ESC European Society of Cardiology

Cardiovascular Research (2022) **118**, 3011–3012
https://doi.org/10.1093/cvr/cvac164

INVITED EDITORIAL

Gene editing for cardiomyopathy takes a step forward

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Received 1 October 2022; editorial decision 6 October 2022; online publish-ahead-of-print 13 October 2022

This editorial refers to 'Gene editing reverses arrhythmia susceptibility in humanized PLN-R14del mice: modelling a European cardiomyopathy with global impact', by Jaydev Dave *et al.*, https://doi.org/10.1093/cvr/cvac021.

A man in his early 40s plays tennis at his club. Suddenly, he falls unconscious. A doctor from the nearby court runs to assist him. The man has no pulse, and the doctor starts resuscitation. A defibrillator is brought in. At the second discharge, the man's heart resumes beating. At hospital admission, the man reports that another member of his family died of sudden cardiac arrest, and another suffers a heart failure despite his young age. A genetic test reveals a pathogenic mutation in one of the genes associated with a condition known as arrhythmogenic cardiomyopathy (ACM). Doctors are pleased about this diagnosis, but the man and one of his children who has inherited the same mutation remain in despair. They are told that an implantable cardioverter defibrillator can prevent further life-threatening episodes of ventricular fibrillation, but also that there are no curative options, except for heart transplantation.

With a few variations, this is a recurrent story in cardiology.[1] In recent years, the definition of ACM has expanded beyond arrhythmogenic right ventricular cardiomyopathy, which is mainly caused by defects in the cardiomyocyte desmosome,[2] to include other genetic cardiomyopathies defined by the prominence of ventricular arrhythmias in the clinical presentation.[1] One of the non-desmosomal genes, the mutations of which can cause a dilated and arrhythmogenic phenotype, is phospholamban (PLN), a 52-amino acid protein that binds the cardiac muscle sarcoplasmic reticulum calcium-ATPase SERCA2a and inhibits its function. Among the ACM-causing mutations in PLN, a three-nucleotide deletion, which determines the absence of arginine 14 in the protein (PLNR14Del), has particular arrhythmogenic potential and results in an increased risk of malignant ventricular arrhythmias in young adult carriers.[3] This mutation is the most frequent single genetic cause of ACM in The Netherlands and has been identified in several other European countries and in North America.[4]

While the mutated genes causing ACM are heterogenous in nature, several of them are transmitted with a dominant or co-codominant mode of inheritance. This renders conventional gene therapy, based on the supplementation of a normal copy of the cDNA, ineffective or challenging, as high levels of the wild-type protein are needed to overcome the heterozygous defect. Essentially, this leaves these ACM patients with no disease-modifying options, except that of inhibiting or inactivating the disease allele. As far as the PLN14Del mutation is concerned, progress in gene inactivation has been recently achieved by antisense oligonucleotides that selectively target the mutation.[5]

A definitive cure for these forms of ACM would be the permanent inactivation of the disease allele using gene editing. Dave *et al.*[6] have now taken an important step towards achieving this ambitious goal. First, the same group.[7] generated a mouse model for the PLN R14del disease, in which the human gene with the mutation was knocked into the genome as a substitute for its mouse version. This mouse model is relevant for pathophysiological studies on the disease but also provides investigators the human sequence surrounding the mutation, in which to develop a gene editing approach that would also work in patients. The authors attempted at correcting the genetic defect by inducing the selective inactivation of the mutant PLN allele. The Cas9 nuclease, when targeted to a given genomic sequence by a specific guide RNA complementary to this sequence, introduces a double-stranded DNA break (DSB); then, this break is repaired in post-mitotic cells by non-homologous end joining (NHEJ), a process that leads to the introduction of small insertions or deletions (INDELs), which inactivate the gene.[8] Mice were injected systemically with an AAV9 vector coding for Cas9 and a single-guide RNA against the PLNR14Del 3 bp deletion. Targeted disease allele discrimination was proved to be effective by this approach, and the overall efficiency was sufficient to prevent the disease phenotype in terms of both dilation and stress-induced arrhythmias. The developed gene editing system did not induce significant mutations at other genome sites.

The state-of-the-art findings in Dave *et al.*[6] support the concept that a CRISPR/Cas9-based strategy is valuable for further preclinical development. However, as it commonly happens in science, they also highlight how gene editing is still in its infancy when applied to the adult heart. A first and foremost problem remains the efficiency of CRISPR/Cas9 delivery. AAV vectors are currently the gold standard for cardiac and muscle gene transfer but require very high titres, and their use can be fraught with serious side effects, as different clinical studies have recently experienced.[9] AAV vectors have the additional problem of expressing their transgene indefinitely. While this is beneficial for gene therapy applications aimed to supplement a missing factor, it can become problematic when the therapeutic effect is wanted for a very limited time as in gene editing. The continued expression of Cas9 can lead to unspecific genomic alterations and, being of prokaryotic origin, elicit an unwanted immune response against the transduced cells.[10] Novel technologies based on the transient administration of the Cas9 mRNA and the CRISPR guide RNA using non-viral methods such as lipid nanoparticles[11] hold great promise to overcome these problems.

In addition to these gene delivery issues, a major limitation of conventional CRISPR/Cas9 is that this technology induces DSBs that are repaired through NHEJ. This is an effective approach for PLN but, in the case of several other cardiac genes, could lead to allele insufficiency. In addition, allele discrimination by the targeting single guide RNA for PLNR14Delta is favoured by the nature of the mutation (a 3 bp deletion) but could be more problematic for single nucleotide point mutations. In these

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conditions, other more precise gene editing approaches can be considered. Among these, prime editing permits the direct insertion of new genetic information within the DNA using a guide RNA that targets a specific site and encodes the desired sequence,[12] while base editing (which would not be applicable to INDELs such as PLNR14Delta) directly introduces single nucleotide variants in DNA.[13] These methods have the additional advantage of not inducing DSBs into the host DNA. However, prime editing efficiency is still low *in vivo* and base editing is limited by the identity of the target sequence. A more versatile approach for perfect gene correction is homologous recombination (HR), in which DSBs are repaired using an exogenously provided template that carries the correct sequence.[14] However, HR is down-regulated in post-mitotic cells and would require prior awakening of the molecular machinery that performs homology-directed repair in cardiomyocytes.

Gene editing for curative purposes, which was not even conceivable 20 years ago, has already became a reality for cells that can be isolated *ex vivo*, such as haematopoietic stem cells or CAR-T cells, and is fast progressing in clinical trials for organs that can be transfected efficiently, such as the liver and the eye.[15] For cardiac diseases, we are still in the process of creating the proper conditions for gene editing to be effective, but our progress is continuous and incremental. The Dave *et al*. paper is another important brick to build our wall of confidence in this technology.

Funding

This work was supported by the British Heart Foundation Programme Grant RG/19/11/34633; the European Research Council Advanced Grant 787971 'CuRE'; the King's College London BHF Centre of Research Excellence grant RE/18/2/34213; by grants 825670 'CardioReGenix' and 874764 'REANIMA' from the European Commission Horizon 2020 programme, along with the continuous support of Fondazione CRTrieste, Trieste, Italy.

Conflict of interest: None declared.

References

1. Laws JL, Lancaster MC, Ben Shoemaker M, Stevenson WG, Hung RR, Wells Q, Marshall Brinkley D, Hughes S, Anderson K, Roden D, Stevenson LW. Arrhythmias as presentation of genetic cardiomyopathy. *Circ Res* 2022;**130**:1698–1722.
2. Corrado D, van Tintelen PJ, McKenna WJ, Hauer RNW, Anastastakis A, Asimaki A, Basso C, Bauce B, Brunckhorst C, Bucciarelli-Ducci C, Duru F, Elliott P, Hamilton RM, Haugaa KH, James CA, Judge D, Link MS, Marchlinski FE, Mazzanti A, Mestroni L, Pantazis A, Pelliccia A, Marra MP, Pilichou K, Platonov PGA, Protonotarios A, Rampazzo A, Saffitz JE, Saguner AM, Schmied C, Sharma S, Tandri H, Te Riele A, Thiene G, Tsatsopoulou A, Zareba W, Zorzi A, Wichter T, Marcus FI, Calkins H, International Experts. Arrhythmogenic right ventricular cardiomyopathy: evaluation of the current diagnostic criteria and differential diagnosis. *Eur Heart J* 2020;**41**:1414–1429.
3. van Rijsingen IA, van der Zwaag PA, Groeneweg JA, Nannenberg EA, Jongbloed JD, Zwinderman AH, Pinto YM, Dit Deprez RH, Post JG, Tan HL, de Boer RA, Hauer RN, Christiaans I, van den Berg MP, van Tintelen JP, Wilde AA. Outcome in phospholamban R14del carriers: results of a large multicentre cohort study. *Circ Cardiovasc Genet* 2014;**7**:455–465.
4. van der Zwaag PA, van Rijsingen IA, Asimaki A, Jongbloed JD, van Veldhuisen DJ, Wiesfeld AC, Cox MG, van Lochem LT, de Boer RA, Hofstra RM, Christiaans I, van Spaendonck-Zwarts KY, Lekanne dit Deprez RH, Judge DP, Calkins H, Suurmeijer AJ, Hauer RN, Saffitz JE, Wilde AA, van den Berg MP, van Tintelen JP. Phospholamban R14del mutation in patients diagnosed with dilated cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy: evidence supporting the concept of arrhythmogenic cardiomyopathy. *Eur J Heart Fail* 2012;**14**:1199–1207.
5. Beverborg NG, Spater D, Knoll R, Hidalgo A, Yeh ST, Elbeck Z, Sillje HHW, Eijgenraam TR, Siga H, Zurek M, Palmer M, Pehrsson S, Albery T, Bomer N, Hoes MF, Boogerd CJ, Frisk M, van Rooij E, Damle S, Louch WE, Wang QD, Fritsche-Danielson R, Chien KR, Hansson KM, Mullick AE, de Boer RA, van der Meer P. Phospholamban antisense oligonucleotides improve cardiac function in murine cardiomyopathy. *Nat Commun* 2021;**12**:5180.
6. Dave J, Raad N, Mittal N, Zhang L, Fargnoli A, Oh JG, Savoia ME, Hansen J, Fava M, Yin X, Theofilatos K, Ceholski D, Kohlbrenner E, Jeong D, Wills L, Nonnenmacher M, Haghighi K, Costa KD, Turnbull IC, Mayr M, Cai CL, Kranias EG, Akar FG, Hajjar RJ, Stillitano F. Gene editing reverses arrhythmia susceptibility in humanized PLN-R14del mice: modelling a European cardiomyopathy with global impact. *Cardiovasc Res* 2022;**118**:3140–3150.
7. Raad N, Bittihn P, Cacheux M, Jeong D, Ilkan Z, Ceholski D, Kohlbrenner E, Zhang L, Cai CL, Kranias EG, Hajjar RJ, Stillitano F, Akar FG. Arrhythmia mechanism and dynamics in a humanized mouse model of inherited cardiomyopathy caused by phospholamban R14del mutation. *Circulation* 2021;**144**:441–454.
8. Bennett EP, Petersen BL, Johansen IE, Niu Y, Yang Z, Chamberlain CA, Met O, Wandall HH, Frodin M. INDEL detection, the 'Achilles heel' of precise genome editing: a survey of methods for accurate profiling of gene editing induced indels. *Nucleic Acids Res* 2020;**48**:11958–11981.
9. Mullard A. Gene therapy community grapples with toxicity issues, as pipeline matures. *Nat Rev Drug Discov* 2021;**20**:804–805.
10. Crudele JM, Chamberlain JS. Cas9 immunity creates challenges for CRISPR gene editing therapies. *Nat Commun* 2018;**9**:3497.
11. Cannata A, Ali H, Sinagra G, Giacca M. Gene therapy for the heart lessons learned and future perspectives. *Circ Res* 2020;**126**:1394–1414.
12. Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, Chen PJ, Wilson C, Newby GA, Raguram A, Liu DR. Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature* 2019;**576**:149–157.
13. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* 2016;**533**:420–424.
14. Xue C, Greene EC. DNA repair pathway choices in CRISPR-Cas9-mediated genome editing. *Trends Genet* 2021;**37**:639–656.
15. Eisenstein M. Base editing marches on the clinic. *Nat Biotechnol* 2022;**40**:623–625.