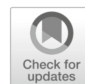

Genome Editing in Dyslipidemia and Atherosclerosis

Zhifen Chen, Constanze Lehertshuber, and Heribert Schunkert

Abstract

Despite successive advancement of genome editing technology with zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), the recent breakthrough in the field has been related to clustered regularly interspaced short palindromic repeats/associated proteins (CRISPR/Cas). The high efficiency and convenience of CRIPSR/Cas systems dramatically accelerate pre- and clinical experimentations of dyslipidemia and atherosclerosis. In this chapter, we review the latest state of genome editing in translational research of dyslipidemia and atherosclerosis. We highlight recent progress in therapeutic development for familial dyslipidemia by genome editing. We point to the challenges in maximizing efficacy and minimizing safety issues related to the once-and-done therapy focusing on CRISPR/Cas systems. We give an outlook on the potential gene targets prioritized by large-scale genetic studies of cardiovascular diseases and genome editing in precision medicine of dyslipidemia and atherosclerosis.

Keywords

Dyslipidemia · Atherosclerosis · Genome editing · CRISPR/Cas · Familial hypercholesterolemia · Genome-wide association study · Precision medicine

1 Dyslipidemia and Atherosclerosis

Atherosclerosis represents the major cause of coronary artery disease and thereby mortality worldwide [1]. The complex etiology of atherosclerosis is initiated by dysfunctional endothelial cells lining the arteries that are no longer capable of appropriately regulating vascular tone and permeability for molecules and cells [2]. Progressive infiltration of lipoprotein particles carrying cholesterol into the vessel wall triggers an inflammatory response mediated by cholesterol-loaded macrophages. Proliferation of smooth muscle cells causes vascular remodeling and ultimately leads to narrowing of the vessel and obstruction of blood flow. Dyslipidemia, a common and strong risk factor for atherosclerosis, describes elevated plasma levels of low-density lipoprotein cholesterol (LDL-C), lipoprotein(a) (Lp(a)), and/or triglyceride-rich lipoproteins (TRLs, VLDL, and IDL) [3] and/or decreased levels of

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high-density lipoprotein cholesterol (HDL-C) [4]. In addition to lifestyle and environmental influences, dyslipidemia is largely determined by genetic factors. Its extreme forms are manifested as familial dyslipidemias caused by gene mutations, including hypercholesterolemia (e.g., *LDLR*, *APOB*, *PCSK9*, *LPA*, and *ANGPTL3*) [5–7], hypertriglyceridemia (e.g., *LPL*, *APOC3*, *APOC2*, *APOA5*, *ANGTPL4*, *GPIHBP1*, and *LMF1*), dysbetalipoproteinemia (e.g., *APOE*), analphalipoproteinemia (e.g., *ABCA1*), LCAT deficiency [8], and combined hyperlipidemia (e.g., *USF1*) [9, 10]. Familial hypercholesterolemia (FH), the most common form of the overall rare dyslipidemias, occurs in 1 out of 200,000–250,000 people heterozygously and in 1 out of 160,000–320,000 people homozygously [11, 12]. To fulfill the pressing need of precision medicine, efforts have been increasingly committed to developing targeted therapies for dyslipidemia and atherosclerosis.

2 Current Therapies of Dyslipidemia and Atherosclerosis

2.1 From Traditional Pharmacology to Targeted Therapy

Pharmacological treatment of dyslipidemia and atherosclerosis predominantly focuses on cholesterol lowering [4]. For many years, statin (inhibiting cholesterol synthesis), ezetimibe (suppressing intestine uptake of cholesterol), and bile acid sequestrants have been the major treatments of the conditions [13, 14]. However, a significant proportion of patients do not achieve guideline-recommended cholesterol levels with these medications. Recently approved bempedoic acid further reduces LDL by about ~20% [15]. PCSK9 monoclonal antibodies [16], another new drug type, enable effective LDL reduction in addition to statin therapy but with high costs, hampering the general use. While small molecules targeting PCSK9 are under investigation to bring down the cost, drugs lowering other causal lipids and their inflammatory responses are on the way to treat the residual cardiovascular risk [17]. Revolutionary discoveries of human genetics in the past decade have been a nutritious ground for novel drug developments [18–20]. Genetic studies of atherosclerosis, coronary artery disease [1], and myocardial infarction (MI) not only nominated but also validated causal genes, pathways, and risk factors for the conditions. For instance, genetic studies supported Lp(a) and TGs as causal risk factors for atherosclerosis, which led to intensive investigations of related genes, such as *LPA*, *APOC3*, *ANGPTL3*, and *ANGPTL4* [3, 21–23]. Based on a better understanding of the affected mechanisms, these genes evolved as novel targets for biological drugs, monoclonal antibodies, and nucleic acid-based therapies [24].

2.2 Nucleic Acid-Based Therapy

Nucleic acid-based therapies were initially designed as replacement for dysfunctional genes by delivery of the correct coding sequence [25]. Recently, this concept has been expanded to include gene silencing by antisense oligonucleotides, or short interfering RNAs (siRNA), transcriptional modulation by microRNAs, and long noncoding RNAs (lncRNA), as well as modification of epigenetics and genome editing [25–27]. For instance, gene supplementation of *LDLR* is currently investigated in a phase 1/phase 2a first-in-man trial (NCT02651675) for homozygous FH due to function loss of the gene [28]. AON (antisense oligonucleotide)- and/or siRNA-based therapies targeting several dyslipidemia genes have been intensively tested in large-scale clinical trials for treating atherosclerotic CAD, such as *APOA*, *PCSK9*, *APOC3*, and *ANGPTL3* [29–34]. LncRNA BM450697 was reported to regulate LDLR via epigenetic-dependent mechanism, and siRNAs targeting the lncRNA enhanced hepatic cholesterol uptake [35]. These novel therapeutic strategies not only expand the druggable genome that previously was largely limited to enzymes, membrane proteins, and circulatory factors but also potentially have advantages of

specificity, efficacy, and safety. However, limited half-lives of nucleic acids, requirement of frequent injection, and medication compliance are general limitations. The limitations are not applicable for gene editing-based therapies that could introduce permanent therapeutic changes to specific gene targets. It is conceivable that in the future, a single administration of such drugs mediates durable cure of dyslipidemias and atherosclerosis.

3 Genome Editing

3.1 Evolution of Genome Editing Technology

Genome editing generally refers to the specific modification of nucleotide sequences (mainly DNA) by enzymic activities (e.g., nucleases and nickase) [36]. In a broader sense, it also includes RNA editing. Nucleases usually cut a nucleotide sequence and create damage (typically a double-strand break (DSB)), whereas nickases introduce single-strand breaks (SSB) [37]. Both DSB and SSB in turn trigger natural genetic repair mechanisms, such as nonhomologous end joining (NHEJ) and homology-directed repair (HDR) enabled by a homologous-armed template [38]. The cellular repairing machinery is hijacked to install precise nucleotide manipulations.

In the late 1970s, the first generation of gene editing tools was engineered based on hybrid proteins including zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) [39–41]. Both types of nucleases rely on a recombinant recognition domain to bind the target DNA sequence. Target-specific ZFN and TALEN engineering involves in tedious designing and screening of the optimal recombinant protein with high binding accuracy and affinity. Of note, TALENs have reached clinical experimentation to generate universal allogeneic CAR T-cells for B-cell lymphoma [42, 43].

Ever since 2012, genome editing has become easier, faster, and more economic, due to the discovery and engineering of RNA-guided gene rewriting technology—the CRISPR/Cas system [44]. The new system holds promise to cure genetic diseases through (1) inactivating detrimental or aberrant gene expression, (2) amending disease-causing or associated mutations, or (3) targeted insertion of therapeutical DNA (Fig. 2a–c). CRISPR/Cas harnesses the marriage of two independent components, the small guide RNA (sgRNA) and a Cas protein. The allocation of two functions of the traditional recombinant nucleases into the nucleotide sequence recognition by a sgRNA and the enzymic cutting by a Cas nuclease dramatically simplified the design and construction of the editing tools. The classic CRISPR/Cas9 system creates DSB and relies on NHEJ for gene knockout and HDR for an error-free DNA retyping. For newer types of CRISPR tools, the nuclease activity of a Cas protein was either inactivated to only bring transcriptional activators or suppressors to the targeted genomic site [46–48] or transformed into nickase tandem to other enzymes, such as deaminases in base editors (BEs) [49]. By directly triggering chemical reaction (deamination) on DNA and converting C to T (CBE) or A to G (ABE), BEs allow gene knockout without DSB and individual nucleotide(s) rewriting independent of a template, which hold promise for therapeutic gene editing with minimum off-target effects. In fact, point mutations represent the most common genetic variations associated with human diseases [50]. Recently, more types of Cas proteins, such as Nme2Cas9 and Cas13, have been discovered, extending the coverage of editable genome and enabling RNA manipulation [51, 52].

3.2 In Vivo Delivery of Genome Editing Systems

Intracellular delivery of gene editing tools has been the most challenging step in vivo. Adeno-associated virus (AAV)-, adenovirus-, and lentivirus-mediated delivery systems have been tested for CRISPR-based gene therapy [53, 54]. Due to lower immunogenicity, non-integrative and high efficiency, AAVs are widely used in CRISPR-based ex vivo and in vivo biological research and therapeutic

development. However, the packaging limit of AAV (~4.7 kb) often hampers its applications. Thus, instead of spCas9 (~4.1 kb), saCas9 (~3.2 kb) is usually employed for AAV-based gene editing, which allows all-in-one CRISPR therapy carrying both saCAS9 and sgRNA sequences on the same vector [55]. Generally, immunogenicity and potential transgene integration are of high concern when viral vectors were chosen for therapeutic development. Therefore, efforts have been exerted in seeking nonviral carriers for CRISPR-mediated gene [53].

Another promising in vivo delivery method involves the encapsulation of CRISPR/Cas into nanocarriers, in the forms of RNA-protein complex (RNP) or coding nucleic acids (DNA plasmids or mRNAs). In particular, delivery by lipid nanoparticles (LNP) achieves efficient targeting of specific tissues and protects the loaded proteins and nucleic acids [56–58]. Advanced LNP technologies for gene editing include self-assembled DNA nanoclews [59], cationic LNP and lipoplexes [60–62], gold nanoparticles [63–65], and zeolitic imidazole frameworks [66]. Most approaches harness electrostatic interactions between guest and host. Despite the promise, delivery of RNPs has been the most challenging due to the strong negative charge of sgRNA, the large size of Cas proteins, and the sensitivity of RNPs to denaturation and degradation during formulation and delivery. To date, the development of stable and organ-specific nanoparticles for delivery of CRISPR toolkits remains elusive.

4 Genome Editing in Dyslipidemia and Atherosclerosis

4.1 Genome Editing: A Driving Force for Dyslipidemia and Atherosclerosis Research

Ever since the applicable invention of CRISPR/Cas9 system in 2012 [67], it has been increasingly used in cardiovascular research (Fig. 1) and fosters delicacy of cellular and animal models for dyslipidemia and atherosclerosis research. Patient-induced pluripotent stem cell (hiPSC) line of carrier of heterozygous p.C310R (c.928 T > C) mutation in *LPL*, encoding lipoprotein lipase, has been reprogramed to model familial hypertriglyceridemia (FHTG). In parallel, researchers generated a mutation-corrected isogenic iPSC line (AHQUi001-A-1) using CRISPR/Cas9 technology [68]. The isogenic pair could differentiate into relevant cell types, such as adipocyte and endothelial cells, and test therapeutic modifications for the patient. Cell banks, such as WiCell, provide as precious resources of isogenic hiPSCs for dyslipidemia

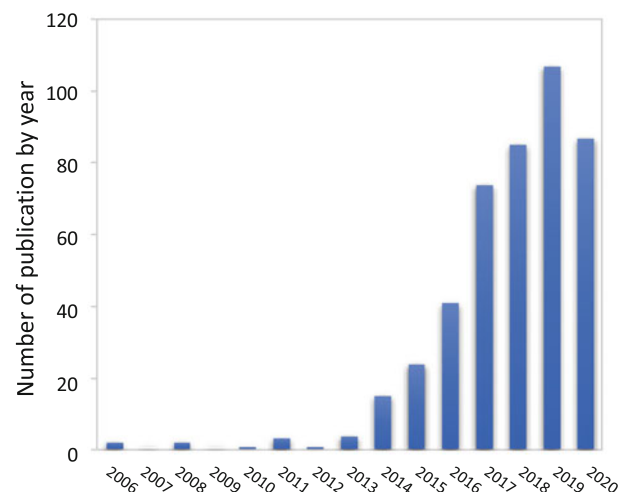

Fig. 1 PubMed search of “gene editing in cardiovascular diseases” for the last 15 years. Numbers of studies by year as indicated, figure adapted from PubMed statistics

and coronary artery disease. Given that the CRISPR/Cas system relies on open chromatin to screen the matched gene code, the efficiency of the gene editing heavily depends on the proliferation and transcription activity of cells. Hyperproliferative cells, such as stem cell and cancer cell, are relatively easy to target with high efficiency. Therefore, gene editing in hiPSC has been valuable in cardiovascular research. It comes with high efficiency for differentiation of many disease relevant cell types that are challenging to access or target, such as hepatocytes, adipocytes, immune cells, endothelium cells, and vascular smooth muscle cells [69].

CRISPR/Cas systems have substantially reduced the time and cost to generate animal models of germline gene knockouts or somatic targeting in vivo. The high efficiency of CRIPSR/Cas allows genetic modification of multiple genes at any time points of an animal's lifespan. The diverse CRISPR tools allow the flexibility in duration of editing, conditional alleles, tissue-specific targeting, and directions of modulation. Yang and Jaenisch et al. have established a protocol to create gene-modified mice by piezo-driven injection of Cas9 mRNA and sgRNA into zygotes. The authors showed that, beginning with target design, the time frame for generation of transgenic mice can be as short as four weeks [70]. Currently, this method and similar others are commonly used for cardiovascular research. For example, Yu and Cowan et al. generated G protein-coupled receptor 146 (GPR146) deficiency mice and showed that the deficiency protected against hypercholesterolemia and atherosclerosis [71]. To establish atherosclerosis mouse models using CRISPR in adult mice, Jarrett et al. performed somatic knockout of *Ldlr* via AAV8 medicated delivery of all-in-one AAV-CRISPR. The approach robustly disrupted *Ldlr* and resulted in severe hypercholesterolemia and atherosclerotic lesions in the mouse aorta [72]. Although the cholesterol increase induced by the somatic *Ldlr* knockdown was not as high as by germline *Ldlr* knockout, it might better model the chronic condition of atherosclerosis which usually develops at higher age [72]. Similar approaches were adopted to generate atherosclerosis animal models in rabbit, pig, and hamster by knocking out *Ldlr*, *Apoe*, or *Lcat* (lecithin-cholesterol acyltransferase) [73–76]. The success of the transgene models, on the other hand, suggested the effectiveness of in vivo CRISPR/Cas system in testing novel gene functions in dyslipidemia and atherosclerosis. Indeed, the novel role of CCC(COMMD-CCDC22-CCDC93) complex in hepatic cholesterol metabolism was explored and confirmed by somatic CRISPR/Cas targeting of *Commd* and *Ccdc22* in mice [77, 78].

However, as for point mutation correction, the editing efficiency of CRISPR/Cas remains low. Omer et al. attempted to correct the loss-of-function mutation E208X in *Ldlr* gene of the mouse liver by AAV-CRISPR/Cas system. The HDR-mediated correction only achieved 6.7% efficiency but resulting in, to some extent, lower serum lipid levels and decreased lesion area [79]. The coming waves of newer types of CRISPR technologies, such as base editor and prime editor, hold potential to improve in this regard.

4.2 Preclinical Investigation of Genome Editing for Dyslipidemia and Atherosclerosis

Gene editing in adult humans, that is, somatic editing, holds the promise to permanently modify one's risk of dyslipidemia and atherosclerosis. In light of the compliance issue with statins, high costs of PCSK9 monoclonal antibodies, and discomfort of lifetime injection of RNA therapies, such once-and-done strategy is attractive. A poll about the acceptance of the gene editing therapy indicated the support from the majority of the participants [80, 81]. Several gene editing strategies against dyslipidemia and atherosclerosis have been intensively investigated in preclinical settings to inactivate pathogenic gene expression, correct disease-causing mutations, mimic atheroprotective effects of natural genetic variations, or insert beneficial transgenes.

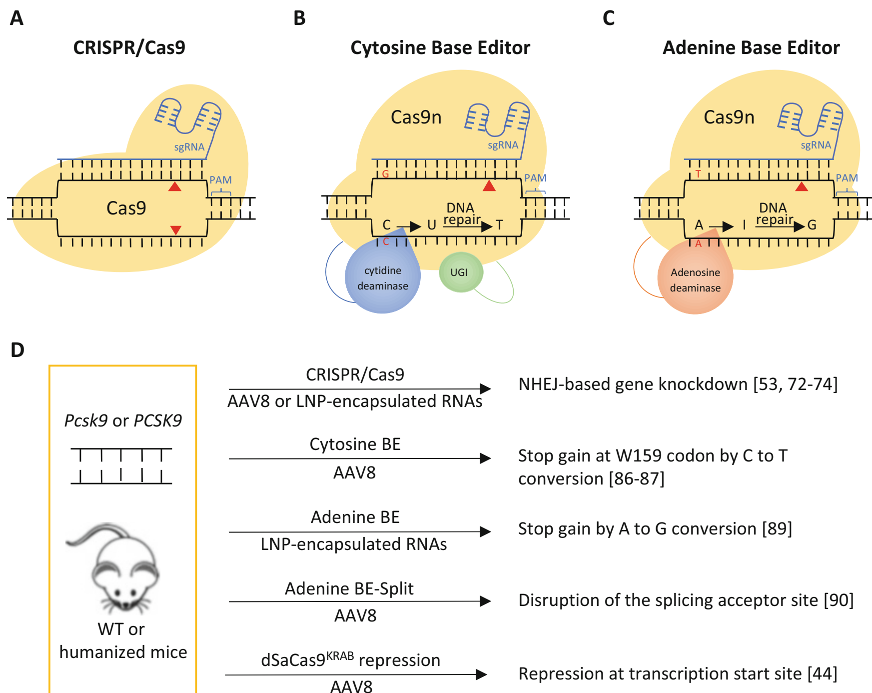

Fig. 2 Therapeutic genome editing of *PCSK9* by CRISPR/Cas and base editing. (**a**, **b**) General overview of DNA editing by CRISPR/Cas9, cytosine base editor (CBE), and adenine base editor (ABE). (**a**) Editing mechanism of CRISPR/Cas9. Cas9 nucleases create double-strand break (DSB) within the guide RNA (gRNA) pairing sequence, usually at 3–4bp ahead of 5′-protospacer adjacent motif [45]. DSB will be fixed through nonhomologous end joining (NHEJ) to create gene knockout or homology directed repair (HDR) to install a genotype or transgene of interest. (**b**) Mechanism of CBE. Cas9 nickase (Cas9n) nicks the top strand, while the cytidine deaminase domain of CBE convers C to U. Uracil glycosylase inhibitor (UGI, an optional component) protects the U intermediate from excision by uracil DNA glycosylase to boost efficiency of base pair editing assisted by nature DNA repair, which ultimately converts a C•G pair to T•A base pair. (**c**) Mechanism of ABE. After DNA nicking, adenosine deaminase domain converts A to inosine intermediate, which will be substituted by G in the subsequent DNA repairing process. ABE replaces A•T to G•C pair. gRNA, guide RNA. (**d**) Overview of strategies investigated to decrease *Pcsk9* or *PCSK9* in vivo. The editing tool is shown above the arrow line and the delivery approach is described below the line. WT, wild type; LNP, lipid nanoparticle; Adenine BE-Split, ABE separated to two domains (split-ABE-Rma573 and split-ABE-Rma674) for virus package, *KRAB* Kruppel-associated box (transcriptional repressor)

The most intensive testing focused on *PCSK9*, given the well-studied biology and rare side effect as a therapeutic target. Gene editing-based therapies allow permanent modification of the culprit and therefore are advantageous as a one shot and one cure for dyslipidemia, especially for FH. Disruption of *Pcsk9* in mice by CRIPSR/Cas9 has been evaluated by AAV- and nanocarrier-based delivery of spCas9 or saCas9 systems [55, 82–87] (Fig. 2d). All led to significant reduction of circulating Pcsk9, plasma total cholesterol (TC), and LDL-C levels. The therapeutic target was further assessed by inactivating the gene using base editing, which result in comparable atheroprotective outcomes [88–91]. A head-to-head comparison of *Pcsk9* gene and base editing in a humanized mouse model showed that the latter introduced no chromosomal translocations, fewer indels, and less new forms of peptides, indicating that it might be a safe strategy for clinical applications [89]. Other gene editing approaches to lower LDL-C level

are pursued, such as CRISPR-/Cas9-based targeting of *Apob* in *Ldlr*$^{-/-}$ mice [55, 72] and replacement of FH mutation of *LDLR* E208X in somatic cells of transgenic mice [55, 79], both of which reduced plasma TC level and atherosclerosis development in mice.

Given that existing lipid-lowering therapies are centered on optimizing cholesterol levels, drugs to reduce levels of non-LDL lipids including TGs and Lp (a) are of an urgent need, particularly for the dyslipidemia patients suffering from obesity, diabetes, or insulin resistance, whose primary risk of atherosclerosis is often related to elevated TGs and other forms of lipids. The attempts beyond LDL-C lowering by gene editing focus on *APOC3* and *ANGPTL3* for reducing TG levels and LPA for decreasing Lp(a).

As naturally occurring loss-of-function (LoF) mutations in *ApoC3* and *ANGPTL3* have found to be atheroprotective [92, 93], CRISPR-/Cas9-mediated inactivation of the two genes was tested to treat hyperlipidemia and atherosclerosis. APOC3, a secretory glycoprotein primarily produced by the liver, inhibits LPL- and hepatic lipase-mediated hydrolysis process of triglycerides in circulation and therefore increases TRL levels. In a human-like animal model (hamster), inactivation of *ApoC3* by CRISPR-Cas9 significantly decreased triglyceride level with no statistical differences in total cholesterol and HDL-C levels, phenocopying APOC3-deficient humans [94]. ApoC3 knockout hamsters also had less atherosclerotic lesions in both thoracic and abdominal arteries, suggesting clinical relevance of APOC3 targeting for the treatment of hypertriglyceridemia and atherosclerosis [95]. In the case of ANGPTL3, an inhibitor of LPL and endothelial lipase, base editing was employed to introduce LoF mutations at Gln-135 site of *Angptl3* in the liver of *Ldlr−/−* mice. This resulted in a median editing rate of 35% in the liver as well as substantially reduced triglycerides (56%) and cholesterol (51%) [96], suggesting a method to treat combined hyperlipidemia and atherosclerosis [96, 97].

LPA, expressed in the liver, encodes for apo (a) that could covalently bound to APOB100, an essential component for both LDL and Lp (a) [98]. Genetic variation of *LPA* was estimated to explain 91% of the variation in Lp(a) levels [22, 99]. Serum Lp(a) level could not be modulated by dietary and lifestyle factors, further necessitating the therapeutic intervention [100]. Lp(a) was also shown as a major carrier of oxidized phospholipids and to induce plaque progression [101–103]. An earlier pioneer study of RNA editing was explored to transform apoB100 mRNA into its truncated form apoB48 by a recombinant adenovirus encoding cytidine deaminase complex (apoBEC-1) to reduce both atherogenic lipoproteins in humanized apoB/apo (a) transgenic mice. This resulted in hepatic editing of human APOB mRNA and reduced plasma levels of human APOB100 and Lp(a). Similar result was observed when the apoB mRNA was edited accordingly in rabbit. These studies demonstrate mRNA editing by apoBEC-1 as a novel approach for lowering plasma concentrations of the atherogenic lipoproteins LDL and Lp(a) [104]. Furthermore, ongoing preclinical studies are investigating the use of base editing to reduce Lp(a) level by inactivating LPA gene.

These proof-of-concept studies demonstrated the feasibility of in vivo gene editing in reducing phenotypes of dyslipidemia and atherosclerosis and triggered industrial interests in developing these further in clinical experimentations. Currently, base editing of *LPA*, *PCSK9*, and *ANGPTL3* are under pharmaceutic development, and ABE-PCSK9 has entered the preclinical toxicology studies. So far, all the tested gene targets address familial dyslipidemia. CRISPR-based therapies could provide personalized treatment for the diseases, which currently cannot be cured. However, whether it could be cost effective to treat nonfamilial forms of dyslipidemia and atherosclerosis should be further investigated. Concerns about its advantage over traditional medications and long-term on- and off-target effects need to be addressed before clinical use. Pilot applications might be firstly available for individuals at high risk for myocardial infarction.

4.3 Further Target Discovery for Dyslipidemia and Atherosclerosis

4.3.1 Gene and Variant Targets Inspired by Human Knockout

Phenotypically healthy humans carrying knockouts of a gene provide evidence that pharmacological knockout of this gene may be safe. For example, LoF variants of *PCSK9* were associated with strikingly low plasma levels of LDL-C, reduced CAD risk [105], and but no apparent adverse health consequences, thus providing reassurance that therapeutic neutralization of *PCSK9* may be safe [106, 107]. Likewise, human knockouts of *ANGPTL3* and *APOC3* led to the development of pre- and clinical drugs for lowering serum levels of cholesterol and triglycerides, and the corresponding alleles related to hypolipidemia are under investigation for treatments using base editing. Increasing discoveries of such "experiments of nature" will be empowered by exome or whole genome sequencing in large-scale biobank cohorts [3]. More gene and allele targets relevant to dyslipidemia and atherosclerosis will emerge [3, 108–111].

4.3.2 Candidate Genes and Variants from Large-Scale Genetic Studies

Genome-wide association studies (GWASs) have discovered over 300 CAD loci and more than 900 loci of blood lipid traits including LDL-C, HDL-C, non-HDL-C, total cholesterol and triglycerides, unveiling novel variants, and genes and pathways underlying dyslipidemia and atherosclerosis with unprecedented speed and mechanistic complexity [20, 112, 113]. GWASs also rediscovered rare variants for dyslipidemia and atherosclerosis, suggesting that beyond these, drug targets are tagged by novel GWAS variants and gene candidates at the many loci associated with lipids and atherosclerosis, especially those loci overlapping for the two traits (Fig. 3). When we explored gene loci shared for CAD and lipids including LDL, TGs, TC, and HDL using the latest statistics of GWAS catalog, EMBL-EBI (2021), we identified 83 loci and classified the mapped genes into related pathophysiological pathways (Fig. 3). Surprisingly, other than the largest portion (~30%) of the genes directly involved lipid metabolism, many genes play roles in known pathways linked to CAD, such as inflammation, angiogenesis, and vascular remodeling. Genes for insulin resistance and glucose metabolism were also identified in our analysis, suggesting that novel genes and pathways for the disease are secondary to dysglycemic regulation. The convenience of CRISPR-based technologies will allow investigation of the novel genetic findings in a high-throughput manner.

Furthermore, by testing causality harnessing genetic information, Mendelian randomization (MR) could identify specific genes as potential therapeutic target and assure efficacy and, importantly, safety before the initiation of drug development [114, 115]. Another genetic approach alerting adverse effect is termed phenome-wide association study (PheWAS), which tests associations of a genetic variant or a gene with hundreds of clinical phenotypes linked to all the organ systems [116, 117]. Using integrative data of individual's genome and electronic health record from large biobank cohort, PheWAS could assess for desirable and adverse clinical outcomes linked to variant and gene of interest. MR and PheWAS provide reassurance for novel gene target selection in pre- and clinical investigations.

4.3.3 Driver Genes and Variants of Systems Genetic Studies

Although compelling efforts have been made to prioritize disease-associated genes utilizing approaches from molecular biology to GWAS, the genetic landscape of atherosclerosis and CAD is not fully elucidated. In the past decade, systems biology based on omic technologies accelerates the understanding of mechanisms underlying complex traits [118, 119]. Systems biology networks, genetic variations, and gene expression with other higher biological layers identify driver variants and genes for complex

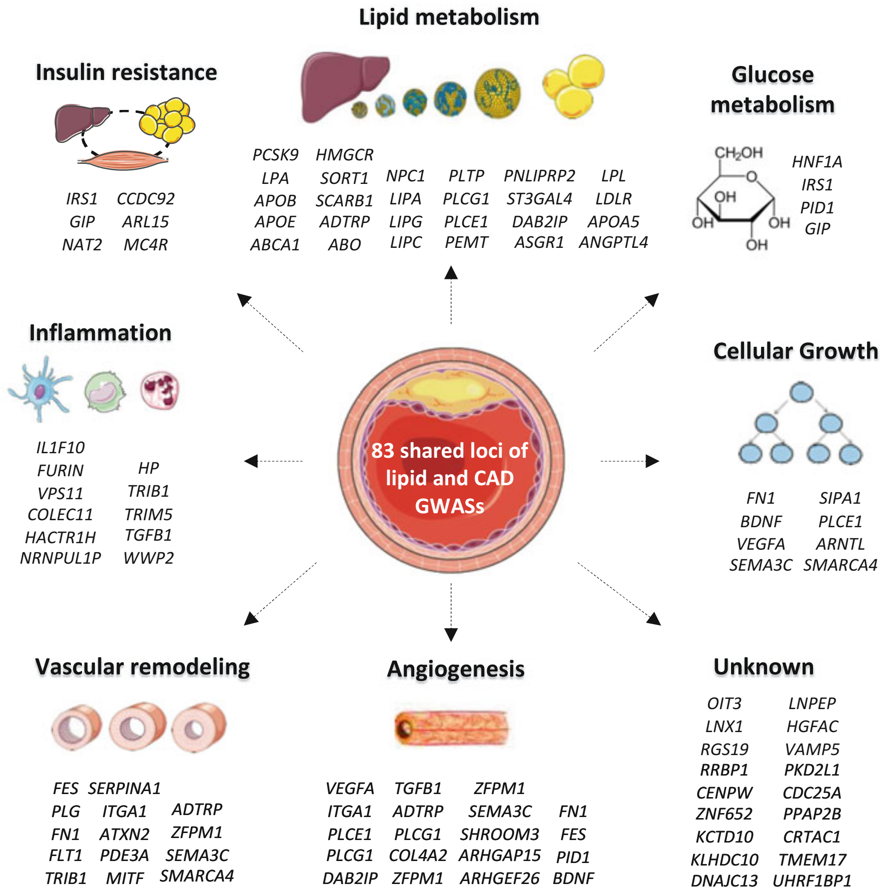

Fig. 3 Genes mapped to 83 shared loci of lipids and CAD GWAS loci and potentially related pathophysiological pathways of atherosclerosis. CAD, coronary artery disease

diseases. Targeting of the key drivers to modulate disease-associated gene or protein networks might enable correction of multiple pathogenic pathways in parallel. Genome editing technologies will play a crucial role in testing related hypothesis and therapeutic potentials [119].

5 Concluding Remarks and Future Perspectives

The possibility of manipulating DNA and RNA has advanced cardiovascular medicine, including understanding gene functions and genetic diseases, as well as the development of novel drug targets. Although the field is still in its infancy, the potentials are exemplified by clinical trials to treat sickle cell disease, to improve effectiveness of chimeric antigen receptor T-cell (CAR-T), or to reverse eye diseases [120]. A clinical trial of base editing targeting *PCSK9* to treat heterozygous familial hypercholesterolemia (HeFH) started in July 2022 (clinicaltrials.gov_NCT05398029). Beyond these examples, many rare genetic disorders, in principle, will be treatable with CRISPR-based therapies.

Despite the exciting progress, many challenges should be tackled before its broader applications. First, tissue-specific delivery of

genome editors has been a long-standing issue. Although AAV systems could allow relatively specific targeting in the liver, brain, muscle, and eye with low immunogenicity [121, 122], they should be further optimized, and many more tissues need to be considered. A new field of research exploiting nanoparticle-based delivery could provide alternative solutions. Second, current genome editing tools strictly rely on specific recognition sequences as well as specific binding sites on the target, such as the protospacer adjacent motif sequences for Cas proteins [45]. The absence of the assisting recognition sequence limits the targeting capability. Therefore, many research teams focus on discovering or engineering editing tools independent of such sequences. Third, substantial variability of editing efficacy depending on genetic loci and cell types was observed, which are partially caused by differences in chromatin accessibility and DNA repairing mechanisms throughout phases of cell cycle. Forth, off-target mutagenesis, although being rare, were detected within sequences of high similarity. While well-designed gRNAs are critical to minimize off-target events, advanced methods have been established to assess unwanted editing in a genome-wide fashion, such as BLISS, GUIDE-Seq, and DISCOVER-seq [123–125]. Finally, a long way has to be gone to fulfill regulatory guidelines and define cost reimbursement for these once-in-a-lifetime therapies. Of note, ongoing therapeutic testing of CRISPR aims to treat patients by modifying their somatic genome. The scientific and social challenges related to human germline editing are discussed elsewhere [126].

Nevertheless, gene editing therapies have to be evaluated carefully case-by-case in extensive pre- and clinical experimentations. Given the recent progress and efforts around the globe to tackle the related issues, genome editing will certainly expand into a new class of therapy to treat many diseases, including dyslipidemia and atherosclerosis.

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