

# Negating the dominant-negative allele: a new treatment paradigm for arrhythmias explored in human induced pluripotent stem cell-derived cardiomyocytes

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Online publish-ahead-of-print 12 April 2013

**This editorial refers to ‘Allele-specific RNA interference rescues the long-QT syndrome phenotype in human induced pluripotent stem cell cardiomyocytes’<sup>†</sup>, by E. Matsa et al., on page 1078**

Congenital long-QT syndrome (LQTS) is a disorder of cardiac repolarization that manifests typically with ventricular arrhythmias such as torsades de pointes in patients with prolonged QT intervals in the ECG and structurally normal hearts. The known mutations affect genes encoding either subunits of cardiac ion channels or proteins involved in their regulation. Matsa and colleagues<sup>1</sup> describe how they succeeded in using human induced pluripotent stem cell (hiPSC) technology not only to model a subtype of LQTS (LQT2), but also to evaluate a novel therapeutic approach using human cardiomyocytes derived from patient-specific hiPSCs.

The authors generated cardiomyocytes from hiPSCs derived from a patient carrying a heterozygous mutation in the *KCNH2* gene, corresponding to an A561T amino acid exchange in the *KCNH2*-encoded protein hERG. Four hERG subunits co-assemble to form the pore of the ion channel generating the delayed rectifier potassium current  $I_{Kr}$ , an important determinant of the cardiac action potential duration. In accordance with the clinical phenotype of the patient, the hiPSC-derived cardiomyocytes displayed prolonged action potentials.

The authors further demonstrated that, similar to other LQT2-causing ion channel mutations,<sup>2</sup> the A561T mutation results in impaired ion channel trafficking: mutated subunits were not glycosylated properly and thus were retained in a perinuclear compartment instead of being integrated into the plasma membrane. Presumably due to co-assembly, the mutation also interfered with normal trafficking of the wild-type subunits in a dominant-negative fashion (Figure 1A).

This dominant-negative mechanism is the key to the novel strategy applied by Matsa and colleagues to rescue the disease phenotype in hiPSC-derived cardiomyocytes: they designed a small interfering RNA (siRNA) that specifically led to a knockdown of the mutated rather than of the wild-type hERG subunit on the mRNA and protein level. Thus, by negating the dominant-negative allele, they allowed more wild-type hERG subunits to co-assemble into tetramers composed only of ‘healthy’ subunits that can reach the plasma membrane, augmenting the  $I_{Kr}$  current and shortening the prolonged action potential (Figure 1B).

This approach is different from the classical concept of gene therapy aiming at replacement of gene function. To treat a disease caused by a dominant-negative mutation by overexpression of the wild-type allele successfully, it would be necessary to express the wild-type allele at higher than normal levels to overcome the dominant-negative effect. By using allele-specific siRNA, depending on the biophysical properties of the mutation, the rescue of functional ion channels can even surpass the level of mRNA and protein knockdown.

In their study, Matsa and colleagues assume that the presence of one mutated subunit in a hERG tetramer is sufficient to prevent its trafficking to the plasma membrane. This seems plausible, considering the marked action potential prolongation found in patient-derived hiPSC cardiomyocytes. Assuming equal expression levels of mutant and wild-type protein, this would result in only 6.25% of the synthesized channel complexes reaching the plasma membrane in patient cells. An allele-specific siRNA knockdown of the mutated allele by ~60% as achieved by the authors would increase this number more than four-fold to 26%.

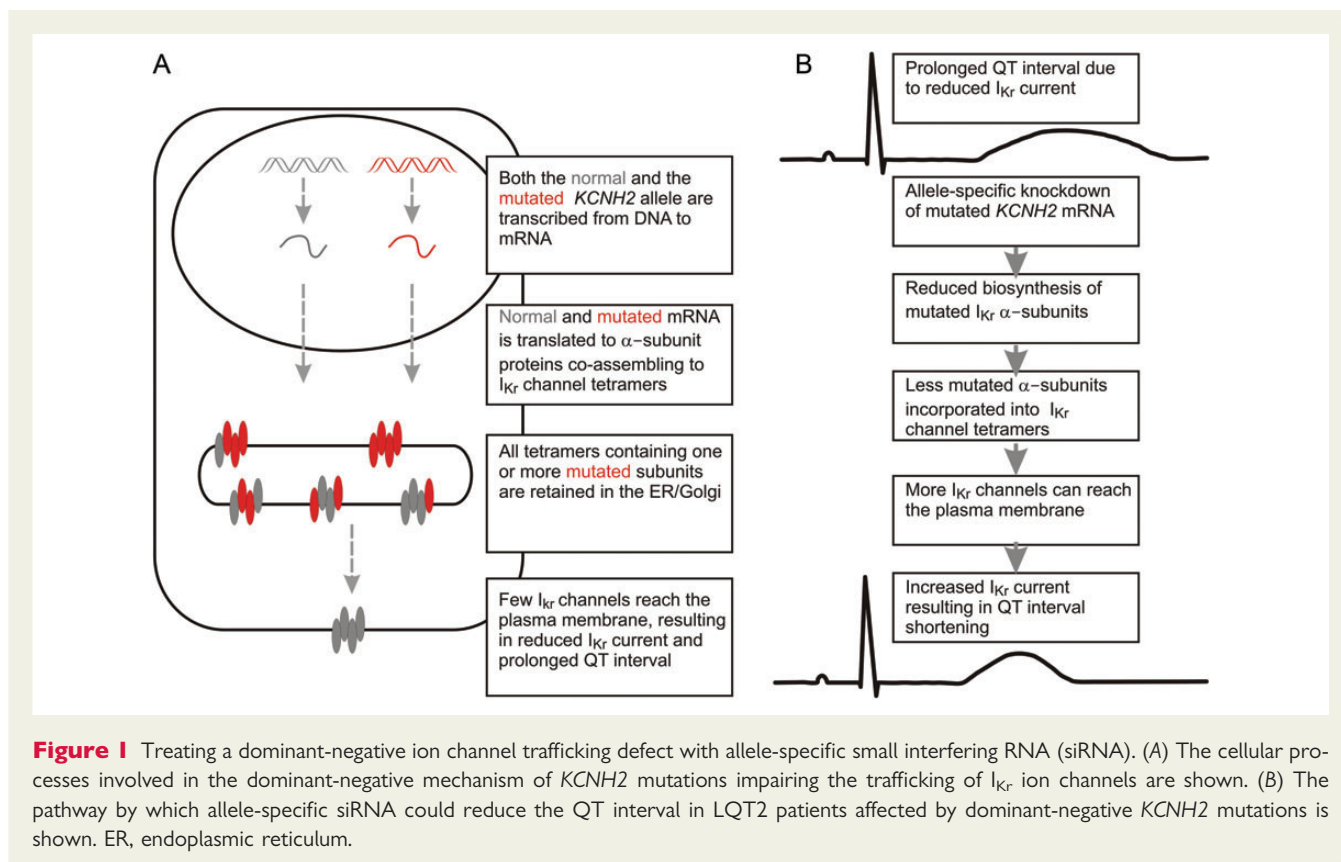
However, the above mechanism does not pertain to all dominant-negative LQT mutations. For example, in LQT1 caused

The opinions expressed in this article are not necessarily those of the Editors of the *European Heart Journal* or of the European Society of Cardiology.

<sup>†</sup> doi:10.1093/eurheartj/eh1067.

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by a dominant-negative R190Q mutation of *KCNQ1* (the gene encoding the  $\alpha$ -subunit of the channel responsible for the  $I_{Ks}$  current), trafficking analysis of co-transfected wild-type and mutated subunits suggested that tetramers containing no more than one mutated subunit can undergo normal trafficking, while tetramers containing two or more mutated subunits are retained in the endoplasmic reticulum.<sup>3</sup> Under these circumstances, the fraction of ion channels that are correctly integrated into the plasma membrane in a heterozygous patient would amount to 31.25%, which could be increased by a 60% knockdown of the mutated allele to 69%. This corresponds to little more than a two-fold increase, which may or may not be clinically relevant if applied to the treatment of patients.

What are the hurdles in the way of translating this methodology to the clinic? At first sight, considering that RNA-based therapies are already being used in clinical studies and even in clinical routine, one might think that this translation can be easily performed. Three RNA-based drugs have been approved for clinical use so far. Fomivirsen is an antisense nucleotide directed against a viral mRNA, and pegaptanib is a modified short RNA molecule binding specifically to vascular endothelial growth factor. Both drugs are applied by injection directly into the vitreous body of the eye to treat ophthalmic cytomegalovirus infection and wet age-related macular degeneration, respectively.<sup>4</sup> By using this route of delivery, it is possible to reach a high concentration of the drug at the desired site of action without much systemic effect. However, applying an RNA-based drug to the heart of patients will probably require more effort. Animal studies suggest

that systemic application of siRNA may be sufficient to reach therapeutic levels in the heart.<sup>5</sup> The only RNA-based drug approved by the Food and Drug Administration (FDA) for systemic use so far is mipomersene, which the Committee for Medicinal Products for Human Use (CHMP) has recently refused European approval due to safety concerns.<sup>6</sup> Mipomersene is an antisense oligonucleotide directed against apolipoprotein B, the main component of LDL cholesterol, and effectively lowers LDL cholesterol levels in patients with familial hypercholesterolaemia.<sup>7,8</sup> Major side effects found in clinical trials were flu-like symptoms, liver toxicity, and an apparently higher rate of serious cardiac events. Whether these side effects are specific to mipomersene or represent a general problem of systemically administered RNA-based drugs remains to be investigated.

Another important issue will be whether a homogenous concentration of the allele-specific siRNA throughout the myocardium can be achieved. In patients with LQTS, the so-called dispersion of repolarization, meaning different action potential durations in adjacent layers of cardiomyocytes, provides an important substrate for the development of re-entrant arrhythmias.<sup>9</sup> If the effect of an siRNA-based therapy across the myocardium is inhomogeneous, it might increase this dispersion, exposing the patient to an even higher risk of arrhythmic episodes.

Notwithstanding whether or not this concept will eventually find its way into the clinic, the work by Matsa and colleagues clearly demonstrates the value of hiPSC-derived cardiomyocytes as a platform to develop novel pharmacological approaches in the field of cardiac arrhythmias. While a heterologous expression

system might allow the study of the biophysical properties or the trafficking of an ion channel, it is not suitable to study the effect of mutations or treatments on the most relevant parameter, which is the duration of the cardiac action potential. Not all components that modulate this parameter in a human cardiomyocyte are known, which makes it hard to predict the effect of specific interventions. For example, the mother of the LQT2 patient, who also carries the heterozygous A561T mutation in the *KCNH2* gene, has no symptoms of LQTS and a much shorter QT interval than her daughter. The use of hiPSC-derived cardiomyocytes offers therefore the unique possibility to study the effect of a disease-causing mutation, and to develop therapies, in a patient-specific genetic background.

**Conflict of interest:** none declared.

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## CARDIOVASCULAR FLASHLIGHT

doi:10.1093/eurheartj/ehf475

Online publish-ahead-of-print 2 December 2013

### Mitral cleft repair by mitraclipping

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We present a case of a 51-year-old male patient with severe mitral regurgitation and a dilated left ventricle with a poor function (LVEF 15%). The mitral regurgitation initially was considered to be due to annular dilatation and malcoaptation. Mitral surgery was considered too riskful. The patient was accepted for mitraclip treatment. In this procedure, a posterior leaflet cleft was objected with three-dimensional trans oesophageal echocardiography. The regurgitation orifice was located at the cleft location, and was the cause of mitral-insufficiency. Mitraclips are made to be placed perpendicular on the leaflets, and cannot be placed on a cleft, which is itself perpendicular on the mitral closure orifice. Because surgical edge-to-edge mitral repair has been successful in a mitral cleft, we decided to do likewise with mitral clips. The first mitraclip was placed posteromedial right along the cleft, but did not reduce the mitral-insufficiency enough. There was a reduction of the mitral-insufficiency in the cleft, but a new mitral-insufficiency emerged in the more anterolateral closure. A second mitraclip was placed close to the cleft on the anterolateral side in a symmetrical fashion. This resulted in two left ventricular inflow orifices, while in between the clips the cleft was closed. Near complete reduction of the mitral-insufficiency was achieved without significant mitral stenosis.

This is the first report of feasibility of a mitraclip closure of a cleft, essentially by placing two mitraclips symmetrically in close proximity at both sides of the cleft perpendicular to the mitral closure orifice.

