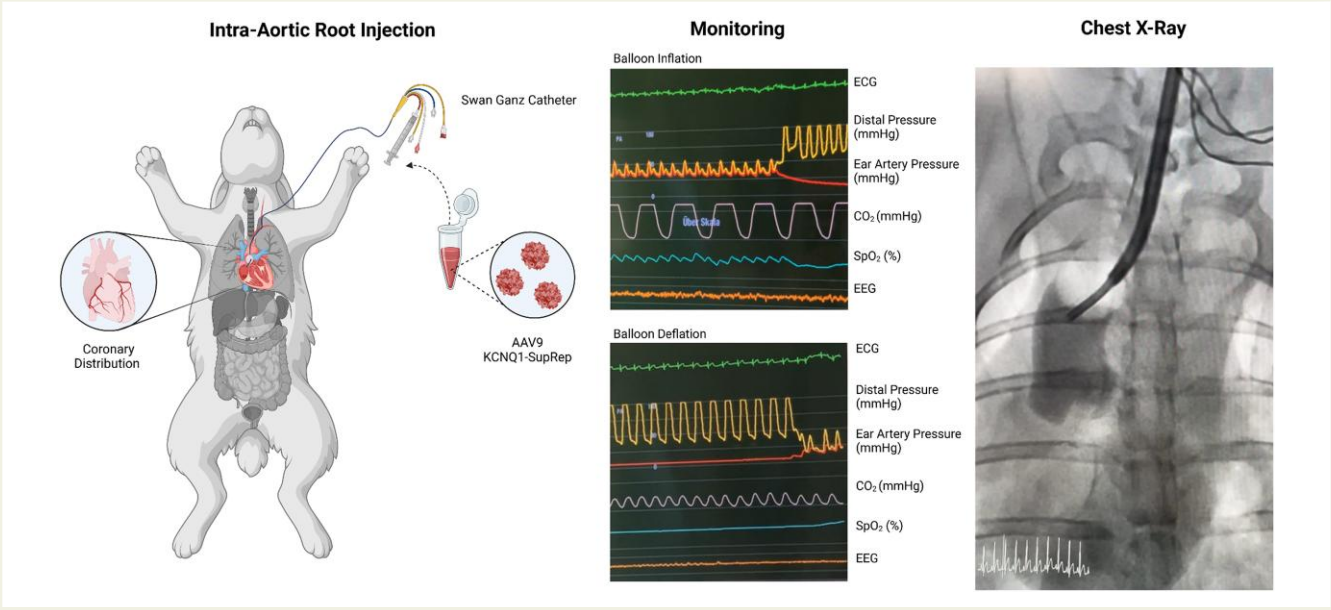


Figure 3 AAV9-KCNQ1-SupRep delivery: route, methodology, and monitoring. AAV9-KCNQ1-SupRep gene therapy was delivered in transgenic LQT1 rabbits via a targeted intra-aortic root injection. Using a Swan–Ganz catheter, the viral construct was perfused during thrice balloon occlusion to obtain a trans-coronary distribution of our gene therapy into the heart. During the surgical procedure, animal vital signs including heart rate (ECG II lead), respiratory rate, oxygen arterial saturation, capnography, invasive blood pressure (in aorta distal to the balloon and in the ear artery), non-invasive Doppler blood pressure, and electroencephalogram (BIS®) were continuously monitored. Chest X-ray imaging was used to guide the catheter and assess the virus perfusion. Figure created using Biorender.com

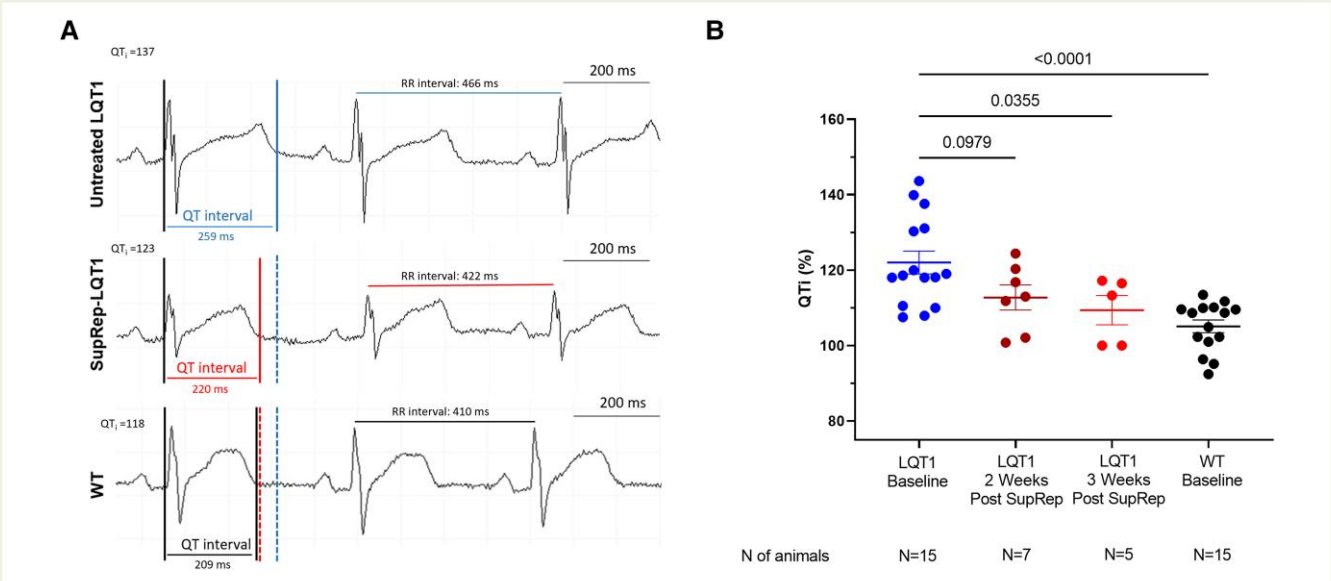


Figure 4 AAV9-KCNQ1-SupRep shortens the pathologically prolonged QT index in treated transgenic LQT1 rabbits. Panel *A* depicts the representative 12-lead ECG traces from LQT1 and WT animals. The QT interval of the UT-LQT1 rabbit is shown in blue, SupRep-treated LQT1 rabbit in red, and WT rabbit in black. Compared to the UT-LQT1 rabbit, the QT interval of the SupRep-treated rabbit is shortened and close to the level of the WT rabbit. (*B*) QTi in LQT1 rabbits before SupRep treatment (baseline, blue), in LQT1 rabbits either 2 (dark red) or 3 weeks (red) post-SupRep treatment, and in WT rabbits at baseline (black) is shown. SupRep treatment significantly shortens the QTi in the treated rabbits close to the level of WT. Results are expressed as mean ± standard error of the mean (SEM). One-way ANOVA (comparing data to LQT1-UT) with *post hoc* Dunnett's multiple comparison was performed. $P < .05$ was considered statistically significant

Table 1 Summary of QT index and action potential duration from LQT1 and wild-type rabbits

Rabbits	QTi (%)	ΔQTi (post–pre-surgery)	QTi *P* value (vs. UT-LQT1)	APD_{90} (ms)	APD_{90} *P* value (vs. UT-LQT1)
UT-LQT1	122 ± 3			525 ± 15	
Sham LQT1	130 ± 9	10 ± 6	*P* = .3886	566 ± 21	*P* = .3200
SupRep LQT1	111 ± 2	−13 ± 4	*P* = .0303	394 ± 15	*P* < .0001
UT-WT	105 ± 2		*P* < .0001	417 ± 14	*P* < .0001

QTi and APD_{90} *P* values were assessed using one-way ANOVA with *post hoc* Dunnett test comparing each group with untreated (UT)-LQT1. LQT1 rabbits treated with SupRep gene therapy (SupRep LQT1) demonstrate a significant reduction in QTi and APD_{90} as compared to UT-LQT1 rabbits. Data presented as mean ± SEM.
APD_{90}, action potential duration at 90% repolarization; LQT1, type 1 long QT syndrome; SupRep, suppression-replacement.

P < .0001; *Figure 4*; *Table 1*). Importantly, there were no significant changes in the QTi (pre- vs. post-treatment) of WT and LQT1 rabbits receiving vehicle/sham treatment with NaCl (see Supplementary data online, *Figure S3*). The QTi of the LQT1 rabbits 2 weeks post-AAV9-KCNQ1-SupRep treatment (113 ± 3%, *P* = .09) showed a trend towards a shorter QTi than the QTi of UT-LQT1 (122 ± 3%) rabbits. Further, the QTi shortening effect in LQT1 rabbits was even more pronounced at 3 weeks post-AAV9-KCNQ1-SupRep treatment (110 ± 4%, *P* = .03) compared to UT-LQT1 (122 ± 3%) rabbits. Importantly, treatment with AAV9-KCNQ1-SupRep shortened the pathologically prolonged QTi in treated LQT1 transgenic rabbits (110 ± 4%) to near WT rabbit QTi levels (105 ± 2%), thus rescuing the pathologic LQT1 ECG phenotype. To confirm that this SupRep effect on cardiac repolarization was not influenced by heart rate changes affecting the QT and QTi, we compared RR intervals across the different treatment groups (see Supplementary data online, *Table S2*). Compared to UT LQT1 rabbits, there was a significant increase in the heart rate (decreased RR interval) among LQT1 rabbits 2 weeks after SupRep gene therapy. However, this effect was not observed in rabbits 3 weeks post-treatment with SupRep, indicating a transient effect possibly related to the previous surgical procedure.

In addition to assessing the QTi, we evaluated the effect of SupRep gene therapy on QT dispersion, a marker of regional heterogeneity of cardiac repolarization. At baseline, the QT dispersion was significantly higher in LQT1 rabbits compared to WT rabbits (*P* = .01). Following treatment with AAV9-KCNQ1-SupRep, QT dispersion decreased in treated LQT1 rabbits, approaching levels observed in WT rabbits (see Supplementary data online, *Figure S4*).

Effect of *in vivo* AAV9-KCNQ1-SupRep at the cellular level in ventricular cardiomyocytes isolated from transgenic LQT1 rabbits

After determining the efficacy of AAV9-KCNQ1-SupRep gene therapy at the whole animal level, we measured the efficacy of the gene therapy at the cellular level by assessing changes at the mRNA level using qPCR and the APD via patch-clamp measurements in VCMs isolated from LQT1 rabbits sacrificed at either 2 or 3 weeks post-AAV9-KCNQ1-SupRep injection (*Figure 5*; *Table 1*). At the gene expression level, we demonstrated a ∼30% reduction in human mutant *KCNQ1* in our SupRep-treated LQT1 rabbits compared to LQT1-sham rabbits (*P* = .02, Supplementary data online, *Figure S5*), which is in line with the percentage reduction previously demonstrated to be effective in mouse models of other genetic arrhythmia disorders and in computational studies.[34,35]

As expected, the APD_{90} of VCMs isolated from UT-LQT1 rabbits (525 ± 15 ms) was not different from VCMs from sham-treated LQT1 rabbits (566 ± 21 ms; *P* = .55) but significantly prolonged compared to VCMs isolated from WT rabbits (417 ± 14 ms, *P* < .0001) (*Figure 5*; *Table 1*). The APD_{90} of VCMs isolated from AAV9-KCNQ1-SupRep-treated LQT1 rabbits at both 2 weeks (402 ± 22 ms, *P* < .0001) and 3 weeks (386 ± 20 ms, *P* < .0001) post-treatment, in contrast, was significantly shorter than the APD_{90} from UT-LQT1 (525 ± 15 ms) and sham-treated LQT1 rabbits (566 ± 21 ms) (*Figure 5*; *Table 1*). The SupRep-induced APD_{90} shortening resulted in levels similar to the APD_{90} of VCMs from WT rabbits (417 ± 14 ms; 2 weeks post-SupRep-LQT1 vs. WT, *P* = .97, 3 weeks post-SupRep-LQT1 vs. WT, *P* = .72).

In addition to the APD, we assessed the incidence of early after depolarizations (EADs) during AP measurements in VCMs isolated from UT LQT1, SupRep-treated LQT1, and WT rabbits (see Supplementary data online, *Figure S6*). Overall, UT LQT1 rabbits had a higher EAD incidence as compared to WT rabbits (14% vs. 3%, *P* = .059). Treatment of LQT1 rabbits with SupRep demonstrated a trend towards reduction in EAD incidence (7%), showing a similar incidence to that observed in WT rabbits.

AAV9-KCNQ1-SupRep-treated transgenic LQT1 rabbits demonstrate WT-like QT/action potential duration response to isoproterenol provocation

During ISO stimulation (10 μM ISO perfusion, 5 mL/h), the sham-treated LQT1 rabbit showed a pronounced QT prolongation with ΔQTi of +24.5 when reaching a 20% increase in heart rate (*Figure 6*). In contrast, the SupRep-treated LQT1 rabbit behaved nearly identical to the WT rabbit during ISO infusion. Specifically, at a 20% increase in heart rate, the WT rabbit and the SupRep-treated LQT1 rabbit showed a ΔQTi of 16.9 and 16.5, respectively.

Due to the initial observations at the whole animal level, we investigated in-depth the effect of β-adrenergic stimulation at the individual VCM level (*Figure 7A*). VCMs isolated from both UT-LQT1 and sham-LQT1 rabbits demonstrated no significant differences in APD_{90} after ISO compared to baseline (UT-LQT1 baseline 499 ± 29 ms, ISO 440 ± 31 ms, *P* = .07; sham-LQT1 baseline 567 ± 23 ms, ISO 529 ± 23 ms, *P* = .051). And notably, ∼15% VCMs isolated from both UT-LQT1 and sham-LQT1 rabbits demonstrated a paradoxical prolongation of the APD_{90} under β-adrenergic stimulation. Ventricular cardiomyocytes isolated from KCNQ1-SupRep-treated LQT1 rabbits, in contrast, demonstrated a significantly shortened APD_{90} (367 ± 15 ms; *P* < .0001) after β-adrenergic stimulation with 10 nM ISO as compared

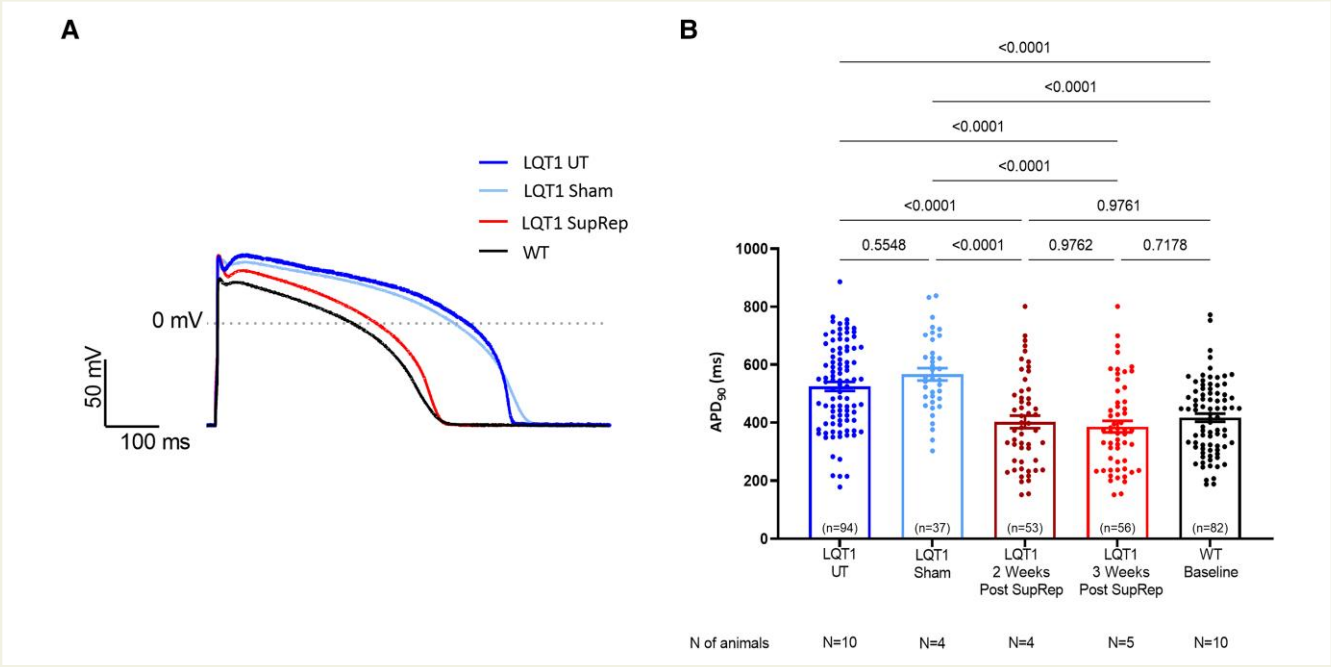


Figure 5 Treatment with KCNQ1-SupRep shortens significantly the pathologically prolonged APD_{90} in transgenic LQT1 rabbits. (*A*) Representative whole cell patch clamp action potential recordings in ventricular cardiomyocytes (VCMs) isolated from UT-LQT1, sham-treated LQT1, 2- or 3-week SupRep-treated LQT1, and UT-WT rabbits. (*B*) Whole cell patch clamp was used for APD_{90} measurements at 37°C at 1 Hz stimulation rate. SupRep-treated LQT1 rabbits demonstrate significantly shortened APD_{90} as compared to the UT or sham-treated LQT1 rabbits. Results are expressed as mean ± standard error of the mean (SEM). One-way ANOVA with *post hoc* Tukey's multiple comparison was performed. $P < .05$ was considered statistically significant

to baseline (475 ± 16 ms) representing a ΔAPD_{90} of 109 ± 17 ms. Similarly, VCMs isolated from WT rabbits demonstrated significantly shortened APD_{90} after ISO (308 ± 21 ms, $P = .001$) compared to baseline (368 ± 19 ms) for ΔAPD_{90} of 60 ± 13 ms.

Next, we assessed the effect of β-adrenergic stimulation with 10 nM ISO on the calcium transient duration at 90% decay ($Ca^{2+}T_{90}$) in isolated VCMs (*Figure 7B*). No significant differences were observed in the $Ca^{2+}T_{90}$ of VCMs isolated from UT-LQT1 rabbits after ISO (baseline 357 ± 15 ms, ISO 380 ± 19 ms, $P = .3$) whereas VCMs isolated from sham-treated LQT1 rabbits demonstrated a significantly increased $Ca^{2+}T_{90}$ after provocation with ISO (baseline 301 ± 23 ms, ISO 384 ± 29 ms, $P = .03$). Ventricular cardiomyocytes isolated from SupRep-treated LQT1 rabbits, in contrast, demonstrated a significant $Ca^{2+}T_{90}$ shortening under β-adrenergic stimulation (baseline 338 ± 13 ms, ISO 293 ± 17 ms, $P = .003$) similar to what was observed in WT VCMs (baseline 344 ± 20 ms, ISO 246 ± 13 ms, $P = .0007$), indicating a normalization of the QT/APD and calcium transient response to ISO in the SupRep-treated LQT1 rabbits.

Immune response to AAV9 gene therapy

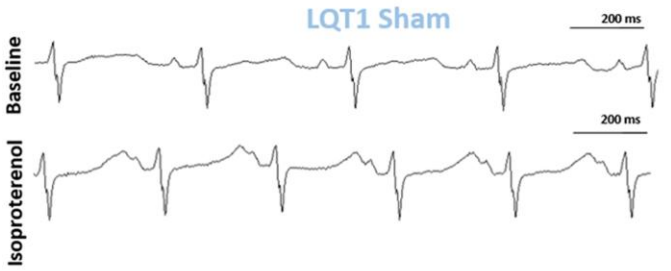
A well-recognized challenge in gene therapy lies in the host's immune response to the viral vector used in the treatment. This includes the development of neutralizing antibodies (NAbs) targeting the vector, which can potentially diminish the therapy's effectiveness.[36] Herein, we examined the immunological reaction in rabbits by assessing AAV9-NAbs levels before and 2–3 weeks after administering AAV9-KCNQ1-SupRep gene therapy. Of note, none of the rabbits showed any AAV9-NAbs prior to treatment. However, all the rabbits displayed detectable titres of AAV9-NAbs 2–3 weeks after SupRep gene therapy. Additionally, we also investigated the effect of SupRep gene therapy on inflammatory changes in the heart. Importantly, we did not observe any histological evidence of increased inflammation in the cardiac tissue during the 2–3-week period following SupRep gene therapy (see Supplementary data online, *Figures S7* and *S8*).

Discussion

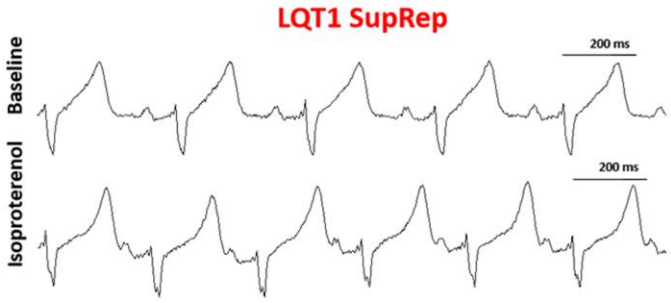
Loss-of-function variants in the *KCNQ1*-encoded I_{Ks} ($K_v7.1$) potassium channel causes LQT1.[37] Though impressive strides have been made in clinical risk stratification and symptom management, no current guideline-directed therapies address the LQT1 pathophysiology at the molecular level. Moreover, although treatments such as beta blockers are effective in LQT1 symptom management, their side-effect profile is not negligible[14] and can lead to patient non-adherence. Beta blocker non-adherence is particularly dangerous as abrupt discontinuation can cause a withdrawal response triggering breakthrough cardiac events.[38–40] Considering the constraints imposed by current treatment modalities, gene therapy could be a potential solution. As demonstrated herein, the efficacy of SupRep gene therapy in ameliorating the pathologic phenotype in LQT1 rabbits (*Structured Graphical Abstract*) is promising for its further development as a therapeutic option not only for LQT1 but also for other genetic conditions mediated by dominant-negative mechanisms.[41]

Current gene therapy strategies

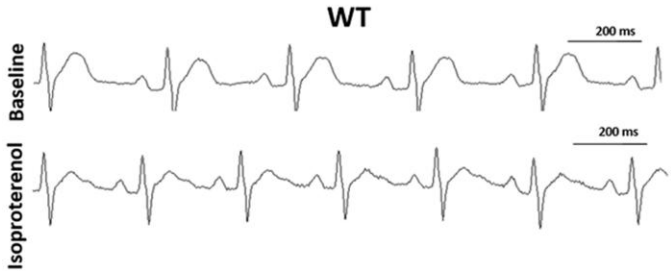
Three main categories of gene therapies are used currently in clinical practice: gene replacement therapy (GRT), gene silencing therapy (GST), and direct-genome editing. In LQTS, GRT may be a viable option for only a small number of individuals. Previously, in a LQTS mouse model, GRT with adenoviral[42] or adeno-associated[43] viral vectors was used to overexpress functional $K_v1.5$ channel subunits and was shown to yield short-term or



	Baseline	Isoproterenol
QT (ms)	297	303
RR (ms)	420	330
QTi (%)	166.6	191.1
ΔQTi		24.5



	Baseline	Isoproterenol
QT (ms)	176	184
RR (ms)	318	257
QTi (%)	112.8	129.3
ΔQTi		16.5



	Baseline	Isoproterenol
QT (ms)	166	174
RR (ms)	356	307
QTi (%)	99.6	116.4
ΔQTi		16.9

Figure 6 AAV9-KCNQ1-SupRep-treated transgenic LQT1 rabbit behaves like WT rabbit under β-adrenergic stimulation with isoproterenol. Depicted are three pairs of ECGs collected at baseline and after isoproterenol (ISO) perfusion in one sham-treated LQT1 rabbit, one SupRep-treated LQT1 rabbit, and one WT rabbit. The AAV9-KCNQ1-SupRep-treated LQT1 rabbit demonstrates a similar ΔQTi (%) as the WT rabbit after provocation with ISO. A 20% heart rate increase was observed in all three animals after stimulation with ISO. Raw values for RR and QT intervals are also provided both at baseline and under β-adrenergic stimulation with isoproterenol

long-term transgene expression, respectively, leading to APD/QT shortening. The major limitation of GRT is the presence of dominant-negative variants whereby the mutated gene product functions as a 'poison peptide' and interferes with the function of the WT gene product. Indeed, this dominant-negative loss-of-function mechanism is the predominant pathophysiological basis for LQT1. Therefore, GRT is insufficient in treating LQT1 in most patients. Recently, some groups have sought to use GST to silence the mutant allele by implementing an allele-specific RNA interference (RNAi) approach to target specific variants in LQTS-causative genes.[44–46] However, there are over 600 LQT1 disease-causative variants with no single variant causing majority of the disease.[4] Thus, variant-specific GST presents an insurmountable hurdle for translation into clinical practice as it necessitates the design of a separate RNAi for each discrete disease-causing variant and renders the treated patient with residual haploinsufficiency which is suboptimal. Lastly, similar to allele-specific GST, direct-genome editing strategy is impractical for a disease like LQTS which contains hundreds of unique disease-causing variants.

Generation of suppression-and-replacement gene therapy

To overcome the limitations of current gene therapy strategies, we developed a novel hybrid gene therapy that combines both GST and GRT into a single 'suppression-and-replacement' gene therapy. Previously, we have shown the efficacy of SupRep gene therapy in correcting the pathologically prolonged APD_{90} in LQT1 and LQT2 patient-specific hiPSC-CMs.[15,16] Here, we show for the first time in an animal model, the efficacy of SupRep gene therapy in correcting the pathologic phenotype in transgenic LQT1 rabbits *in vivo* and on the organ level.

SupRep gene therapy offers several advantages over traditional GRT and GST. First, the shRNA was designed to target a region of *KCNQ1* that is devoid of common polymorphisms and known pathogenic variants, thus allowing the generalizability of this therapy to virtually all patients with LQT1 and eliminating the need for multiple RNAi. Second, the replacement arm of this therapy consists of a uniquely designed

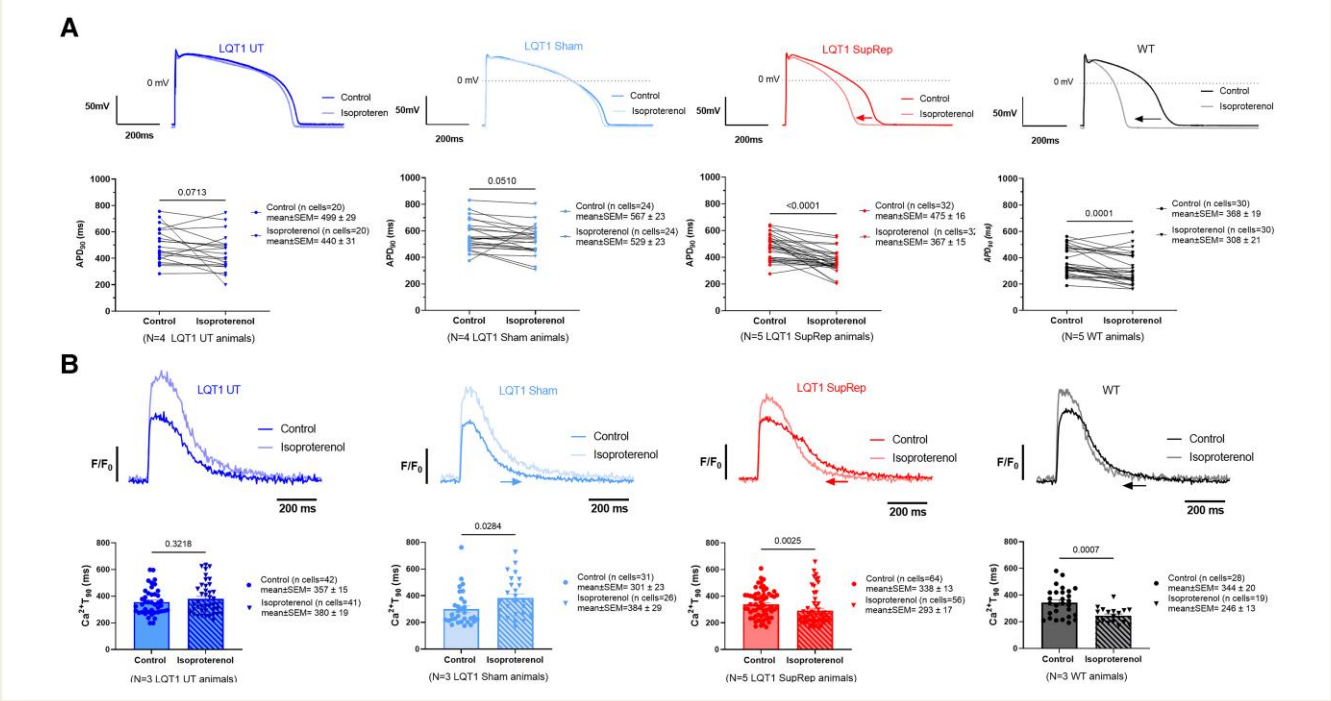


Figure 7 Ventricular cardiomyocytes isolated from SupRep-treated LQT1 rabbits demonstrate WT-like APD_{90} and $Ca^{2+}T_{90}$ under β-adrenergic stimulation. (*A*) (Top) Representative whole cell patch clamp action potential (AP) traces and (bottom) statistical analysis of the APD_{90} at baseline (control) and under isoproterenol provocation. Indicated are individual ventricular cardiomyocytes (VCMs) and their changes. Unlike VCMs isolated from sham and UT-LQT1 rabbits, VCMs isolated from SupRep-treated LQT1 rabbits and WT rabbits demonstrated a significant shortening in APD_{90} at 1 Hz stimulation rate following β-adrenergic stimulation. Paired Student's *t*-test was performed. (*B*) (Top) Representative calcium transient recordings and (bottom) $Ca^{2+}T_{90}$ statistical analysis at baseline (control) and under isoproterenol provocation. VCMs isolated from SupRep-treated LQT1 and WT rabbits showed a $Ca^{2+}T_{90}$ shortening after β-adrenergic stimulation. Unpaired Student's *t*-test or Mann–Whitney was performed. $P < .05$ was considered statistically significant

cDNA that is impervious to knockdown by the shRNA and rescues the disease beyond haploinsufficiency. Thus, SupRep overcomes the limitations of the currently available gene therapy approaches.

In creating the KCNQ1-SupRep gene therapy, we enhanced the cardiac specificity of the candidate therapeutic by using a cTnC promoter to drive the expression of the replacement *KCNQ1* transgene and we encapsulated the gene therapy construct in a cardiotropic AAV9 viral vector to minimize expression of the construct outside the heart. Additionally, AAVs are currently the preferred viral vectors in clinical trials due to their non-integrating nature, lower immunogenicity, and potential indefinite persistence in non-dividing cells such as cardiomyocytes, raising the possibility of one-time dosing.[47]

For this first-in-animal study, we developed a sophisticated intra-aortic root injection technique during balloon occlusion of the proximal aorta to facilitate distribution via the coronaries to maximize delivery of the therapy to the heart. One advantage of distribution via the coronaries is the achievement of a more homogeneous and global transgene delivery across the myocardium in the territory of the targeted coronary vessel.[48] Furthermore, this is accomplished while utilizing a significantly smaller quantity of AAV9 (1E10 vg/kg) as compared to other animal trials involving intravenous cardiac gene therapy.[49,50]

Choosing the ideal animal model

In the current study, in addition to optimizing the gene therapy construct, viral vector, and route of administration, selection of the appropriate animal model was of paramount importance. For many diseases, mice are the first choice of an animal model and mice-derived data has even been IND-enabling for first-in-human trials of gene therapy for AAV9-*MYBPC3* and AAV9-*PKP2* (https://www.tenayatherapeutics.com/our-programs/; https://rocketpharma.com/clinical-trials/pkp2-acm/; https://www.lexeotx.com/programs/cardiac-programs/arrhythmogenic-cardiomyopathy/). However, this is not the case for LQTS. While mouse models of LQTS have been developed, it is important to note that their cardiac repolarization relies on other potassium currents than the I_{Kr} and I_{Ks} currents found in humans.[51,52] In contrast, rabbit cardiac electrophysiology is remarkably similar to that of humans and rabbits do rely on I_{Kr} and I_{Ks} as their main repolarizing currents, making them an ideal animal model for studying KCNQ1/I_{Ks}-mediated diseases such as LQT1.[18] In 2008, Brunner *et al.*[17] established a transgenic LQT1 rabbit by overexpressing the human dominant-negative mutant KCNQ1-Y315S into rabbit embryos. These transgenic rabbits recapitulate the LQT1 phenotype on the cellular, whole heart, and animal levels. Thus, it was a logical choice to test our hybrid gene therapy in this LQT1 rabbit model with the human KCNQ1-Y315S variant as the pre-human, potential IND-enabling 'clinical trial' for evaluating KCNQ1-SupRep gene therapy.

Efficacy of SupRep gene therapy in transgenic LQT1 rabbits

Here, we provide compelling evidence for the favourable efficacy of AAV9-KCNQ1-SupRep gene therapy in transgenic LQT1 rabbits. It is estimated that for every 10 ms increase in the QTc value, there is a corresponding ~5%–7% increase in the incidence of ventricular arrhythmias.[53] Thus, significant shortening of the QTc in patients with LQTS

reduces the risk for life-threatening cardiac events. LQT1 rabbits treated with SupRep gene therapy demonstrated pronounced (13 ± 4%) shortening in the QT index and the APD_{90} (394 ± 15 ms) bringing both parameters near the level of WT rabbits. The decrease in the QT index and the cellular APD in SupRep-treated rabbits provides promising evidence of the effectiveness of SupRep gene therapy in mitigating the pathological phenotype of LQT1 rabbits. Moreover, compared to UT LQT1 rabbits, SupRep-treated LQT1 rabbits demonstrated diminished QT dispersion and a trend towards reduced EAD incidence, further supporting its protective effects.

Clinically, the majority of LQT1 patients experience potentially fatal arrhythmias during exercise. This vulnerability is primarily due to the crucial role of I_{Ks} in repolarization reserve during physical activity.[54] Under normal circumstances, β-adrenergic stimulation enhances I_{Ks}, thus shortening APD. In LQT1, however, this sympathetic activation of I_{Ks} is impaired and individuals with LQT1 often exhibit paradoxical prolongation of the APD/QT and arrhythmias during catecholamine infusion.[24] In the past, the epinephrine/isoproterenol stress test was even used to unmask patients with otherwise concealed LQT1 and LQT2.[55,56] In this study, the SupRep-treated LQT1 rabbits exhibited a response similar to that of WT rabbits under β-adrenergic stimulation with ISO, offering additional evidence supporting the efficacy of SupRep gene therapy in mitigating the pathological LQT1 phenotype both at rest and during adrenergic stress.

Limitations and outlook

As the first ever gene therapy proof-of-concept study in a medium-sized animal model of LQTS, this manuscript has several limitations which will be addressed in future investigations before advancing this gene therapy for patients.

First, while KCNQ1-SupRep is designed to minimize heterogeneity in transduction by using a single suppression-and-replacement vector, some level of heterogeneity is expected due to challenges in delivery to all cardiomyocytes. High heterogeneity could lead to repolarization dispersion and arrhythmias, while moderate cell-to-cell APD heterogeneity may be compensated through electrotonic coupling of cells via gap junctions. Moreover, experimental and modelling studies have demonstrated that only 20%–40% of all cardiomyocytes need to be corrected by the gene therapy—a similar % as observed in our study—to rescue the phenotype and prevent arrhythmia formation.[34,35] In the present study, the absence of arrhythmias in our SupRep-treated rabbits hints at a potential compensation. Furthermore, we noted a favourable decrease in QT dispersion among the SupRep-treated LQT1 rabbits compared to their baseline levels, approaching levels observed in WT rabbits. This indicates that despite a reduction of the mutant KCNQ1 by only 30%, there is no evidence of a potentially adverse increase in regional heterogeneity. Nevertheless, further research such as evaluation of APD spatial heterogeneity using repolarization maps is needed to fully understand these dynamics and to obtain direct evidence for anti-arrhythmic effects *in vivo*, which is challenging due the rarity of spontaneous TdP and the lack of arrhythmia inducibility by programmed ventricular stimulation,[11,57,58] thus warranting large cohorts of LQTS animals. Furthermore, although surrogate methods were used to determine potential heterogeneity of transduction, future studies should directly quantify the transduction rate in VCMs isolated from AAV9-KCNQ1-SupRep-treated rabbits.

Second, i.v. administration of SupRep gene therapy as least invasive delivery method will most likely be helpful, but not crucial, before progressing to clinical trials. However, substantial progress in enhancing cardiac tropism is necessary to enable heart-specific targeting and minimize off-target delivery. One potentially promising approach could involve utilization of the novel 'MyoAAV' capsid,[59] which has recently shown increased efficiency in gene therapy delivery with targeted expression in muscles, including skeletal and cardiac tissues. Nevertheless, current first-in-human gene therapies for patients with other genetic heart diseases are utilizing i.v. administration for their AAV9-based GRTs.[60]

Third, in the present study, we utilized a cTnC promoter to drive the replacement shIMM cDNA, while *KCNQ1* suppression was achieved using a ubiquitous U6 promoter-driven shRNA. To mitigate potential off-target effects, future studies should use a cardiac-specific promoter for both suppression and replacement.

Fourth, we designed a unique shRNA and shIMM cDNA for the *KCNQ1* SupRep construct used in this study, which achieved even greater knockdown efficiency *in vitro* than the one originally used.[15] However, given that the human and rabbit *KCNQ1* do not share 100% nucleotide identity, we were unable to find a sequence with 100% identity to the rabbit *KCNQ1*. Given that the degree of *in vivo* knockdown was 30% for the markedly overexpressed human LQT1-causative *KCNQ1*-Y315S transcript, we anticipate even less knockdown of the endogenous rabbit alleles by this shRNA. Accordingly, the presence of endogenous rabbit KCNQ1/K_v7.1 channels likely contributed to the APD and QT normalization. As the human mutant *KCNQ1*-Y315S pore mutant has a dominant negative effect and is markedly overexpressed in the transgenic LQT1 rabbit heart,[17] a simple down-regulation of 30% of the human mutant *KCNQ1* transgene in the presence of the residual endogenous rabbit *KCNQ1*, however, would not suffice to completely rescue the phenotype, indicating that a combination of persistent rabbit *KCNQ1* and the *KCNQ1* shIMM cDNA most likely contributes to the rescue, although herein we did not directly demonstrate the functionality of the shIMM-encoded K_v7.1 channels. Given these considerations and the greater unmet therapeutic need for patients with *KCNH2*-mediated LQT2/SQT1, we have also designed a novel *KCNH2* SupRep construct,[16] in which the suppression arm has 100% nucleotide identity with the rabbit's endogenous *KCNH2* alleles and a safety/efficacy trial in rabbit models of *KCNH2*-mediated short QT syndrome (SQT1)[26] is already underway.

Fifth, in the current study, a stimulation rate lower than the physiological *in vivo* heart rate (~3 Hz) was used to assess APD differences at the cellular level. In future studies, it will be important to demonstrate the gene therapy effect also at physiologic heart rates.

Sixth, dose escalation and survival studies will help elucidate the long-term safety and efficacy of this gene therapy. These studies will also help to determine whether SupRep gene therapy could potentially be a one-time dose therapeutic in patients. Prior research has demonstrated the ability of AAVs to persist indefinitely in non-dividing cells like cardiomyocytes,[61] and to sustain favourable electrophysiological effects on the APD in a mouse model of LQTS for over 6 months,[43] suggesting the feasibility of a single treatment approach. However, this aspect requires further exploration since mice data cannot be used to derive robust conclusions for other species. In the current study, we examined the development of NAbs in AAV9-KCNQ1-SupRep-treated rabbits 2–3 weeks after gene therapy administration and the development of AAV9-NAbs over time did not affect the therapeutic efficacy of SupRep gene therapy. This underscores the advantages of utilizing SupRep as a one-time dose treatment in individuals lacking pre-existing AAV9-NAbs, without compromising efficacy, potentially even in the absence of immunosuppressants.

Although compared to sham (NaCl) treated and UT rabbits, SupRep-treated LQT1 rabbits showed significant shortening of the QTi and APD, in future studies, it will be important to also test the effect of a sham AAV aortic injection on QT/APD.

Lastly, it will be essential to assess the safety of SupRep gene therapy in species that resemble humans even more closely such as non-human primates to ensure its suitability for human use.

Conclusions

Herein, we provide the first proof-of-concept gene therapy for treating LQT1 in a medium-sized animal model. In transgenic LQT1 rabbits, KCNQ1-SupRep gene therapy effectively shortened the QT interval and APD_{90} to levels close to those of WT rabbits, both at baseline and after provocation with isoproterenol. If intravenous administration of KCNQ1-SupRep gene therapy yields comparable therapeutic efficacy, these promising results strongly support the ongoing development of this hybrid gene therapy for patients with LQT1.

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Supplementary data

Supplementary data are available at *European Heart Journal* online.

Declarations

Disclosure of Interest

M.J.A. is a consultant for Abbott, BioMarin Pharmaceuticals, Boston Scientific, Bristol Myers Squibb, Daichii Sankyo, Illumina, Invitae, Medtronic, Tenaya Therapeutics, and UpToDate. M.J.A. and Mayo Clinic have license agreements with AliveCor, Anumana, ARMGO Pharma, Pfizer Inc., and Thryv Therapeutics. S.B., D.J.T., and C.S.J.K. have a royalty/intellectual property relationship with Pfizer. N.C., M.Be., L.K., J.O., J.P., and G.B. were employed by Pfizer at the time of this study. Other authors declare no conflicts relevant to this manuscript.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Ethical Approval

All animal experiments were performed in compliance with European Union (EU) legislation (directive 2010/63/EU) and the Swiss Animal Welfare Ordinance, after approval by the Cantonal Veterinary Office and the Animal Welfare Officer (Kanton Bern, approval number BE55-21).

Pre-registered Clinical Trial Number

None supplied.

References

1. Schwartz PJ, Stramba-Badiale M, Crotti L, Pedrazzini M, Besana A, Bosi G, *et al.* Prevalence of the congenital long-QT syndrome. *Circulation* 2009;**120**:1761–7. https://doi.org/10.1161/CIRCULATIONAHA.109.863209
2. Schwartz PJ, Ackerman MJ. The long QT syndrome: a transatlantic clinical approach to diagnosis and therapy. *Eur Heart J* 2013;**34**:3109–16. https://doi.org/10.1093/eurheartj/eht089
3. Amin AS, Tan HL, Wilde AA. Cardiac ion channels in health and disease. *Heart Rhythm* 2010;**7**:117–26. https://doi.org/10.1016/j.hrthm.2009.08.005
4. Huang H, Kuenze G, Smith JA, Taylor KC, Duran AM, Hadziselimovic A, *et al.* Mechanisms of KCNQ1 channel dysfunction in long QT syndrome involving voltage sensor domain mutations. *Sci Adv* 2018;**4**:eaar2631. https://doi.org/10.1126/sciadv.aar2631
5. Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL, *et al.* Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. *Circulation* 2000;**102**:1178–85. https://doi.org/10.1161/01.CIR.102.10.1178
6. Shalaby FY, Levesque PC, Yang WP, Little WA, Conder ML, Jenkins-West T, *et al.* Dominant-negative KvLQT1 mutations underlie the LQT1 form of long QT syndrome. *Circulation* 1997;**96**:1733–6. https://doi.org/10.1161/01.CIR.96.6.1733
7. Synková I, Bébarová M, Andršová I, Chmelikova L, Švecová O, Hošek J, *et al.* Long-QT founder variant T309I-Kv7.1 with dominant negative pattern may predispose delayed afterdepolarizations under β-adrenergic stimulation. *Sci Rep* 2021;**11**:3573. https://doi.org/10.1038/s41598-021-81670-1
8. Thomas D, Wimmer AB, Karle CA, Licka M, Alter M, Khalil M, *et al.* Dominant-negative I(Ks) suppression by KCNQ1-deltaF339 potassium channels linked to Romano–Ward syndrome. *Cardiovasc Res* 2005;**67**:487–97. https://doi.org/10.1016/j.cardiores.2005.05.003
9. Wang Z, Tristani-Firouzi M, Xu Q, Lin M, Keating MT, Sanguinetti MC. Functional effects of mutations in KvLQT1 that cause long QT syndrome. *J Cardiovasc Electrophysiol* 1999;**10**:817–26. https://doi.org/10.1111/j.1540-8167.1999.tb00262.x
10. Boulet IR, Raes AL, Ottschytsch N, Snyders DJ. Functional effects of a KCNQ1 mutation associated with the long QT syndrome. *Cardiovasc Res* 2006;**70**:466–74. https://doi.org/10.1016/j.cardiores.2006.02.006
11. Zeppenfeld K, Tfelt-Hansen J, de Riva M, Winkel BG, Behr ER, Blom NA, *et al.* 2022 ESC guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. *Eur Heart J* 2022;**43**:3997–4126. https://doi.org/10.1093/eurheartj/ehac262
12. Monnig G, Kobe J, Loher A, Eckardt L, Wedekind H, Scheld HH, *et al.* Implantable cardioverter-defibrillator therapy in patients with congenital long-QT syndrome: a long-term follow-up. *Heart Rhythm* 2005;**2**:497–504. https://doi.org/10.1016/j.hrthm.2005.02.008
13. Moss AJ, Zareba W, Hall WJ, Schwartz PJ, Crampton RS, Benhorin J, *et al.* Effectiveness and limitations of beta-blocker therapy in congenital long-QT syndrome. *Circulation* 2000;**101**:616–23. https://doi.org/10.1161/01.CIR.101.6.616
14. Martinez K, Bains S, Giudicessi JR, Bos JM, Neves R, Ackerman MJ. Spectrum and prevalence of side effects and complications with guideline-directed therapies for congenital long QT syndrome. *Heart Rhythm* 2022;**19**:1666–72. https://doi.org/10.1016/j.hrthm.2022.06.008
15. Dotzler SM, Kim CSJ, Gendron WAC, Zhou W, Ye D, Bos JM, *et al.* Suppression-replacement KCNQ1 gene therapy for type 1 long QT syndrome. *Circulation* 2021;**143**:1411–25. https://doi.org/10.1161/CIRCULATIONAHA.120.051836
16. Bains S, Zhou W, Dotzler SM, Martinez K, John Kim CS, Tester DJ, *et al.* Suppression and replacement gene therapy for KCNH2-mediated arrhythmias. *Circ Genom Precis Med* 2022;**15**:e003719. https://doi.org/10.1161/CIRCGEN.122.003719
17. Brunner M, Peng X, Liu GX, Ren XQ, Ziv O, Choi BR, *et al.* Mechanisms of cardiac arrhythmias and sudden death in transgenic rabbits with long QT syndrome. *J Clin Invest* 2008;**118**:2246–59. https://doi.org/10.1172/JCI33578
18. Nerbonne JM. Molecular basis of functional voltage-gated K^+ channel diversity in the mammalian myocardium. *J Physiol* 2000;**525**:285–98. https://doi.org/10.1111/j.1469-7793.2000.t01-1-00285.x
19. Bentzen BH, Bahrke S, Wu K, Larsen AP, Odening KE, Franke G, *et al.* Pharmacological activation of Kv11.1 in transgenic long QT-1 rabbits. *J Cardiovasc Pharmacol* 2011;**57**:223–30. https://doi.org/10.1097/FJC.0b013e318203a44d
20. Castiglione A, Hornyik T, Wulfers EM, Giammarino L, Edler I, Jowais JJ, *et al.* Docosahexaenoic acid normalizes QT interval in long QT type 2 transgenic rabbit models in a genotype-specific fashion. *Europace* 2022;**24**:511–22. https://doi.org/10.1093/europace/euab228
21. Giannetti F, Barbieri M, Shiti A, Casini S, Sager PT, Das S, *et al.* Gene- and variant-specific efficacy of serum/glucocorticoid-regulated kinase 1 inhibition in long QT syndrome types 1 and 2. *Europace* 2023;**25**:euad094. https://doi.org/10.1093/europace/euad094
22. Pacak CA, Sakai Y, Thattaliyath BD, Mah CS, Byrne BJ. Tissue specific promoters improve specificity of AAV9 mediated transgene expression following intra-vascular gene delivery in neonatal mice. *Genet Vaccines Ther* 2008;**6**:13. https://doi.org/10.1186/1479-0556-6-13
23. Shimizu W, Tanabe Y, Aiba T, Inagaki M, Kurita T, Suyama K, *et al.* Differential effects of beta-blockade on dispersion of repolarization in the absence and presence of sympathetic stimulation between the LQT1 and LQT2 forms of congenital long QT syndrome. *J Am Coll Cardiol* 2002;**39**:1984–91. https://doi.org/10.1016/S0735-1097(02)01894-6

24. Tomson TT, Arora R. Modulation of cardiac potassium current by neural tone and ischemia. *Card Electrophysiol Clin* 2016;**8**:349–60. https://doi.org/10.1016/j.ccep.2016.01.007
25. Ackerman MJ, Khositseth A, Tester DJ, Hejlik JB, Shen WK, Porter CB. Epinephrine-induced QT interval prolongation: a gene-specific paradoxical response in congenital long QT syndrome. *Mayo Clin Proc* 2002;**77**:413–21. https://doi.org/10.1016/S0025-6196(11)62209-X
26. Odening KE, Bodi I, Franke G, Rieke R, Ryan de Medeiros A, Perez-Feliz S, *et al.* 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. *Eur Heart J* 2019;**40**:842–53. https://doi.org/10.1093/eurheartj/ehy761
27. Kopton RA, Buchmann C, Moss R, Kohl P, Peyronnet R, Schneider-Warme F. Electromechanical assessment of optogenetically modulated cardiomyocyte activity. *J Vis Exp* 2020. https://doi.org/10.3791/60490
28. Liu GS, Thornton J, Van Winkle DM, Stanley AW, Olsson RA, Downey JM. Protection against infarction afforded by preconditioning is mediated by A1 adenosine receptors in rabbit heart. *Circulation* 1991;**84**:350–6. https://doi.org/10.1161/01.CIR.84.1.350
29. Odening KE, Hyder O, Chaves L, Schofield L, Brunner M, Kirk M, *et al.* Pharmacogenomics of anesthetic drugs in transgenic LQT1 and LQT2 rabbits reveal genotype-specific differential effects on cardiac repolarization. *Am J Physiol Heart Circ Physiol* 2008;**295**:H2264–72. https://doi.org/10.1152/ajpheart.00680.2008
30. Wanderer S, Waltenspuel C, Gruter BE, Strange F, Sivanrupan S, Remonda L, *et al.* Arterial pouch microsurgical bifurcation aneurysm model in the rabbit. *J Vis Exp* 2020. https://doi.org/10.3791/61157
31. Terentyev D, Rees CM, Li W, Cooper LL, Jindal HK, Peng X, *et al.* Hyperphosphorylation of RyRs underlies triggered activity in transgenic rabbit model of LQT2 syndrome. *Circ Res* 2014;**115**:919–28. https://doi.org/10.1161/CIRCRESAHA.115.305146
32. Odening KE, Choi BR, Liu GX, Hartmann K, Ziv O, Chaves L, *et al.* Estradiol promotes sudden cardiac death in transgenic long QT type 2 rabbits while progesterone is protective. *Heart Rhythm* 2012;**9**:823–32. https://doi.org/10.1016/j.hrthm.2012.01.009
33. Liu GX, Choi BR, Ziv O, Li W, de Lange E, Qu Z, *et al.* Differential conditions for early after-depolarizations and triggered activity in cardiomyocytes derived from transgenic LQT1 and LQT2 rabbits. *J Physiol* 2012;**590**:1171–80. https://doi.org/10.1113/jphysiol.2011.218164
34. Liu MB, Priori SG, Qu Z, Weiss JN. Stabilizer cell gene therapy: a less-is-more strategy to prevent cardiac arrhythmias. *Circ Arrhythm Electrophysiol* 2020;**13**:e008420. https://doi.org/10.1161/CIRCEP.120.008420
35. Navarrete-Welton A, Kim TY, Bronk P, Qu Z, Koren G, Choi B-R. Low-efficiency gene editing is capable of suppressing arrhythmogenesis in long QT syndrome type II: computer simulation study. *Circulation* 2023;**148**:A12241. https://doi.org/10.1161/circ.148.suppl_1.12241
36. Freitas MV, Francio L, Haleva L, Matte UDS. Protection is not always a good thing: the immune system's impact on gene therapy. *Genet Mol Biol* 2022;**45**:e20220046. https://doi.org/10.1590/1678-4685-gmb-2022-0046
37. Wang Q, Curran ME, Splawski I, Burn TC, Millholland JM, VanRaay TJ, *et al.* Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nat Genet* 1996;**12**:17–23. https://doi.org/10.1038/ng0196-17
38. Lederballe Pedersen O, Mikkelsen E, Lanng Nielsen J, Christensen NJ. Abrupt withdrawal of beta-blocking agents in patients with arterial hypertension. Effect on blood pressure, heart rate and plasma catecholamines and prolactin. *Eur J Clin Pharmacol* 1979;**15**:215–7. https://doi.org/10.1007/BF00563108
39. Gilligan DM, Chan WL, Stewart R, Oakley CM. Adrenergic hypersensitivity after beta-blocker withdrawal in hypertrophic cardiomyopathy. *Am J Cardiol* 1991;**68**:766–72. https://doi.org/10.1016/0002-9149(91)90651-Z
40. Vincent GM, Schwartz PJ, Denjoy I, Swan H, Bithell C, Spazzolini C, *et al.* High efficacy of beta-blockers in long-QT syndrome type 1: contribution of noncompliance and QT-prolonging drugs to the occurrence of beta-blocker treatment "failures". *Circulation* 2009;**119**:215–21. https://doi.org/10.1161/CIRCULATIONAHA.108.772533
41. Bains S, Giudicessi JR, Odening KE, Ackerman MJ. State of gene therapy for monogenic cardiovascular diseases. *Mayo Clin Proc* 2024;**99**:610–29. https://doi.org/10.1016/j.mayocp.2023.11.003
42. Brunner M, Kodirov SA, Mitchell GF, Buckett PD, Shibata K, Folco EJ, *et al.* In vivo gene transfer of Kv1.5 normalizes action potential duration and shortens QT interval in mice with long QT phenotype. *Am J Physiol Heart Circ Physiol* 2003;**285**:H194–203. https://doi.org/10.1152/ajpheart.00971.2002
43. Kodirov SA, Brunner M, Busconi L, Koren G. Long-term restitution of 4-aminopyridine-sensitive currents in Kv1DN ventricular myocytes using adeno-associated virus-mediated delivery of Kv1.5. *FEBS Lett* 2003;**550**:74–8. https://doi.org/10.1016/S0014-5793(03)00822-6
44. Matsa E, Dixon JE, Medway C, Georgiou O, Patel MJ, Morgan K, *et al.* Allele-specific RNA interference rescues the long-QT syndrome phenotype in human-induced pluripotency stem cell cardiomyocytes. *Eur Heart J* 2014;**35**:1078–87. https://doi.org/10.1093/eurheartj/eht067
45. Lu X, Yang X, Huang X, Huang C, Sun HH, Jin L, *et al.* RNA interference targeting E637K mutation rescues hERG channel currents and restores its kinetic properties. *Heart Rhythm* 2013;**10**:128–36. https://doi.org/10.1016/j.hrthm.2012.09.124
46. Cocera-Ortega L, Wilders R, Kamps SC, Fabrizi B, Huber I, van der Made I, *et al.* shRNAs targeting a common KCNQ1 variant could alleviate long-QT1 disease severity by inhibiting a mutant allele. *Int J Mol Sci* 2022;**23**:4053. https://doi.org/10.3390/ijms23074053
47. Penaud-Budloo M, Le Guiner C, Nowrouzi A, Toromanoff A, Chérel Y, Chenuaud P, *et al.* Adeno-associated virus vector genomes persist as episomal chromatin in primate muscle. *J Virol* 2008;**82**:7875–85. https://doi.org/10.1128/JVI.00649-08
48. Scimia MC, Gumpert AM, Koch WJ. Cardiovascular gene therapy for myocardial infarction. *Expert Opin Biol Ther* 2014;**14**:183–95. https://doi.org/10.1517/14712598.2014.866085
49. Ruppert T, Heckmann MB, Rapti K, Schultheis D, Jungmann A, Katus HA, *et al.* AAV-mediated cardiac gene transfer of wild-type desmin in mouse models for recessive desminopathies. *Gene Ther* 2019;**27**:516–24. https://doi.org/10.1038/s41434-020-0147-7
50. Ni L, Scott L Jr, Campbell HM, Pan X, Alsina KM, Reynolds J, *et al.* Atrial-specific gene delivery using an adeno-associated viral vector. *Circ Res* 2019;**124**:256–62. https://doi.org/10.1161/CIRCRESAHA.118.313811
51. Salama G, London B. Mouse models of long QT syndrome. *J Physiol* 2007;**578**:43–53. https://doi.org/10.1113/jphysiol.2006.118745
52. Nerbonne JM. Studying cardiac arrhythmias in the mouse–a reasonable model for probing mechanisms? *Trends Cardiovasc Med* 2004;**14**:83–93. https://doi.org/10.1016/j.tcm.2003.12.006
53. Lester RM, Paglialunga S, Johnson IA. QT assessment in early drug development: the long and the short of it. *Int J Mol Sci* 2019;**20**:1324. https://doi.org/10.3390/ijms20061324
54. Jost N, Papp JG, Varro A. Slow delayed rectifier potassium current (IKs) and the repolarization reserve. *Ann Noninvasive Electrocardiol* 2007;**12**:64–78. https://doi.org/10.1111/j.1542-474X.2007.00140.x
55. Vyas H, Hejlik J, Ackerman MJ. Epinephrine QT stress testing in the evaluation of congenital long-QT syndrome: diagnostic accuracy of the paradoxical QT response. *Circulation* 2006;**113**:1385–92. https://doi.org/10.1161/CIRCULATIONAHA.105.600445
56. Shimizu W, Antzelevitch C. Differential effects of beta-adrenergic agonists and antagonists in LQT1, LQT2 and LQT3 models of the long QT syndrome. *J Am Coll Cardiol* 2000;**35**:778–86. https://doi.org/10.1016/S0735-1097(99)00582-3
57. Odening KE, Kirk M, Brunner M, Ziv O, Lorvidhaya P, Liu GX, *et al.* Electrophysiological studies of transgenic long QT type 1 and type 2 rabbits reveal genotype-specific differences in ventricular refractoriness and His conduction. *Am J Physiol Heart Circ Physiol* 2010;**299**:H643–55. https://doi.org/10.1152/ajpheart.00074.2010
58. Bhandari AK, Shapiro WA, Morady F, Shen EN, Mason J, Scheinman MM. Electrophysiologic testing in patients with the long QT syndrome. *Circulation* 1985;**71**:63–71. https://doi.org/10.1161/01.CIR.71.1.63
59. Tabebordbar M, Lagerborg KA, Stanton A, King EM, Ye S, Tellez L, *et al.* Directed evolution of a family of AAV capsid variants enabling potent muscle-directed gene delivery across species. *Cell* 2021;**184**:4919–38. e22. https://doi.org/10.1016/j.cell.2021.08.028
60. Argiro A, Bui Q, Hong KN, Ammirati E, Olivotto I, Adler E. Applications of gene therapy in cardiomyopathies. *JACC Heart Fail* 2024;**12**:248–60. https://doi.org/10.1016/j.jchf.2023.09.015
61. Brommel CM, Cooney AL, Sinn PL. Adeno-associated virus-based gene therapy for lifelong correction of genetic disease. *Hum Gene Ther* 2020;**31**:985–95. https://doi.org/10.1089/hum.2020.138