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A CRISPR Approach to the Study and Treatment of Cardiovascular Disease

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Personalized medicine is based on the hypothesis that the best care considers the differences between individuals in reaching a diagnosis and treatment strategy. At one of the most basic levels, our differences are encoded in our DNA. Changes in DNA sequence within exon boundaries can create variations in amino acid sequence in our proteins; intergenic variations can lead to altered levels of gene expression by impacting promoter or repressor activity that alters gene transcription. Next generation DNA sequencing (at the whole exome or whole genome levels) has made it possible to comprehensively assess individual genetic variations. To assess the causality of identified genetic variants with a defined phenotype, it is important to test the impact of reversing the putative causal variant(s) to the common DNA sequence(s) found in the population. Until recently, accomplishing this level of gene editing was costly, expensive and technically challenging. The advent of gene editing with the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) Cas-9 technique has proved to be a transformative technology that has accelerated gene editing for novel diagnostic and therapeutic approaches.

In this issue, Rezaei and colleagues provide a comprehensive yet succinct review of CRISPR/Cas9, its function and utility in numerous applications and model systems (Rezaei et al., 2019). They begin by providing a brief background of CRISPR history, first discussing how CRISPR system was initially identified as a defense system in *Escherichia coli* and other bacteria and archaea. They introduce the six types of CRISPR systems, then focus the reader on the type II CRISPR/Cas9 combination that currently dominates research done in this area. Next they provide an overview of how CRISPR/Cas9 works in the cell. In short, CRISPR/Cas9 takes advantage of a cell's own DNA repair mechanism. Cas9, with the help of a single guide RNA (sgRNA) and a protospacer adjacent motif (PAM) sequence is able to

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identify specific sequences in the genome that are chosen by the investigator as their location of interest. Double-stranded cleavages created by Cas9 in the genome are repaired by one of two mechanisms: Non-homologous End Joining (NHEJ) is the most common yet most error-prone method; and Homology Directed Repair (HDR), which requires the use of a template stand, but which is much more precise and allows for more exact modifications in the genome. Lastly, they sum up the pros and cons of each of the repair methods as well as some insights into expected outcomes.

After providing this background, they introduce a range of applications for utilizing CRISPR/Cas9 technology in the cardiovascular space, beginning with *in vivo* and *ex vivo* gene therapy methods. *Ex vivo* studies involve modification of the genes in a specific cell population that can be removed from the patient that can be edited with the CRISPR technique and then reintroduced into the patient. *In vivo* methods are more complex, and require introduction of the genome editing technology directly into the patient. The authors note that there is a much greater chance of therapeutic success for monogenic conditions, where a single copy of a mutated gene can cause disease, relative to the more complex (but more common) polygenic disorders where multiple loci in the genome may contribute to the disease process. At this point they provide a variety of specific examples of mutations that have corrected using this technology, including one in which a mutation in the MYBPC3 gene that causes hypertrophic cardiomyopathy was corrected in a human embryo.

For the *in vitro* approaches, this review summarizes the challenges associated with the use of Zinc Finger Nucleases (ZFNs) and Transcription activator-like effector nuclease (TALEN) technologies for creating isogenic lines of patient-derived inducible pluripotent stem cells (iPSC) for modeling of diseases including Barth Syndrome, lipid disorders, electrophysiologic disorders and valvular heart disease. They note that whereas TALENs use 15 genes in somatic cell or iPSC cultures to produce mutated alleles, CRISPR-Cas9 is a simpler and more direct approach to these types of experimental problems. They discuss the creation of Cas9 guide RNAs to target genes of interest for similar goals, and the potential to use CRISPR/Cas9 to evaluate the impact of miRNAs on cardiac cell differentiation.

The authors present an *in vitro* study that documents use of CRISPR-Cas9 to edit Duchenne's Muscular Dystrophy (DMD) mutations in 3-dimensional engineered heart muscle, with the encouraging analysis that correction of only a subset (~30%) of the cardiac myocytes was sufficient to restore dystrophin expression and contractile function to near normal levels. In the same section, they discuss the use of CRISPR/Cas9 to simplify the process of transposing relatively large sections of DNA for manipulating the host genome, and the application of this technique for modifying four cardiac genes (ADRB2, GRK5, RYR2 and ACTC1).

Rounding out the discussion of *in vitro* applications, the authors describe a study conducted by Hai, et al. who used CRISPR/Cas 9 to delete the *Soat1* locus in mouse macrophages. *Soat1* is a segment of chromosome 1, exon 2 that encodes Acetyl-coenzyme A: cholesterol acetyltransferase 1 (ACAT1), a gene that esterifies free cholesterol. ACAT1 helps control cholesterol levels in macrophages and other cells. CRISPR deletion of the *Soat1* region

in mice enabled the authors to conclusively prove that ACAT1 was responsible for the cholesterol modifying effects of this quantitative trait locus (QTL) (Hai et al., 2018).

Rezaei and colleagues conclude with a discussion of *in vivo* genome editing options. They describe disease correction of the previously mentioned DMD in preclinical mice. NHEJ repair was able to cleave or repair the mutated exon in dystrophin gene which resulted in improved muscle function. Additionally, they have identified several potentially catastrophic consequences that could occur in the setting of therapeutic genome editing of the cardiovascular system. Examples include cases in which correction methods that use NHEJ can cause tumor formation, cellular dysfunction, unintentional cleavage of the healthy allele, and limitations with respect to the impact of checkpoints in the cell cycle and efforts related to use of CRISPR in terminally differentiated cells, including cardiomyocytes.

We commend the authors of this thoughtful review for providing a strong introduction to the background, theory and use of this powerful new technology that seems likely to advance our understanding of cardiovascular genetics and the field of personalized medicine.

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References cited

- Hai Q, Ritchey B, Robinet P, et al.: 2018. Quantitative Trait Locus Mapping of Macrophage Cholesterol Metabolism and CRISPR/Cas9 Editing Implicate an ACAT1 Truncation as a Causal Modifier Variant. *Arteriosclerosis, thrombosis, and vascular biology* 38:83–91.
- Rezaei H, Khadempour S, Farahani N, et al.: 2019. Harnessing CRISPR/Cas9 technology in cardiovascular disease. *Trends Cardiovasc Med*.