Current Status of Genome Editing in Cardiovascular Medicine

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Abstract During the past decades, numerous genetic mutations have been implicated in the pathogenesis of cardiovascular diseases (CVDs). With the launching of the Precision Medicine Initiative in January 2015, emerging technologies such as induced pluripotent stem cells (iPSCs) and genome editing are well positioned to provide powerful tools to correlate genotypes with phenotypes. These new technologies are helping to identify specific mutations associated with human CVDs. Patient-specific iPSC-derived cardiomyocytes (iPSC-CMs) offer an exciting experimental platform to model CVDs, study the molecular basis of cardiovascular biology, accelerate predictive drug toxicology tests, and advance potential regenerative therapies. By harnessing the power of genome engineering, scientists are uncovering the molecular mechanisms underlying the pathogenesis of CVDs, which will pave the way for the development of new personalized prediction, prevention, and treatment of diseases.

Keywords Genetic cardiovascular diseases • Cardiovascular disease modeling • Induced pluripotent stem cells • Genome engineering • Precision medicine

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K. Turksen (ed.), *Genome Editing*, DOI 10.1007/978-3-319-34148-4_7

Abbreviations

AAV	Adeno-associated virus
APD	Action potential duration
ARVD/C	Arrhythmogenic right ventricular dysplasia/cardiomyopathy
BAV	Bicuspid aortic valve
BTHS	Barth syndrome
CHD	Coronary heart disease
CPVT	Catecholaminergic polymorphic ventricular tachycardia
CRISPR/Cas	Clustered regularly interspaced short palindromic repeat (CRISPR)/ Cas (CRISPR-associated)
CVD	Cardiovascular disease
DCM	Dilated cardiomyopathy
DSB	Double-strand break
EAD	Early afterdepolarization
FH	Familial hypercholesterolemia
GWAS	Genome-wide association studies
HCM	Hypertrophic cardiomyopathy
HDR	Homology-directed repair
iPSC	Induced pluripotent stem cell
iPSC-CM	Induced pluripotent stem cell-derived cardiomyocyte
iPSC-EC	Induced pluripotent stem cell-derived endothelial cell
iPSC-SMC	Induced pluripotent stem cell-derived smooth muscle cell
KCNH2	Potassium channel voltage-gated eag related subfamily H, member 2
KCNQ1	Potassium channel voltage-gated KQT-like subfamily Q, member 1
LDL-C	Low-density lipoprotein cholesterol
LDLR	Low-density lipoprotein receptor
LQTS	Long-QT syndrome
LVNC	Left ventricular non-compaction
N1	Notch1
NHEJ	Nonhomologous end-joining
PCSK9	Proprotein convertase subtilisin/kexin type 9
PLN	Phospholamban
RCM	Restrictive cardiomyopathy
SaCas9	*Staphylococcus aureus* Cas9
SpCas9	*Streptococcus pyogenes* Cas9
TALEN	Transcription activator-like effector nuclease
TAZ	Tafazzin
TTN	Titin
VUS	Variant of uncertain significance
ZFN	Zinc-finger nuclease

Cardiovascular Disease Genomics

Precision Medicine

Cardiovascular disease (CVD) is a major health problem that affects more than 85 million individuals in the United States alone [1]. One of the key goals in biomedical research currently is to identify the specific genes and variants associated with CVDs in humans. Such anticipated outcomes promise to transform human health by enabling more personalized prediction, prevention, and treatment of cardiovascular diseases on an individual level. This precision medicine approach is principally based on the ability to diagnose and stratify patients into different treatment groups by correlating a patient's genotype with the associated cellular phenotype, which will indicate how the genetic differences among individuals could influence their responses to therapies [2]. However, realizing the potential to treat individual patients requires the development of an accurate and cost-effective diagnosis system and reliable disease models.

The Genetics of Cardiovascular Disease

Human cardiovascular diseases include a wide range of disorders, including congenital heart diseases, cardiomyopathies, vasculature, and electrical conduction disorders. Recent advances have shown that genetics are significant in conferring risk for these disorders [3]. More than 1000 genetic mutations in more than 100 genes have been associated with inherited CVDs [3, 4], including dilated and hypertrophic cardiomyopathy (DCM and HCM, respectively) [5–7], arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) [5, 8], long-QT syndromes (LQTS) [9, 10], aortic aneurysms [11], and hypercholesterolemia [12].

In 1975, Goldstein and colleagues identified a homozygous missense mutation in the low-density lipoprotein receptor (LDLR) gene in a patient with familial hypercholesterolemia (FH), representing the first demonstration of a causal genetic variant in Mendelian CVD [13–16]. Since then, many Mendelian forms (monogenic disorders) of CVD have been successfully identified by direct DNA sequencing or linkage analysis (Fig. 1) [4, 17]. However, this specific pattern of inheritance is rare and constitutes a minority of cases. Most of the common CVDs involve multiple genes, and their inheritance patterns can be variable and complex [18]. One of the main challenges in genetic research is to identify the genes that contribute to complex diseases. To this end, the publication of the first draft of the human genome in 2001 provided a valuable resource of detailed information about the structure, organization, and function of the nearly complete set of human genes [19, 20].

A decade later, the genome-wide association studies (GWAS), which examined genetic variants to determine the disease-causing variants between case and control subjects, have identified hundreds of loci associated with CVDs and traits [21]. Although

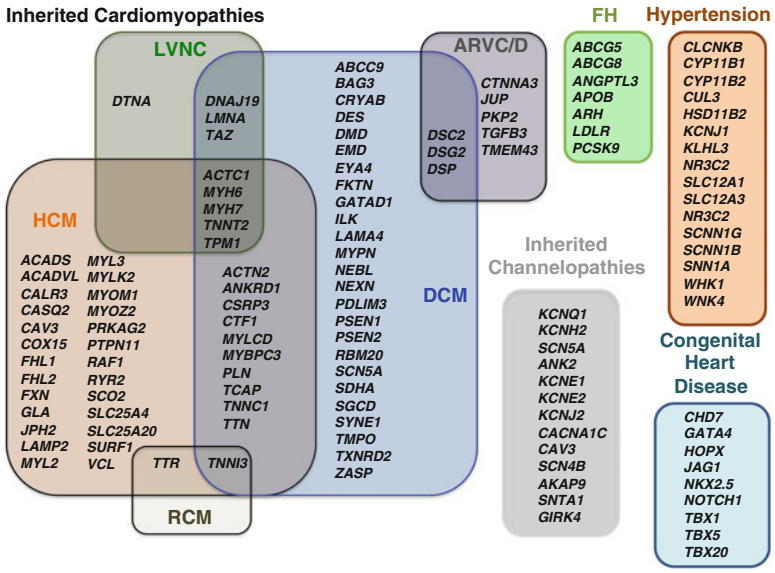

Fig. 1 The genetic basis of Mendelian cardiovascular diseases. Mutations in more than 100 genes have been associated with Mendelian cardiovascular diseases. Inherited cardiomyopathies, characterized by significant overlap, include hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), left ventricular non-compaction (LVNC), and arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D). *FH* familial hypercholesterolemia

a plethora of newly discovered loci associated with cardiovascular risk factors and disease have been reported, the application of these findings to diagnosis, risk prediction, prevention and treatment of disease is still in its infancy and requires further research.

In the span of just a few years, rapid advances in next generation sequencing technology, either targeted or genome-wide, have identified and will continue to discover numerous novel genes associated with CVD. Targeted sequencing is now used to sequence candidate regions of the human genome. In a recent study, Wilson et al. demonstrated that cardiomyopathy-associated gene mutations can be identified with high fidelity using a high-throughput, clinical-grade next-generation targeted sequencing assay, providing a powerful new tool for CVD variant discovery [17]. Genome-wide DNA sequencing consists of whole-exome and whole-genome sequencing. In whole-exome sequencing, rare genetic variants of CVD can be identified by sequencing the protein-coding regions in large cohorts with a strong evidence of heritability. Because the majority of genomic content is constituted of noncoding regions, whole-genome sequencing is a comprehensive approach to identifying novel variants in both coding and noncoding regions [19, 20]. Data from the ENCODE project suggest that 37 % of the total human genome might have a function and is probably regulated in a tissue-specific manner [22]. Recent studies by Cordell et al. demonstrated that mutations in the noncoding genomic regions are strongly associated with multiple congenital heart diseases, including tetralogy

of Fallot [23, 24]. Therefore, as whole-genome sequencing becomes more widely utilized, more pathogenic variants associated with coding and noncoding RNAs will be uncovered.

Disease Modeling with Human Pluripotent Stem Cells

The molecular mechanisms underlying the pathogenesis of CVDs remain poorly understood despite tremendous advances in genetics. Disease models have been and will continue to provide important insights into the molecular basis of cardiovascular biology and disease. Transgenic animal models and heterologous cell systems have proven to be highly valuable in understanding of human CVDs [25]. However, considerable differences exist between animal models and human cells, and therefore human-based models are particularly important for cardiovascular research [26].

The recent discovery of the human iPSC technology [27–30], and improvements in the differentiation method of iPSCs into disease-relevant cell types such as cardiomyocytes (iPSC-CMs) [31–34], smooth muscle cells (iPSC-SMCs) [35, 36], and endothelial cells (iPSC-ECs) [37, 38], now provide an unprecedented opportunity for the generation of human patient-specific cell-based models for disease modeling, personalized drug screening, and regenerative approaches [39]. Indeed, significant progress has been made in iPSC-CM technology, which has been used to model monogenic diseases in vitro [40, 41]. Diseases such as LQT (LQTS1 [42–44], LQTS2 [45–47], LQTS3 [48], LQTS8/Timothy syndrome [49]), catecholaminergic polymorphic ventricular tachycardia (CPVT) [50–51], ARVC/D [52–53], HCM [54], and DCM [55–59] have been further elucidated using iPSC-CMs. In principle, this technology provides a means by which a patient's pathophysiology can be studied in vitro. However, the extent to which studies using patient-derived iPSCs will offer any advantage in understanding CVD pathogenesis is yet to be determined.

In addition, the interpretation of any phenotypes observed in a patient's iPSC-derived cells can only be understood via comparison with appropriate control cells. The iPSC-based disease models do not account for possible confounders of genetic background differences between patient iPSCs that might be responsible for the phenotypic differences. Even in studies where healthy siblings have been used as controls for disease patients, the phenotypic differences observed could be the result of natural variance in the genome, rather than in the putative disease-associated mutations [60]. Thus, the ideal comparison would be between cell lines that differ only in the genetic variant (i.e., isogenic cell lines). One way to ensure a better comparison would be to use isogenic wild-type control and mutated cell lines derived by site-specific genome editing from the same parental cell line (Fig. 2).

Site-specific genome-editing technology enables targeted double-strand breaks (DSBs) of the DNA at chromosomal loci of interest [61, 62]. DSBs subsequently recruit endogenous repair machinery for either nonhomologous end-joining (NHEJ) or homology-directed repair (HDR) pathways. The NHEJ

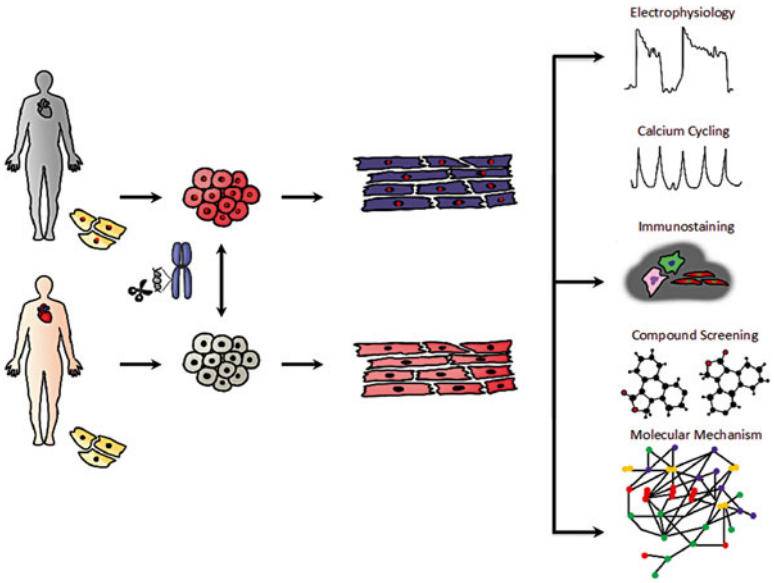

Fig. 2 Utilizing induced pluripotent stem cells (iPSC) and genome-editing technologies to model cardiovascular diseases. The genetic variability among human iPSCs can affect the outcome in modeling experiments. Site-specific genome engineering could in principle eliminate the variation arising from the iPSC line derivation and the genetic background. Isogenic iPSC lines (wild-type control and mutated cell lines) can be generated using the parental iPSC lines derived from healthy controls or patients. Generation of isogenic iPSC-CMs can be used to establish whether the observed in vitro phenotypes are the direct results of the disease-causing variant independently of the genetic background noise. Functional assays can be performed to identify disease-related molecular mechanisms. Additionally, this approach can be utilized in compound screening assays to find novel therapies and to determine the susceptibility of the genetic variant to drug-induced cardiac toxicity and arrhythmias. iPSCs induced pluripotent stem cells; iPSC-CMs induced pluripotent stem cell-derived cardiomyocytes

pathway generates random insertions or deletions at the site of DSBs, whereas HDR employs homologous donor DNA sequences from sister chromatids, homologous chromosomes, or exogenous DNA templates to produce precise genetic alteration. NHEJ and HDR are utilized for different aspects of genome engineering: NHEJ is specifically for gene inactivation, whereas HDR is for precise gene insertions, corrections, deletions, or base substitutions.

To date, four major classes of programmable nucleases, including meganucleases and their derivatives [63–66], zinc-finger nucleases (ZFNs) [67–76], transcription activator-like effector nucleases (TALENs) [77–85], and the clustered regularly interspaced short palindromic repeat (CRISPR)/Cas (CRISPR-associated) (CRISPR/Cas) system [86–94] have been developed to enable site-specific genome engineering in a precise and predictable manner (Table 1). Indeed, genes have been inserted into specific loci, and gene mutations have been introduced or corrected in human iPSC-based cardiovascular disease models. An increasing number of studies utilize genome editing and iPSC technologies to not only study the biological mechanisms of genetic CVD but also to provide personalized therapies for these diseases (Table 2).

Table 1 Comparison of three classes of site-specific nucleases

	ZFNs	TALENs	CRISPR/Cas
DNA-binding determinant	Zinc-finger proteins	Transcription activator-like effectors	crRNA or sgRNA
Nucleases	*Fok*I	*Fok*I	Cas
Success rate[a]	Low (~20–30 %)	High (>90 %)	High (>90 %)
Average disruption rate[a,b]	Low (~10 %)	High (~20 %)	High (~20 %)
Length of target site	18–36 bp	30–40 bp	23 bp
Restriction in target site	G-rich	Start with T and end with A	End with NGG or NAG sequence
Off-target effect	High	Low	Variable
Size	~1 kb × 2	~3 kb × 2	3.3 kb (SaCas9) or 4.2 kb (SpCas9) + 0.1 kb (sgRNA)

CRISPR/*Cas* clustered regularly interspaced short palindromic repeat (CRISPR)/Cas (CRISPR-associated), *crRNA* CRISPR RNA, *sgRNA* single chain guide RNA, *SaCas9 Staphylococcus aureus* Cas9, *SpCas9 Streptococcus* pyogenes Cas9, *TALEN* transcription activator-like effector nuclease, *ZFN* zinc-finger nuclease

[a]The success rates are based on studies using HEK293 cells [61, 120–125]

[b]The average disruption rate is based on the frequency of nonhomologous end-joining at the nuclease target site

Table 2 Summary of major efforts using genome-editing technology to model and treat cardiovascular diseases

Disorder	Study	Gene	Platform	Findings
Disease modeling of genetic cardiovascular diseases				
BTHS	Wang et al. [97]	*TAZ* (c.517delG, c.328 T>C)	CRISPR/Cas9-mediated NHEJ and HDR (random insertion and gene mutation, respectively)	Immature cardiolipin content, abnormal mitochondrial functions, impaired sarcomere organization, and depressed contractile stress generation
DCM	Karakikes et al. [56]	*PLN* (p.R14del)	TALEN-mediated HDR (gene correction)	Calcium-handling abnormalities and abnormal *PLN* protein distribution
	Hinson et al. [57]	*TTN* (p.W976R, c.V6382fs, p.A22352fs, p.P22582fs, c.N22577fs, c.T33520fs)	CRISPR/Cas9-mediated HDR (gene correction, mutation)	Sarcomere insufficiency, impaired responses to mechanical stress and beta-adrenergic signaling with A-band mutations

(continued)

Table 2 (continued)

Disorder	Study	Gene	Platform	Findings
LQTS1	Wang et al. [42]	*KCNQ1* (p.R190Q, p.G269S, p.G345E)	ZFN-mediated HDR (gene insertion)	Prolonged APD and EADs
LQTS2	Wang et al. [42]	*KCNH2* (p.A614V)	ZFN-mediated HDR (gene insertion)	Prolonged APD and EADs
	Bellin et al. [100]	*KCNH2* (p.N996I)	Conventional homologous recombination	Reduced cell membrane *KCNH2* channels, decreased IKr, prolonged APD
BAV	Theodoris et al. [102]	*N1* (p.R1108X, p. H1505del)	TALEN-mediated HDR (gene correction)	Epigenetic dysregulation resulting in derepression of pro-osteogenic and pro-inflammatory gene networks
Personalized therapy of genetic cardiovascular diseases				
FH	Ding et al. [111]	*PCSK9*	CRISPR/ Cas9-mediated NHEJ (exon 1) delivered by adenovirus	Decrease plasma *PCSK9* levels and plasma LDL-C
	Ran et al. [118]	*PCSK9*	CRISPR/ Cas9-mediated NHEJ (exon 1 and 5) delivered by AAV	Decrease plasma *PCSK9* levels and total cholesterol level

AAV adeno-associated virus, *APD* action potential duration, *BAV* bicuspid aortic valve, *BTHS* Barth syndrome, *CRISPR*/*Cas* clustered regularly interspaced short palindromic repeat (CRISPR)/ Cas (CRISPR-associated), *DCM* dilated cardiomyopathy, *EAD* early afterdepolarization, *FH* familial hypercholesterolemia, *HDR* homology-directed repair, *KCNH2* potassium channel, voltage-gated eag-related subfamily H, member 2, *KCNQ1* potassium channel, voltage-gated KQT-like subfamily Q, member 1, *LDL-C* low-density lipoprotein cholesterol, *LQTS* long-QT syndrome, *NHEJ* nonhomologous end-joining, *N1* notch1, *PCSK9* proprotein convertase subtilisin/kexin type 9, *PLN* phospholamban, *TALEN* transcription activator-like effector nuclease, *TAZ* tafazzin, *TTN* titin, *ZFN* zinc-finger nuclease

Disease Modeling of Genetic Cardiovascular Disease

Inherited Cardiomyopathies

Exciting progress has been made in defining the etiology of inherited cardiomyopathies, including HCM, DCM, restrictive cardiomyopathy (RCM), left ventricular non-compaction cardiomyopathy (LVNC), and ARVC/D [40, 95]. To date,

numerous mutations in more than 50 genes that are associated with the pathogenesis of inherited cardiomyopathies have been discovered [17]. Although molecular analysis efforts have revealed important insights regarding the role of genetics in cardiomyopathies, the underlying molecular mechanisms of HCM, DCM, RCM, LVNC, and ARVC/D remain unclear.

In recent years, the iPSC-CM technology has been used to model inherited cardiomyopathies [5, 41]. However, one of the major limitations still remains the variability resulting from the genetic background differences between iPSC lines. In a study by Wang et al., human iPSC-CMs were generated from two patients with Barth syndrome (BTHS), an inherited X-linked cardiac and skeletal mitochondrial myopathy caused by mutation of the gene encoding for tafazzin (*TAZ*) [96, 97]. The study used CRISPR/Cas9-mediated NHEJ to mutate *TAZ* and demonstrated that the mutated isogenic iPSC-CMs exhibited similar phenotypes as BTHS patient-specific iPSC-CMs, including immature cardiolipin content, abnormal mitochondrial functions, impaired sarcomere organization, and depressed contractile stress generation.

Genome-editing technology has also been utilized to study the pathogenesis of familial DCM. In a study by Karakikes et al., the p.R14del mutation in the coding region of the phospholamban (*PLN*) gene was corrected by TALEN-mediated homology-directed repair (HDR) in patient-specific iPSCs [56]. After differentiation into cardiomyocytes, the DCM phenotype was ameliorated in TALEN-corrected iPSC-CMs when compared to the isogenic *PLN* mutated cells, including alleviation of Ca^{2+}-handling abnormalities, electrical instability, and abnormal cytoplasmic distribution of the *PLN* protein.

Most recently, Hinson et al. utilized iPSCs and genome-editing technologies to evaluate the pathogenicity of titin (*TTN*) gene variants [57]. Their study used CRISPR/Cas9-mediated homologous recombination to introduce and correct either missense or frameshift mutations in several loci of the *TTN* gene, including four mutations affecting the A-band and two mutations impacting the I-band. By combining functional analyses with RNA sequencing of isogenic lines, they demonstrated that mutations in the A-band domain of the *TTN* cause DCM, whereas truncations in the I-band are better tolerated. This study also showed that the pathogenesis of *TTN*-induced DCM is associated with sarcomere insufficiency, as well as impaired responses to mechanical stress, and abnormal beta-adrenergic signaling.

Inherited Channelopathies

Long-QT syndrome (LQTS) is an inherited or acquired cardiac arrhythmic disease, predisposing patients to life-threatening ventricular arrhythmias and sudden cardiac death [9, 10]. Mutations in 13 genes have been implicated in the pathogenesis of familial LQTS [98]. The potassium channels, voltage-gated KQT-like subfamily Q, member 1 (*KCNQ1*; LQTS1), and voltage-gated eag-related subfamily H, member

2 (*KCNH2*; LQTS2) are the most common mutated genes associated with LQTS. Because both *KCNQ1* and *KCNH2* function as homotetramers, the mutated monomer displays a dominant-negative effect by impairing the tetramerization of wild-type monomers [42].

In the past 5 years, LQTS1 [42–44], LQTS2 [45–47], LQTS3 [48], and LQTS8/Timothy syndrome [49] have been modeled by iPSC-CMs [9, 99]. However, this approach can still be somewhat limited because it is difficult to obtain patient samples with the desired genetic variants. A study by Wang et al. represented an exciting first step in producing human cardiomyocytes that recapitulated LQTS by inserting the mutated genes in the safe-harbor locus (*AAVS1*/*PPP1R12C*) [42]. Their study utilized ZFNs to insert an expression cassette encoding a pathogenic variant of *KCNQ1* and *KCNH2* into wild-type iPSCs. The *KCNQ1*-mutated and *KCNH2*-mutated iPSC-CMs showed prolonged action potential duration (APD) and calcium-handling abnormalities when compared to the isogenic control iPSC-CMs. This study demonstrated an alternative approach to using actual patient samples and represents a novel way to study genetic variants that are known to display dominant negative effects.

Another study by Bellin et al. utilized a conventional HDR strategy (without using site-specific nucleases) to generate isogenic mutated and wild-type lines of a heterozygous missense *KCNH2* p.N996I mutation [100]. Correction of the mutation restored the electrical current conducted by the HERG channel (IKr) and the action potential duration in iPSC-CMs. As expected, introduction of the same genetic mutation reduced the IKr and prolonged the action potential duration in iPSC-CMs. Their study demonstrated that the isogenic mutated iPSC-CMs expressed fewer *KCNH2* channels at the cell membrane than the isogenic wild-type iPSC-CMs. Further treatment with the proteasome inhibitors, lactacystin and leupeptin, increased the protein levels of *KCNH2* on the cell membrane in the mutated iPSC-CMs, which may suggest a role of proteasomes in the pathogenesis of LQTS2.

Inherited Valvulopathies

In the past decade, congenital defects of the aortic valve known as bicuspid aortic valve (BAV) have been associated with genetic variants of a membrane-bound transcription factor, NOTCH1 (*N1*) [101]. BAV occurs in 1–2 % of the population and involves the formation of two valve leaflets instead of the normal three leaflets. Although the mechanism remains largely unknown, BAV is a major risk factor for early aortic valve calcification, a condition that impairs the opening of the valve and is responsible for more than 100,000 valve transplants annually in the United States.

A study by Theodoris et al. recruited two families carrying a *N1* heterozygous nonsense mutation, which is suspected to cause congenital BAV [102]. Their study utilized TALEN-mediated HDR to correct the *N1* mutation in patient-specific iPSC lines. Comparing the isogenic mutated and control human iPSC-ECs, the *N1* mRNA levels were found to be reduced by 30–40 % in the isogenic mutated iPSC-ECs, which indicates that the *N1* mutation displays a haploinsufficient effect on the

pathogenesis of BAV. After exposing the isogenic iPSC-ECs to shear stress, the *N1* isogenic mutated cells demonstrated epigenetic dysregulation, resulting in derepression of latent pro-osteogenic and pro-inflammatory gene networks.

Personalized Therapy of Genetic Cardiovascular Diseases

Challenges in Therapeutic Genome Engineering

Although different cell types have distinct abilities to repair DSBs, the phase of the cell cycle primarily governs the choice of whether the NHEJ or HDR pathways are utilized. NHEJ dominates DNA repair during the G_1-, S-, and G_2-phases, whereas HDR is limited to the late S- and G_2-phases [103, 104]. This difference in cellular activity makes it more challenging to treat diseases that require HDR-mediated DSB repair (gene correction or gene insertion) than those requiring NHEJ-mediated repair (gene inactivation). Several studies have demonstrated that chemical or genetic interruption of the NHEJ pathway can favor HDR; however, such manipulations can be difficult to employ and are harmful to cells [105, 106].

The potential use of genome-editing technology in cardiovascular therapy can be divided into two approaches: those carried out in vitro or those in vivo (Fig. 3a, b, respectively). With established in vitro approaches, the editing pro-

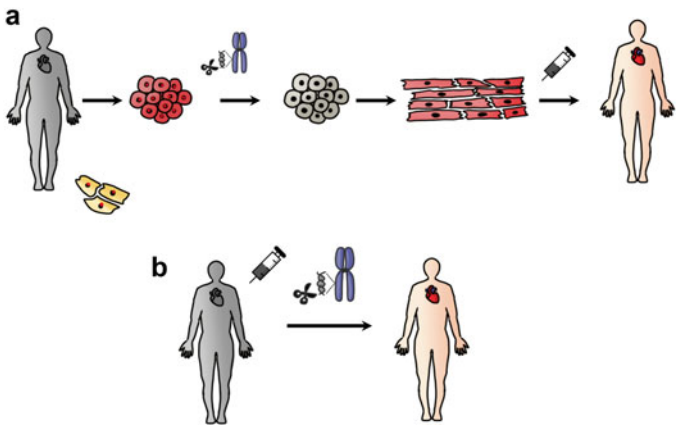

Fig. 3 Personalized therapy of genetic cardiovascular disease (CVD). The potential use of genome-editing technology in cardiovascular therapy can be divided in two approaches: those carried out in vitro or those in vivo. (**a**) With established in vitro approaches, the editing process is achieved via inpatient-specific iPSC lines in culture, and these iPSC lines are subsequently differentiated into the human cell type of interest by using established differentiation protocols. These edited cells can be delivered to patients to treat specific CVDs. (**b**) In vivo genome-editing therapy is achieved by delivering programmable nucleases to patients to correct or disrupt the mutations of interest

cess is achieved in human iPSC lines in culture, and these iPSC lines are subsequently differentiated into the human cell type of interest, such as iPSC-CMs or iPSC-ECs, by using established differentiation protocols [31–34, 37, 38]. These corrected cells can be delivered to patients to treat specific CVDs. By contrast, in vivo genome-editing therapy is still very much a work in progress. For instance, one limitation is that adult cardiomyocytes are arrested in the G_0-phase, in which the HDR mechanism is inactive and NHEJ is very limited. Furthermore, adult cardiomyocytes have a very low rate of replication [107], so that to achieve therapeutic effects, the efficiency of modification must be quite high. Nonetheless, in vivo genome-editing therapy is technically more feasible in certain cell types, including hepatocytes and satellite cells, mainly because these types of cells replicate better and theoretically could outcompete the native diseased cells [108–110].

Current Targets for In Vivo Genome-Editing Therapy

The concentration of low-density lipoprotein cholesterol (LDL-C) in the blood is among the most established causal risk factors for coronary heart disease (CHD) [111]. Pharmacological agents that reduce LDL-C levels, namely statins, are currently the most effective means of reducing this coronary heart disease risk. However, a large proportion of patients are intolerant to statin therapy. Proprotein convertase subtilisin/kexin type 9 (*PCSK9*) has been identified as the cause of autosomal dominant FH [112]. *PCSK9* is specifically expressed in and secreted from the liver and functions as an antagonist to the LDLR. Therefore, *PCSK9* has now emerged as a promising therapeutic target for the prevention of CHD.

Studies have shown that individuals with loss-of-function mutations in *PCSK9* experienced a significant reduction of LDL-C levels and consequently CHD risk [113–116]. As might be expected, gain-of-function mutations elevate LDL-C levels, leading to early-onset CHD in patients diagnosed with FH [112]. In 2015, a *PCSK9*-targeting monoclonal antibody (alirocumab) was approved by the U.S. Food and Drug Administration for the treatment of FH [117]. Although this antibody has been shown to be effective for the treatment of FH, its effects on LDL-C are transient. Patients must receive injections every few weeks to maintain the desired level of *PCSK9*-targeting monoclonal antibody.

A study by Ding et al. utilized CRISPR/Cas9-mediated NHEJ to permanently disrupt the mouse *PCSK9* gene in vivo [111]. They demonstrated that after the administration of adenovirus to express a CRISPR/Cas9 targeting *PCSK9* in mice, at least 50 % of the *PCSK9* alleles in the liver were altered; this resulted in a significant decrease in plasma *PCSK9* levels, as well as an increase in hepatic LDLR levels and a reduction of plasma LDL-C level by 35–40 %. Their study is the first to demonstrate the potential of genome engineering in vivo for the prevention of CVD.

More recently, a study by Ran et al. demonstrated that Cas9 from *Staphylococcus aureus* (SaCas9) can alter the genome with an efficiency similar to that of Cas9 from *Streptococcus pyogenes* (SpCas9), while being encoded by a gene that is more than 1 kilobase (kb) shorter [118]. The smaller SaCas9 (~3.3 kb) enabled in vivo genome engineering using adeno-associated virus (AAV) that would otherwise be prohibited by the AAV's restrictive cargo size (~4.5 kb) [119]; the study then utilized CRISPR/SaCas9 to target the *PCSK9* gene in the mouse liver. As in the previous study [111], more than 40 % of gene modification was observed, accompanied by significant reductions in serum *PCSK9* and total cholesterol levels. Assessment of off-target effects by targeted deep sequencing did not show any indel formations. This study suggested that in vivo genome editing using the novel CRISPR/SaCas9 has the potential to be both highly efficient and specific.

Conclusions and Future Perspectives

With the launching of the Precision Medicine Initiative, rapidly emerging technologies such as iPSCs and genome editing are well positioned to provide powerful tools for studying genotype–phenotype associations, for predicting the cardiovascular risks of individual patients and their responses to therapies [2]. The iPSC technology is revolutionary and continues to evolve. As it becomes easier to edit mutations in iPSCs, it will become feasible to test genetic variants of uncertain significance (VUS), and to assess the importance of genetic modifiers on disease penetrance [9] (Fig. 4). The genome-editing technology presents a novel and rapidly advancing technology with exciting applications. However, significant challenges remain, including enhancing specificity and minimizing off-target effects, increasing efficiency, and improving the selection of targeted sites and delivery methods, and especially for in vivo genome engineering. Further refinements are needed to fully exploit the potential of genome editing to be a vital tool of future precision medicine treatment for CVD.

We envision that the use of genome engineering to generate human cell-based disease models will become a standard approach in the laboratory, allowing researchers to decipher the molecular mechanisms of genetic variants and unlock the secret of CVDs. Nevertheless, many obstacles remain unresolved at this point. Population-based data sets will be necessary to identify novel genetic variants that are contributors to CVDs. Bioinformatics will be an important tool to determine the casual relationship between genotypes and phenotypes, as most of the diseases in question will be polygenic. Finally, in the genetic diagnosis aspect, research should focus on improving the accuracy, flexibility, turnaround, and cost of the next-generation sequencing.

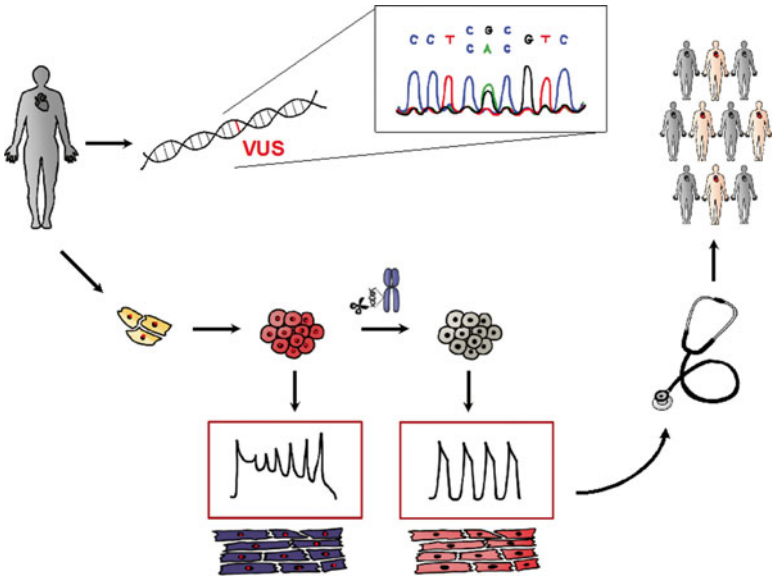

Fig. 4 Future perspectives of genome-editing technologies in cardiovascular research. Clinical genetic testing attempts to identify a rare variant in genes associated with CVDs. When a known pathogenic variant is identified, this may guide the clinical diagnosis, and genetic screening can be offered to family members to identify those at risk for developing the disease. When a variant of uncertain significance (VUS) is identified, a genome-corrected iPSCs can be developed, and functional assays can be done on the isogenic iPSC-CMs to detect the phenotypic abnormalities between isogenic wild-type control, and mutated iPSC-CMs; this may help to reclassify the VUS as possibly disease causing and place it in a similar category as a pathogenic variant. This technique can also be utilized to identify novel genetic modifiers or variants that may be associated with variability in drug responses

Acknowledgments We gratefully acknowledge Joseph Gold, Jade Chao, and Blake Wu for critical reading, and Varachaya Khwanjaipanich for preparing the illustrations. This work was supported from the National Institutes of Health (NIH) R01 HL113006, R01 HL123968, R01 HL125627, and R01 HL128170, and American Heart Association Established Investigator Award, Burroughs Wellcome Fund (J.C.W.), NIH K99 HL104002, AHA 15BGIA22730027, and Stanford CVI Seed Grant (I.K.), and German Research Foundation (T.S.).

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