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INVITED REVIEW ARTICLE

Integration of Transformative Platforms for the Discovery of Causative Genes in Cardiovascular Diseases

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Abstract

Cardiovascular diseases are the leading cause of morbidity and mortality worldwide. Genome-wide association studies (GWAS) are powerful epidemiological tools to find genes and variants associated with cardiovascular diseases while follow-up biological studies allow to better understand the etiology and mechanisms of disease and assign causality. Improved methodologies and reduced costs have allowed wider use of bulk and single-cell RNA sequencing, human-induced pluripotent stem cells, organoids, metabolomics, epigenomics, and novel animal models in conjunction with GWAS. In this review, we feature recent advancements relevant to cardiovascular diseases arising from the integration of genetic findings with multiple enabling technologies within multidisciplinary teams to highlight the solidifying transformative potential of this approach. Well-designed workflows integrating different platforms are greatly improving and accelerating the unraveling and understanding of complex disease processes while promoting an effective way to find better drug targets, improve drug design and repurposing, and provide insight towards a more personalized clinical practice.

Keywords GWAS · RNAseq · iPSC · Organoids · Multi-omics · Animal models

Introduction

Cardiovascular diseases (CVDs) and stroke are the leading cause of morbidity and mortality in the USA and worldwide [1]. In the decade between 2006 and 2016, we saw an increase of 14.5% (95% CI, 12.1–17.1%) in deaths attributed to CVDs, translating in approximately 17.6 million (95% CI, 17.3–18.1 million) deaths worldwide in 2016. According to the 2019 American Heart Association report [2], heart disease was at the top amongst the 10 leading causes of death in the USA in 2016. In spite of advancements in diagnosis and treatment, the average annual direct and indirect cost of CVD and stroke continues on a steep increase in the USA, going from $103.5 billion in 1996 to 1997 to an estimated $351.2 billion in 2014 to 2015. Essential risk factors contributing to CVDs include behavioral (smoking, physical activity, diet, and obesity) and health factors (hypertension, cholesterol—specifically low-density lipoprotein cholesterol, LDL-c-, diabetes, and glucose control). Nonetheless, the prevalence of CVDs in adults over 20 years old remains at about 48% (with a 9% prevalence for coronary artery disease, heart failure, and stroke when excluding hypertension) [2]. Within this bleak scenario, it is clear that understanding the genetic individual risk, beyond the modifiable risk factors, can contribute substantially to the fight against CVDs globally at the individual level through novel approaches in personalized medicine.

Genetic variants can predispose individuals to specific diseases and Genome-wide association studies (GWAS) have proven powerful epidemiological tools to identify the variants associated with human traits. Stemming from the revolution brought about by the human genome sequencing in 2001 [3], the short period spanning 2005–2007 ended a decade of research that went from associations mostly at the chromosome level [4–6] to the development of GWAS, thus allowing the study of genetic mutations and individual variants at the loci level in genes and their association with CVDs. The added boost came in the form of new sequencing technologies and

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assembly algorithms for rapid turnaround times, reduced costs, the ever-improving analysis tools, and the accessibility to data through consortia and repositories. Those, together with the increased public awareness and acceptance, have established GWAS as a mainstream technology. The specifics of GWAS approaches, methodologies, and current advances and limitations will be further addressed in the accompanying reviews in this issue.

GWAS have identified hundreds of loci associated with CVDs, including over 118 loci associated with lipids as a main driver of CVDs. Most GWAS studies have yet to produce clinical interventions, though, likely due to two key limitations: (1) because of the resolution limit, GWAS may not always pinpoint a specific gene; (2) GWAS alone cannot assign the causality and underlying mechanism [7]. Therefore, a number of emerging technologies, used in conjunction with GWAS, allow research investigators in multidisciplinary teams to accelerate the discovery and characterization of causal genes and/or variants for CVDs and provide insight on their underlying mechanisms towards accelerating clinical translation (Fig. 1). Generally speaking, GWAS followed by functional studies has identified that changes in mRNA expression, promoter function, enhancer/repressor functions, chromatin remodeling, changes in protein function, including changes in coding regions, or expression of non-coding RNAs, appear to underlie disease-associated variants (Fig. 2). In this review, we focus on underscoring the value of technologies used in conjunction with GWAS to accelerate translation of the GWAS findings to the clinical setting. We will summarize the most widely used amongst those enabling technologies to illustrate how, per se or in combination in platforms involving multiple approaches, they contribute mechanistic insight and promote clinical translation of GWAS data in the cardiovascular field. We will point some key limitations and provide insight on emerging opportunities from the perspective of their integration with GWAS. Our objective here is to bring greater awareness of the power of multidisciplinary approaches involving some of the highlighted technologies to complement the findings of GWAS and accelerate translation to clinical settings towards personalized medicine. Consequently, the limited number of works cited were chosen to serve as descriptive examples of the use of those technologies in conjunction with GWAS in the CVD field, beyond the specifics of their individual biological implications (which we recognize would deserve a separate review in most cases). With the increasing volume of literature available, we have to apologize in advance to all the research teams whose essential contributions we will not be able to mention. For a list of abbreviations and acronyms, please, see Table 1.

Emerging Platforms for Advancement of Functional Studies in Relation to GWAS

RNA Sequencing and Single-Cell RNA Sequencing

RNAseq

In the late 2000s, the advent of "bulk" RNA sequencing (RNAseq) constituted a major breakthrough [8–10], helping address some of the limitations of painstaking and expensive technologies, including microarrays [11, 12] and SAGE [13], Because of its intrinsic depth and quantitative nature, the development of ever-improving methodologies and analysis methods, and the reduced costs, RNAseq is becoming widely used. RNAseq of a specific population of cells or tissue in given conditions provides quantitative data on the average gene expression, allowing comparative studies of the transcriptome in the biological samples. It has provided signature gene expression of genes across species and tissues, and in diseases affecting a given tissue or isolated cell type. It has also aided in the identification of novel transcripts, imbalance in allelic expression, alternative splicing, and gene fusion events. Different platforms for sequencing and analysis are available and continue to be further refined, with their description, protocols, and specific advantages and limitations for each platform falling beyond the scope of this review (for a comparative in-depth review, please, see [14]).

RNAseq contributed early on to understand the functional roles of variants found through GWAS affecting gene expression. In a multidisciplinary pioneering approach, involving the development of new tools for paired-end RNAseq alignment and allele-specific analysis, Heap, G.A. et al. [15] uncovered allelic expression imbalance for specific exonic variants, thus linking GWAS findings with variations in gene expression upon activation of primary CD4+ T cells of heterozygous individuals. Since then, this technology has been widely used as a complement to GWAS findings to make causal inferences regarding the effects of candidate variants and haplotypes on inherited regulation of gene expression, known as expression quantitative trait loci (eQTLs).

RNAseq has also been applied to uncover specific regulatory variants, as exemplified by the work of Alloza, I. et al. [16]. Through reanalysis of RNAseq of VSMC isolated from carotid plaques from 7 asymptomatic and 7 symptomatic patients, the team uncovered 700 genomic variants associated with symptomatology ($p < 0.05$) and further validated a cis-quantitative trait locus variant in the *BRUCE/Apollon* gene, BIRC6 (exonic SNP rs35286811), as a proof-of-concept. While underscoring the power of this approach, this work shed new light on the molecular players involved in the transition from a stable to unstable plaque, an area of increasing interest in atherosclerosis research towards potential clinical intervention.

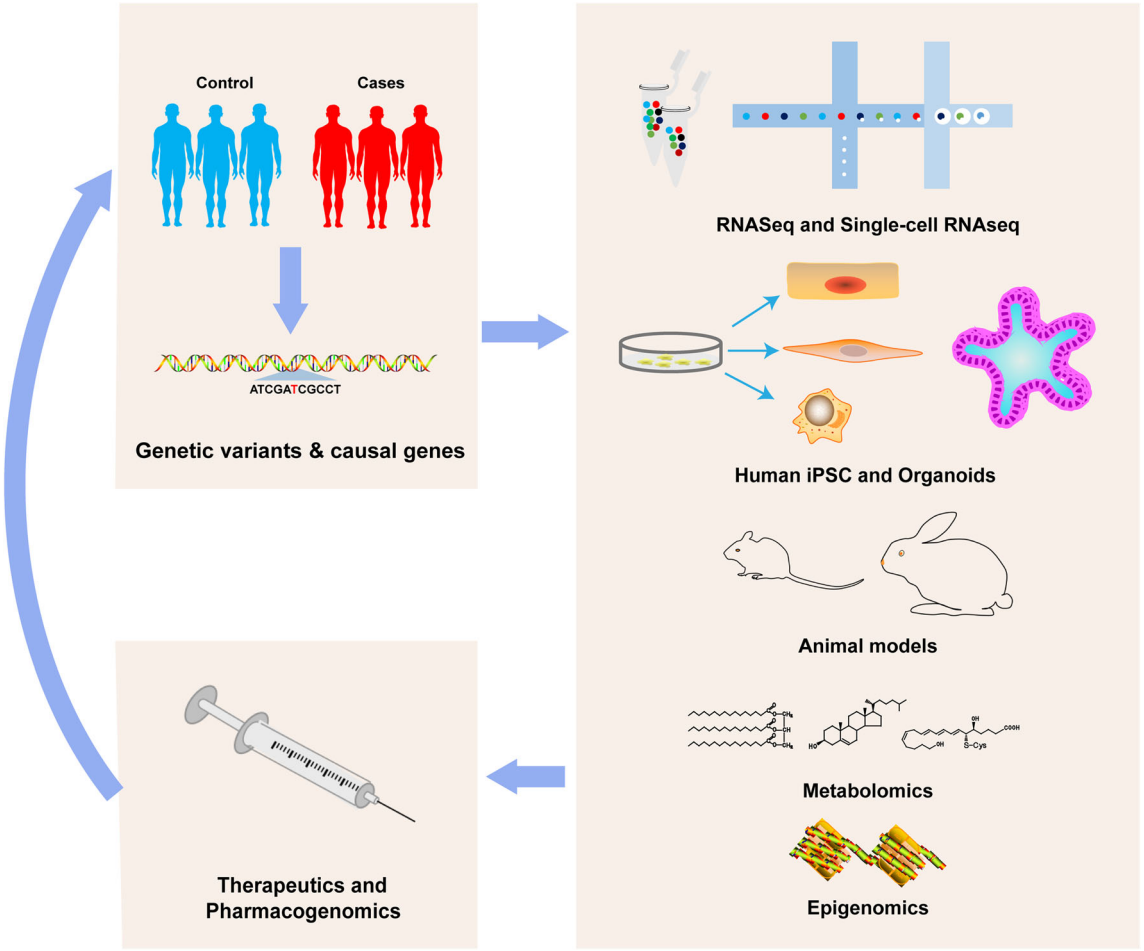

Fig. 1 Transformative technologies used in conjunction with GWAS for functional studies of genes and variants towards therapeutic interventions. GWAS help in identifying variants associated with disease. The biological function of these genes and variants are further studied through integration of the GWAS findings with multiple technological platforms, including RNAseq and scRNA-seq, iPSC and organoids, animal models, metabolomics, and epigenomics. The findings from these studies inform the field of drug development and pharmacogenomics to enable therapeutic interventions towards implementation of personized medicine

In order to identify novel potential therapeutic targets that may reduce the risk of CVDs without increasing the risk of liver disease, diabetes, or other metabolic disorders, we recently undertook a GWAS involving 70,000 participants of the Nord-Trøndelag Health Study [17]. We identified a STOP-gain variant in ZNF529, ZNF529:p.K405X, as associated with decreased low-density lipoprotein cholesterol (LDL-c) ($p = 1.3 \times 10{-}8$) and showing no association with liver enzymes or non-fasting blood glucose. Since this gene is of unknown function and is not present in rodents, we used RNAseq combined with siRNA-mediated knockdown of ZNF529 in human hepatoma cells to interrogate its potential roles. We found increased LDL receptor expression, which is consistent with reduced LDL-c and a likely protective effect in CVD. Although further mechanistic studies should follow, these initial findings underscore the potential for therapeutic targeting of ZNF529 in CVDs.

Limitations and Opportunities In spite of its increased and successful use as an enabling technology in conjunction with GWAS, overall RNAseq has the limitation that cells in a tissue are analyzed in bulk, not allowing for the study of heterogeneous systems at the cell type level. Even after isolation of individual cell types in a tissue, as in the first 2 examples above, bulk RNAseq still misses the stochastic nature of gene expression at the cellular level. Finally, most disease-associated risk loci are located in genomic non-coding regions, which contributes to the complications in identifying their target genes, the affected cell types, and regulatory mechanisms which sometimes can influence multiple genes simultaneously (Fig. 2).

scRNA-seq

Some of the intrinsic limitations of bulk RNAseq can be addressed through single-cell RNA sequencing (scRNA-seq)

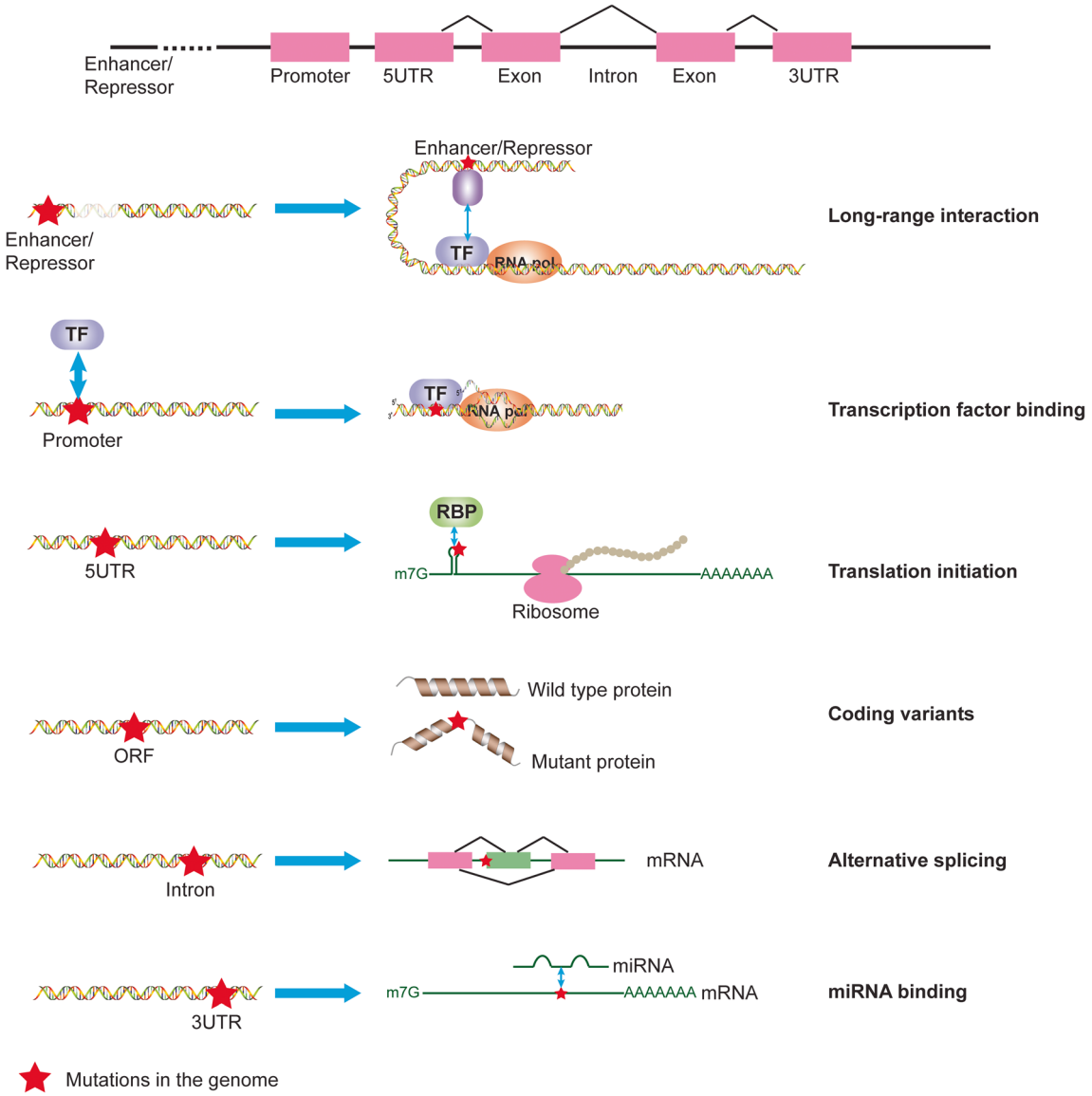

Fig. 2 Functional characterization of genetic variants. Illustration of possible mechanisms behind how genetic variants may affect gene expression and function, based on the location of the variants. A variant in the enhancer or repressor region could affect gene transcription by long-range interaction; a variant in the promoter could affect transcription factor binding and ensuing transcription; a variant in the 5' untranslated region (5UTR) could affect RNA-binding protein (RBP)-RNA interaction and mRNA translation; a non-synonymous variant in the coding region could alter the amino acid sequence and, consequently, the protein function; a variant in the 3' untranslated region (3UTR) could affect RNA stability and translation by disrupting miRNA binding

which provides the transcriptome information at the single-cell resolution in tissues and organs. This allows visualization of the heterogeneity of gene expression associated with the diverse cell populations and the study of their changes during organ development [18] or disease [19]. Currently, there are numerous platforms for scRNA-seq and the choice should be driven by the scientific questions to be addressed [20, 21]. Compared with traditional immunostaining or flow cytometry, scRNA-seq allows to simultaneously assess the expression profiles of thousands of genes and, consequently, has enabled the comprehensive study of the heterogeneity of cells and inter-cell communication [22]. scRNA-seq has provided an unprecedented understanding of the different cell types involved in complex diseases, including the identification of different sub-types and their contribution to cardiovascular pathologies. In atherosclerosis, numerous studies have identified the dynamics changes and unsuspected richness of sub-populations in endothelial cells, macrophages, and smooth muscle cells through scRNA-seq from cells isolated from the atherosclerotic plaque from both human [22, 23] and mice [24–26].

Of relevance here, it is worth highlighting the recent convergence of studies using scRNA-seq and GWAS, which makes the case for implementing their concurrent use. For

Table 1 Abbreviations and acronyms

Abbreviation	Full name	Abbreviation	Full name
ACTN2	Actinin alpha 2	IL2RG	Interleukin 2 receptor subunit gamma
ADCY10P1	Adcy10 pseudogene 1	iPSC	Induced pluripotent stem cells
AHA	American Heart Association	iPSCORE	IPSC collection foromic research
ANRIL	Cyclin-dependent kinase inhibitor 2b antisense rna 1	JAK2	Janus kinase 2
ApoAI	Apolipoprotein a1	KLF14	Krüppel-like factor 14
ApoAII	Apolipoprotein a2	KLHL35	Kelch-like family member 35
APOB	Apolipoprotein b	KO	Knock-out
ApoCIII	Apolipoprotein c3	LDL-c	Low-density lipoprotein cholesterol
APOE	Apolipoprotein e	LDLR	Low-density lipoprotein receptor
APP	Amyloid beta precursor protein	LIPC	Lipase C, hepatic type
ASXL1	Additional sex combs like transcriptional regulator 1	lncRNA	Long non-coding RNA
ATP2B2	ATPase plasma membrane Ca2+ transproting 2	MCAD	Acyl-CoA dehydrogenase medium chain
BAC	Bacterial artificial chromosome	MS	Mass spectrometry
BIRC6	Baculoviral iap repeat-containing 6	NAMs	Non-alcoholic steatohepatitis-associated macrophages
BMI	Body mass index	NASH	Non-alcoholic steatohepatitis
CASR	Calcium sensing receptor	NMR	Proton nuclear magnetic resonance spectroscopy
CDH23	Cadherin related 23	NOG	NOD/Shi-scid IL2RGnull
CETP	Cholesteryl ester transfer protein	OTCD	Ornithine transcarbamylase deficiency
CFTR	Cystic fibrosis transmembrane conductance regulator	PCSK9	Proprotein convertase subtilisin/kexin type 9
CFTR-ΔF508	CFTR deletion of phenylalanine at position 508	pQTLs	Protein quantitative trait loci
CHD	Coronary heart disease	PRKDC	Protein kinase, dna-activated, catalytic subunit
CHIP	Clonal hematopoiesis of indeterminate potential	PTPRN2	Protein tyrosine phosphatase receptor type n2
CNN2	Calponin 2	mQTL	Quantitative genetic control of metabolism
CpG	Cytosine-phosphate-guanine	RAG1	Recombination-activating gene 1
CRISPR	Clusters of regularly interspaced short palindromic repeats	RAG2	Recombination-activating gene 2
CVD	Cardiovascular disease	RNAseq	RNA sequencing
DNMT3A	DNA methyltransferase 3 alpha	SAGE	Serial analysis of gene expression
eQTLs	Expression quantitative trait loci	SCAD	Acyl-CoA dehydrogenase short chain
EWAS	Epigenome-wide association studies	scRNA-seq	Single-cell RNA sequencing
FADS1	Fatty acid desaturase 1	SERPINB9	Serpin family b member 9
FOXN1	Forkhead box n1	siRNA	Small interfering rna
FOXP2	Forkhead box p2	TALEN	Transcription activator-like effector nucleases
GADD45G	Growth arrest and DNA damage inducible gamma	TET2	Tet methylcytosine dioxygenase 2
GCV	Ganciclovir	Tg	Transgenic
GenIE	Genome engineering-based interrogation of enhancers	TK-NOG	NOG mouse expressing a thymidine kinase transgene
GUCA1B	Guanylate cyclase activator 1b	TMAO	Trimethylamine-n-oxide
GWAS	Genome-wide association studies	TMAVA	N,n,n-trimethyl-5-aminovaleric acid
HDL	High-density lipoprotein	TP53	Tumor protein p53
HDL-c	High-density lipoprotein cholesterol	TREM2	Triggering receptor expressed on myeloid cells 2
HLA	Major histocompatibility complex, class I	TRIB1	Tribbles pseudokinase 1
HPCAL1	Hippocalcin like 1	TWAS	Transcriptome-wide association study
HSVtk	Herpes simplex virus-1 thymidine kinase	VSMC	Vascular smooth muscle cells
HTT	Huntingtin	WHHL rabbits	Watanabe heritable hyperlipidemic rabbits
HuGX	Human genes on the X chromosome	ZFN	Zinc finger nuclease
HuR	Human antigen r	ZNF529	Zinc finger protein 529

instance, the macrophage is the major immune cell type in the atherosclerotic plaque. Using scRNA-seq, Cochain, C. et al. [24] first described macrophages expressing high levels of triggering receptor expressed on myeloid cells 2,

TREM2^{high}, confirmed in subsequent studies of atherosclerosis [23, 24, 27], obesity [28], fibrosis in liver cirrhosis [29], and non-alcoholic steatohepatitis (NASH)–associated macrophages (NAMs) [27]. TREM2^{high} appears to define a population of anti-inflammatory macrophages as well as a foam cell-like population (expressing both TREM2 and CD9) displaying a fibrosis-promoting phenotype, which suggests that the latter may represent a plaque stabilizing macrophage population [30]. Remarkably, in independent GWAS studies, *TREM2* emerged as associated with neuroinflammatory diseases, including Alzheimer's disease, dementias, and ischemic stroke [31–34]. Nonetheless, beyond the finding that a common *TREM2* variant is associated with the levels of C-reactive protein, a risk factor for CVD, in Hispanics and African Americans [35], how *TREM2* variants may contribute to various intrinsically inflammatory CVDs and through which cell types and underlying mechanisms, remains to be addressed. This will require further GWAS studies to assess *TREM2* association with CVDs, beyond atherosclerosis, combined with genetically modified mouse models, such as myeloid-specific *TREM2* knock-out and transgenic mice, and other enabling technologies as those discussed herein, to dissect the biology of *TREM2* in CVD and address the effects of the identified variants to evaluate its potential value for therapeutic targeting.

Limitations and Opportunities scRNA-seq has provided an unprecedented understanding of the different cell types involved in diseases, including the identification of different sub-types and their contribution to cardiovascular pathologies. Compared to RNAseq, scRNA-seq has intrinsically less depth, missing lower expression genes, and can miss cell types less represented in the mixture. scRNA-seq has yet to realize its full potential in association with GWAS studies. A pressing challenge is how to integrate the scRNA-seq data from different platforms and different research teams in order to leverage the ever-growing datasets for meta-analysis to use in conjunction with findings from GWAS. This will require improvement on how to isolate the true biological difference from the technology bias and batch effects among different experiments [36]. Several integration methods are already available in the field, such as Harmony [32], Seurat [37], MNN correction [38], and LIGER [39]. A recent benchmark publication comparing these methods shows that none is clearly superior under all scenarios and that researchers need to choose the method on a case-by-case fashion [40]. Finally, recent advances aimed at uncovering spatial information in tissues and organs through several spatially resolved transcriptomic technologies [41] should be highlighted, including laser-capture microdissection [42], RNA image [43, 44], and spatial barcoding [45, 46]. Spatial transcriptomic techniques help in revealing cellular heterogeneity utilizing spatial labels in complex organs, and in building a 3D transcriptome landscape, as illustrated by the publication of a "3D cell atlas of the developing human heart" [47]. These technologies could help understand whether GWAS variants can alter spatial transcription within specific cell populations in organs and tissues to an unprecedented degree of resolution, thus creating new opportunities for mechanistic studies at the individual cell level.

Human-Induced Pluripotent Stem Cells and Organoids

Induced Pluripotent Stem Cells

Human primary cells are valuable resources to study cardiovascular diseases, although it is usually very difficult to obtain cells from healthy individuals or patients for in vitro studies. Induced pluripotent stem cells (iPSC) can be derived from mature somatic cells and have the capacity to differentiate into numerous cell types for research or therapeutic purposes [48, 49]. Human iPSC can be derived from readily available skin or blood cells and, through well-established differentiation approaches, they can serve as an unlimited source for various cell types (for recent cardiovascular relevant reviews and references to specific protocols therein [50–54]).

Differentiation into lineages of relevance to diseases is uniquely poised to address directly the sometimes conflicting results between mouse and clinical studies, which arise mostly from fundamental species-specific differences and the limitations of other in vitro systems. Human iPSC derived from CVD patients or healthy donors enabled addressing the contributions of individual genes in complex regions or the role of non-coding variants identified in GWAS. For example, the 9p21 region is an area with poor synteny in rodents, which prevents using that animal model to study that region. The 9p21 region was identified a decade ago as carrying risk alleles for CVD[55]. Yet, it lacks known coding genes albeit containing the terminal exons of the long non-coding RNA (lncRNA) ANRIL[56]. To address the role of ANRIL in vascular smooth muscle cells, Lo Sardo, V. et al. [57] derived iPSC from individuals carrying either risk or non-risk alleles and edited out of the haplotype region in both groups using transcription activator-like effector nucleases (TALEN). Following the generation of vascular smooth muscle cells from the engineered iPSC, this study identified this lncRNA as a causal locus in this region altering VSMC phenotypes through overexpression of lncRNA ANRIL. Because of its pleiotropic effects on different aspects of CVDs, including endothelial dysfunction, macrophage recruitment to lesions and glucose, and lipid metabolism, ANRIL is being considered a target candidate for cardiometabolic diseases.

Large-scale collections of induced pluripotent stem cells (iPSCs) can accelerate functional studies on the effects of genetic variation in health and disease. One relevant resource

already available to rapidly deploy the iPSC enabling technology in conjunction with population genetics is the iPSCORE (iPSC Collection forOmic Research)[58]. IPSCORE is a collection of 222 iPSC lines from ethnically diverse representative individuals from several multigenerational families and ages carrying risk and non-risk genotypes for 95% of GWAS variants associated with human phenotypes. It was characterized through high-throughput RNA-sequencing and genotyping arrays. As proof-of-concept that the genetic background can remain associated with molecular phenotypes in iPSC-derived cells, the iPSCORE team demonstrated that iPSC-derived cardiomyocytes depict gene expression patterns driven by the genetic background in the iPSC from a three-generational family of participating individuals. The iPSCORE is a prototype resource to examine the molecular and physiological impact of genetic variants across a variety of derived cell types, and, conversely, to uncover which are the functional variants underlying a variety of GWAS phenotypes.

The potential of iPSC as a complement to GWAS will be further realized through combination with other high-throughput technologies. This is clearly illustrated by the recent report by Mirauta, B.A. et al. [59] using a combination of matched quantitative proteomics (Tandem Mass Tag Mass Spectrometry), transcriptomics (RNAseq), and genomic sequencing data from 202 iPSC lines, derived from fibroblasts of 151 donors from the Human Induced Pluripotent Stem Cells Initiative (HipSci project: http://www.hipsci.org) [60]. This work identified 654 protein quantitative trait loci (pQTLs) in those iPSCs, including disease-linked variants in protein-coding sequences and variants with trans-regulatory effects (Fig. 2). A highlight of this work is that the pQTL included GWAS variants that could not have been detected at the mRNA level. Although how these pQTLs and the associated variants contribute to the phenotype in vitro after differentiation into various lineages in the context of specific diseases remains to be addressed, these types of complex studies create reference datasets that constitute valuable resources for ensuing functional studies and will undoubtedly have a fundamental impact as templates towards personalized medicine applications.

Limitations and Opportunities Obvious limitations of this technology include the efficiency of current protocols for differentiation into specific—still limited—cell types and that differentiation may be partial. Since the initial development of human iPSC from fibroblasts [48], new host cells for induction of iPSCs have been identified. Those include peripheral and cord blood cells and adipocyte stem cells, amongst the easiest to obtain. Additionally, a repertoire of new approaches have been developed beyond the use of the original four genes [50, 55, 56] and to increase the efficiency of iPSC yields (for a recent review [61]). The cell of origin and, of high relevance to the use of iPSC in conjunction with GWAS studies, the genetic background of the donor may affect the efficiency of differentiation or lead to heterogeneity or incomplete reprogramming, suggesting that epigenetic memory may contribute to iPSC development. The use of iPSC for functional studies prepared from patients carrying specific mutations identified in GWAS creates a unique requirement. It necessitates the creation of isogenic controls via genome editing as the standard to account for human-to-human variation and the polygenic nature of most diseases, as opposed to comparison to iPSC from individuals lacking the mutation. On the other hand, this approach can provide a better cell-based platform for drug screening and other preclinical applications. Additionally, renewed efforts are on-going towards direct reprogramming of fibroblasts to specific cell types [62–64]. Streamlined research platforms that will support expedited and simplified high-throughput workflows will help to fully realize the potential of iPSC in conjunction with GWAS. Accordingly, the field is experiencing accelerated progress and creating new and exciting opportunities. Without entering into details, human iPSCs as models for vascular biology are being used towards organ-on-chip approaches (for a recent review [65]) and iPSC-based modeling of aortic disease could be leveraged for "clinical-trials-in-a-dish," as recently proposed by Davaapil, H. et al. [66], towards the implementation of precision medicine. Additionally, Cooper, S.E. et al. [67] described an arrayed CRISPR screening method on iPSC, named Genome engineering-based Interrogation of Enhancers (GenIE), to identify likely causal variants with effects on transcription or splicing when introduced in their endogenous genomic locations. In summary, human iPSCs are an attractive platform to obtain hard-to-access human cell types, provide enough cells for disease modeling in vitro, and determine the functional consequences of specific GWAS findings in given cell lineages, and are uniquely positioned to advance the clinical translation of GWAS.

Organoids

In spite of all the advantages of human iPSCs for in vitro manipulation, they cannot recapitulate the higher-level 3D structure and cell-cell communication among different cell types in organs. The development of organoids, defined as "a self-organizing 3D structure which mimics the original *in vivo* architecture of organs or tumors and can be derived from different sources" [68], can help overcome some of those limitations.

The liver is the major organ for the production of lipoproteins and cholesterol biosynthesis and liver pathophysiology is intrinsically linked to CVD. Lipid-lowering drugs inhibiting cholesterol biosynthesis proved to be very effective in reducing CVD risk and many CVD-associated mutations were identified in the lipid metabolism and lipoprotein pathways

(e.g., LDLR, APOE, APOB, PCSK9) [69, 70]. Thus, the liver organoid model is of unique value to study liver biology in relation to cardiovascular genetics [71]. The generation of human liver organoid from iPSC was achieved in 2013 [72]. This system has been further improved in the following years [73], including the incorporation of hepatobiliary structures [74] in the organoid. Liver organoid technology was used to study monogenic diseases, including Wilson's disease [75], α1-antitrypsin deficiency [75], and Alagille syndrome [76], as well as cancer [68] and lipotoxicity. Of relevance here, Abbey, D. et al. [77] recently reported a reproducible and scalable protocol for the generation of hepatic 3D organoids from human iPSC using short exposure to non-engineered matrices for use in exploring human hepatocyte biology and the functional role of genes and variants identified in GWAS studies. Specifically, through gene editing, they created isogenic human iPSC deleted for the *TRIB1* gene, previously associated with metabolic traits, including non-alcoholic fatty liver disease and plasma lipids, of relevance to CVDs. Remarkably, 2D cultures showed maturation defects in the TRIB1-deficient cells, that were rescued in the 3D cultures, which also preserved a lipid-related phenotype that more faithfully recapitulated the human findings. This study clearly supports the need to use 3D cultures to better mimic human liver lipid metabolism in vitro for functional studies.

Functional murine [51] and human [78] heart organoids have been successfully established as well. Improvement of cardiac organoids [79] and the concept of "heart-on-a-chip" [80, 81] are areas of intensive research with specific methodologies presented elsewhere [82]. Human heart organoids were utilized to mimic infarct in vitro [83] and also provided a novel platform to test the biology of genetic mutations [84] and drug cardiotoxicity [85]. With over 1500 mutations in at least 11 different genes identified as potentially causative of hypertrophic cardiomyopathy [86], a list that continues to grow [87], organoids hold unrivaled promise to address functional effects associated with mutations. In the context of this review, the recent work by Prondzynski, M. et al. [88] highlights the feasibility and clinical significance of integrating heart organoids with GWAS studies and sets a seminal precedent for precision medicine in cardiac disease. Analysis of human iPSC established from a family affected by hypertrophic cardiomyopathy was used to derive cardiomyocytes and engineered heart tissues which recapitulated several disease-specific traits, such as hypertrophy, altered calcium response, hypercontractility, and sarcomeric disarray associated with the ACTN2 (c.740C>T; dbSNP ID: rs755492182) mutation of the patients. Furthermore, testing the efficacy of Diltiazem, an L-type calcium-channel inhibitor, in this in vitro system, informed its administration to affected family members to improve the electrical phenotype, illustrating a direct clinical intervention outcome from this approach.

Limitations and Opportunities As illustrated in the examples above, organoids have been successfully employed for drug screenings as they closely recapitulate patient responses and can be used in xenotransplants in mice orthotopically, subcutaneously, or in the kidney capsule for further functional assays. Relaying on iPSC-derived organoids to further GWAS findings benefits from their advantages including the ability to derive iPSC from patients. This allows to model organoids with genetic heterogeneity, create clonal organoids or even genetically modified organoids by ever-improving CRISPR technologies [89], and the use of organoids to study polygenic diseases. An alternative source of cells for 3D organoid studies could be resident precursor cells, as suggested by the 3D cultures of adipose tissue derived from stromal vascular cells [90]. Blood vessel organoids are a rapidly emerging technology [91, 92], holding hope for their use in conjunction with GWAS.

However, it is likely that genetically engineered organoids may not fully mimic DNA methylation and other epigenetic patterns present in the original tissues or the responses from cross-talk with other cell types in the complex tissues under study [93], including the contribution of recruited inflammatory cells, a common element in cardiometabolic diseases. Furthermore, they are limited in size with physiological consequences associated with access to oxygen and nutrients. The recent development of functional blood vessel networks contributing to oxygenation within organoids may help achieve larger organoid sizes and reduce cellular stress to improve tissue maturation. Together with the co-culture with cells of relevance to the disease beyond the primary cell type of interest in a given tissue, these advances will further propel the field allowing better leverage of organoids for GWAS studies. In summary, iPSC and organoids represent relatively new technologies that have yet to be further embraced for the study of newly identified genes or variants from GWAS, particularly in the cardiovascular field. As the differentiation and 3D assembly technologies continue to advance, organoids are likely to become the gold standard to systematically study the genetic spectrum of CVD-associated variants and their biological and physiological consequences and to accelerate personalized clinical interventions.

Animal Models for GWAS-Associated Studies

Knock-Out and Knock-In Mouse Models

Mice are relatively low-cost with many transgenic (Tg) and knock-out (KO) lines available, making mice still the most used animal model in academic research. Human and mouse share about 85% identity at the nucleotide and protein levels

and commercially available mouse lines have been established that recapitulate key aspects of human CVD. Thus, for instance, both *ApoE* and *Ldlr* knock-out mice have been widely used to study atherosclerosis in mice and recently a novel genetic mouse model was reported to better recapitulate characteristics of human aortic aneurysm [94], adding to a number of widely used experimental models of aneurysm [95]. In its simplest and most straight forward fashion, KO and Tg overexpression of candidate genes in wild-type or genetic models of CVD have been routinely used to study the function and molecular mechanisms underlying the contribution of suspected causative genes (or genetic variants) when conserved across species and involved in highly preserved, often well-characterized, pathways. Therefore, there is ample literature and examples of their successful implementation since the early days of GWAS [96–98].

We offer one example from our team that took us from GWAS findings to a potential candidate for drug repurposing. In 2010, our colleagues found that KLF14, a maternally imprinted gene, was associated with HDL-C and CVD in both sexes while showing female-specific association with triglycerides in a GWAS involving more than 10,000 individuals of European ancestry [99]. We generated *Klf14* KO mice and, in conjunction with in vitro approaches, demonstrated that Klf14 promotes ApoA-I transcription, increases HDL-C, and protects against atherosclerosis in the mouse. In addition, we found that perhexiline, a drug currently used in some countries for congestive heart treatment, is a novel KLF14 activator and can reduce atherosclerosis in mice [73, 100]. Although the basis for the gender differences in triglycerides remains under study, our multidisciplinary approach could promote the repurposing of perhexiline for the treatment of atherosclerosis.

We recently witnessed also how GWAS, in turn, guided the discovery of novel pathways in CVD. In 2017, a large whole-exome sequencing performed by Jaiswal, S. et al. [101, 102] on human peripheral-blood cells proposed a link between clonal hematopoiesis of indeterminate potential (CHIP) with early-onset of atherosclerosis. Mutations in DNMT3A, TET2, ASXL1, and JAK2 were associated with coronary heart disease, and thus could define a new pathway contributing to atherosclerosis and CVD. Since then, people have generated different mouse models to recapitulate this phenotype and study the mechanisms of how CHIP influences atherosclerosis [102, 103]. Those studies showed that bone marrow cells carrying CHIP-related mutations will undergo clonal expansion and express higher levels of inflammatory genes. In fact, the presence of CHIP in cells from peripheral blood signals a doubling in coronary artery disease risk, thus bringing the original findings from GWAS to the discovery of a new pathway and marker of CVD risk.

Limitations and Opportunities In spite of the success of these approaches, not all genes and target sites for mutations can be found in the mouse genome and for a given gene, species-specific regulatory elements or physiological roles may exist. Additionally, recognized metabolic differences exist between mice and human, including lipid metabolism in the liver and blood, that have hindered or, at times, even misled translational efforts [104–106]. To overcome those limitations, at least 2 different strategies have been used to humanize mouse models in order to study in vivo non-conserved genes or variants, as we discuss below.

Humanized Mouse Genomes Through Knock-In or Transgenic Technologies

Genomic humanized mice involve knocking-in of human sequences to replace the endogenous mouse sequence. Compared with classical transgenic mice involving human genes—which usually integrate the human coding sequence at random and carry an investigator-defined promoter—these humanized mice are considered more physiologically relevant, since they maintain expression from preserved regulatory elements in the cognate promoter and can mimic expression patterns in vivo [107]. Multiple genomic humanized mouse models have been generate, including *HLA* [108], *FOXP2* [109], *APP* [110], *APOE* [111–114], *CETP* [115], *TP53* [116], and *HTT* [117].

Limitations and Opportunities These mouse models can still be affected by inherent problems of transgenesis, including variability in expression due to random integration and copy numbers, disruption or gene silencing of endogenous genes at the integration site, and lack of reproducibility of results between different founders or in different mouse backgrounds. In addition, they suffer from intrinsic species-specific differences in physiology (Table 2), a limitation of virtually all animal models, which can confound the functional findings. Finally, the use of this type of humanized mouse models in conjunction with findings from GWAS has been limited and mostly restricted to variants in the coding region of genes of interest. This is likely due to low efficiency for gene editing associated with the size of the knock-in fragments, which prevents the replacement of an entire genomic locus to include human regulatory elements. The High-throughput Human Genes on the X Chromosome (HuGX) has the potential to overcome this limitation is. This approach can be applied to human genes able to complement a mouse null allele and for which a bacterial artificial chromosome (BAC) exists. Recombination can be used to create a BAC carrying regulatory regions and/or individual human variants of interest followed by BAC knock-in into the mouse genome and functional studies [118]. Although BAC knock-in implementation in the cardiovascular field in conjunction with GWAS appears to be lagging, this approach was successful in generating BAC Tg mice for cardiovascular disease [119]. It is anticipated that

Table 2 Comparison of mouse and rabbit models for atherosclerosis studies

	Mouse	Rabbit	Human
Cost	$	$$$	-
Time to sexual maturity	6–8 weeks	4–4.5 month	10–20 years
Major plasma lipoprotein	HDL dominant	LDL dominant	LDL dominate
Liver ApoB mRNA editing	Yes	No	No
CETP activity	No	Yes	Yes
Response to statin drugs	No	Yes	Yes
Hepatic lipase	High; mainly in circulation	Low; liver-bound; hepatotoxicity following prolonged cholesterol feeding	High; liver bound
Response to cholesterol diet	Resistant (wild type)	Sensitive; with some individuals resistant	Sensitive
Macrophage VLDL receptor	Yes	Yes	Low
Atherosclerosis model	*Ldlr* KO, *Apoe* KO, *APOE*3*-Leiden mice	Watanabe rabbits; Apoe KO rabbits. High-cholesterol diet (0.2–0.5%) for WT	-
Atherosclerotic plaque pathology	Foam cells with necrotic core	Foam cells with fatty streak and macrophage, advanced lesion with calcification	Early stage: fatty streak Advanced: necrotic core, ulceration, rupture, and thrombosis
Coronary lesion	No	Yes	Yes

BAC Tg mice could prove a useful tool for functional study of variants identified in GWAS as associated with human CVDs [120–122].

Humanized Mouse Models Through Transplantation of Human Cells

Knock-in of human genes in the mouse helps overcome some inter-species differences. Nonetheless, genes absent in mice or with different alternative splicing and regulatory elements create a barrier for their study in animal models. The same applies to a large number of long non-coding RNA (lncRNAs), which are largely species-specific. To increase the translational potential of studies in mice, efforts have been made to humanize the mouse models through the transplantation of human cells. Different models have been developed to humanize the immune system for CVD studies [123, 124]. Here, we will focus on humanized liver mouse models since the liver is one of the most important organs for lipid metabolism and GWAS has identified numerous CVD-associated variants which are in lipid-related genes.

Well-established differences in lipid metabolism between mouse and human prompted the development of technologies for humanizing mouse livers. Chimeric mice with repopulated human hepatocytes in the TK-NOG background are emerging as the model of choice. They express the Herpes Simplex Virus-1 Thymidine Kinase (HSVtk) transgene (TK) driven by the mouse albumin enhancer/promoter in the immunodeficient NOD/Shi-scid *IL2R*gnull (NOG) mouse. Brief exposure to a non-toxic dose of ganciclovir (GCV) causes ablation of the mouse liver cells. Transplanted human liver cells can rescue the injury, are stably maintained within the liver without further GCV treatment (humanized TK-NOG), recapitulate the human transcriptome, and sustain mice survival for approximately 8 months [125–127]. Their usefulness in conjunction with GWAS is exemplified by the study of long non-coding RNAs (lncRNAs). LncRNAs are transcripts of at least 200 nucleotides long lacking predicted coding potential. Most human lncRNAs are not conserved in rodents, which hampers functional studies in vivo. GWAS have identified a plethora of human lncRNA in association with disease, including CVDs (for a recently compiled database [128]). Ruan, X. et al. [124] recently reported an "integrated practical pipeline" to study the physiological function of human lncRNAs non-conserved in mice. Briefly, human lncRNAs differentially regulated in metabolic diseases in the liver, as identified by GWAS, were further designated as potentially functional using epigenetic markers and 3-D chromatin interactions, liver enrichment, co-expression functional prediction, and in vivo regulation by metabolic milieu. The liver-specific humanized TK-NOG mouse model was used to address the regulation and function of non-conserved human lncRNAs. As a proof-of-concept, they provide experimental evidence that the obesity-associated lncRNA, LINC01018, through interaction with the RNA-binding protein Human antigen R (HuR), functions in regulating the expression of genes involved in fatty acid oxidation in humanized livers. Recently, Sugahara, G. et al. [129] described a humanized liver mouse

model utilizing human primary hepatocytes from individual patients, taking this technology a step closer to personalized clinical applications. Ornithine transcarbamylase deficiency (OTCD) is a metabolic and genetic disease of the hepatic urea cycle. Using primary human hepatocytes isolated from two patients and a healthy control, they achieved over 80% replacement rates after implementation of a serial transplantation protocol, with the resulting highly humanized OTCD mice presenting a pathology similar to OTCD patients and allowing to address the effects of allopurinol in this preclinical model. Similar approaches could be readily adapted for the functional study of specific risk-alleles identified in GWAS as associated with liver lipid metabolism.

Limitations and Opportunities One caveat when using humanized mice for these types of studies is evident from the recent data of Jiang, C. et al. [127] derived from comparative transcriptomic analysis of human, mice, and humanized mice livers. This work uncovered a high percentage of genes showing opposite regulation between species, which persists under well-matched conditions through cell-autonomous differences. These caveats should be carefully considered in the process of mouse-to-human translation of findings, even in the liver of humanized mouse models. Those findings also underscore the fundamental need to optimize the replacement rates of mouse hepatocytes. Additionally, the humanized mice showed human-like plasma lipid profiles although failed to develop atherosclerosis [130], likely reflecting species-specific differences or as a consequence of intrinsically impaired immune-mediated contribution to the disease in the NOG background. As technologies continue to advance, it is foreseeable that human iPSC, engineered through gene editing, in combination with further optimization of differentiation protocols and liver organoids production, might be eventually used to provide tissue-specific humanized mouse models for mechanistic studies of GWAS variants and preclinical evaluation of potential therapeutics.

Rabbit Models

The rabbit, although less commonly used as a model, has a unique value for CVD research, having made considerable contributions to the field. A seminal example is the use of the Watanabe heritable hyperlipidemic (WHHL) rabbits, a model of familial hypercholesterolemia, to confirm the efficacy of statin drugs in lowering plasma lipids and reducing atherosclerosis [131]. The statin drugs were ineffective in mice and rats, likely reflecting that mice have differences in lipid profiles and metabolic pathways compared with human, while rabbits provided a more suitable preclinical model. We provided a thorough comparison of mouse, rabbit, and human models in Fan J. et al. [104] and we summarize it here in Table 2.

Currently, and of relevance to CVD, there are several rabbit models for atherosclerosis studies including the WHHL rabbits [132, 133], *ApoE* KO rabbit [134], and *apoA* transgenic rabbits [135]. With the advent and improvement of genomic editing tools (ZFN, TALEN, CRISPR/Cas9) [136], genome-edited rabbits are becoming increasingly attractive models for CVD. At the Center for Advanced Models for Translational Sciences and Therapeutics (CAMTraST, https://camtrast.med.umich.edu/) of the University of Michigan, we created a platform to generate knock-out and transgenic rabbits to serve the research community [136, 137]. CETP is a key intravascular regulator of cholesterol transfer between LDL and HDL and was first associated with plasma lipid metabolism at the onset of population genetic studies [138] and later with coronary heart disease [139] and metabolic syndrome [140, 141]. Mice do not express the *CETP* gene, and studies from CETP Tg mice are controversial regarding their ability to recapitulate the human CETP biology, which is beyond the scope of this review. To address those limitations, we created a *CETP* KO rabbit [106] and demonstrated that these rabbits have higher plasma HDL, matching the human genetic findings [142, 143]. On high-cholesterol high-fat diet, they showed reduced plasma total cholesterol in association with decreased ApoB-containing particles and were protected against diet-induced atherosclerosis. Therefore, rabbits can provide useful models to understand simultaneously the contribution of *CETP* variants to atherosclerosis and statin responses, as well as to explore the potential of CETP inhibition for the treatment of human atherosclerosis. Beyond CVDs, recently Lu, Y. et al. [144] used rabbits to address the effects of *GADD45G* variants on craniofacial abnormalities, further underscoring the unique value of this model for functional studies of GWAS findings.

Limitations and Opportunities As an enabling technology for understanding human variation in conjunction with GWAS, rabbits as a model are currently lagging behind overall and in the CVD field in particular, likely associated with costs and the need for dedicated facilities and personnel. Inter-institutional collaborative work can help overcome those limitations. For instance, in addition to the *CETP* KO rabbits, we have generated *CFTR* [124] and *ApoCIII* [145] KO rabbits, human *ApoAII* knock-in and Tg rabbits [146, 147], immunodeficiency rabbits (*RAG1/RAG2*, *FOXN1*, *PRKDC*, and *IL2RG* knock-out) [148], and *CFTR-ΔF508*[149]. As the use of rabbits continues to expand through the improvement of efficient tools for gene editing, we anticipate that genome-edited rabbit models will gain wider use as emerging platforms in the CVD field to help bench-to-bedside translation of human GWAS findings.

Metabolomics

The perturbation of metabolism is a common feature underlying multiple cardiovascular diseases [150]. Recent

technological advances in metabolite profiling, using high-throughput mass spectrometry (MS)– and proton nuclear magnetic resonance spectroscopy (NMR)–based technologies, facilitated the untargeted metabolite profiling in human and mouse biological samples, particularly serum metabolite concentrations [151], as a readout of biological processes associated with diseases. This provides phenotypic data for GWAS investigating the quantitative genetic control of metabolism (mQTL) and informs follow-up functional studies to explore the effects of genetic variation on gene regulatory networks associated with the pathogenesis of complex diseases, including cardiovascular and metabolic disorders.

Several studies have demonstrated that the blood metabolic profile may predict the future CVD risk, including accumulation of succinate [152], branched-chain amino acids [151, 153], urea cycle metabolites [154], short-chain dicarboxylacylcarnitines [155], medium-chain acylcarnitines [155], fatty acids [155], amino acids [156], and metabolites associated with the gut microbiome, like trimethylamine-N-oxide (TMAO)[157], and N,N,N-trimethyl-5-aminovaleric acid (TMAVA) [158]. Recently, we used metabolomics combined with a meta-analysis of liver transcriptomics to uncover suppression of glycine biosynthetic genes in NAFLD and association with fatty liver, leading to the discovery of glycine-based potential therapies for fatty liver disease [159].

Paving the way towards integration of metabolomics with GWAS, Gieger, C. et al. [160] used quantitative measurement of 363 metabolites in serum of 284 male participants of the KORA (Cooperative Health Research in the Region of Augsburg, Germany) study and determined that 12% of the observed variance in metabolic differences was explained by frequent SNPs and 28% of the variance could be explained by metabolite ratios as proxies for enzymatic activity, leading to the identification of four causal genetic variants in the *FADS1*, *LIPC*, *SCAD*, and *MCAD* loci. Meanwhile, Shah, S. et al. [161] demonstrated a high heritability for amino acid changes (arginine, ornithine, alanine, proline, leucine/isoleucine, valine, glutamate/glutamine, phenylalanine, and glycine) in premature coronary artery disease. Additionally, the integration of GWAS and Metabochip meta-analysis identified 97 BMI-associated loci [162]. Metabolomics has, therefore, established itself as integral to advance functional characterization of GWAS findings.

Limitations and Opportunities The increasing relevance of metabolomics in the study of cardiometabolic diseases is highlighted by the 2017 Scientific Statement of the American Heart Association [163] recognizing the contributions of metabolomics and its potential to provide "new insights into the factors that contribute to complex human diseases such as cardiovascular disease" while recognizing that "current challenges facing the field of metabolomics include scaling throughput and technical capacity for metabolomics approaches, bioinformatic and chemoinformatic tools for handling large-scale metabolomics data, methods for elucidating the biochemical structure and function of novel metabolites, and strategies for determining the true clinical relevance of metabolites observed in association with cardiovascular disease outcomes." As an increasing body of literature demonstrates the power of integrating GWAS with metabolomics at different levels [164–170], the AHA charge that "Progress made in addressing these challenges will allow metabolomics the potential to substantially affect diagnostics and therapeutics in cardiovascular medicine" continues to be relevant. Echoing the AHA statement, we advocate further support for the improvement of this powerful technology and the undertaking of metabolomics (and proteomics) studies in conjunction with GWAS as a powerful approach to accelerate the bench-to-bedside translation of genetic findings and promote the practice of precision medicine based on individual genetic and metabolic variability and the discovery of novel disease biomarkers and therapeutic targets [171].

Epigenomics

Epigenetic modifications, per se through interaction with the environment (internal and external milieu) or as a consequence of genetic variation, are recognized drivers of health and disease. Nonetheless, they are not captured in epidemiological studies and may play a relevant role in understanding the manifestation of disorders that appear to have weak GWAS association, including cardiometabolic diseases. In fact, epigenetic changes independent of genetic variation have been often associated with chronic diseases, including CVD. The systematic and intentional undertaking of comprehensive epigenome-wide association studies (EWAS), defined as the determination and compilation of genome-wide quantifiable epigenetic marks (such as DNA methylation and histone acetylation) at the individual level to derive associations between epigenetic variation and a particular identifiable phenotype/trait, is the next logical step towards personalized medicine. An increasing number of EWAS datasets relevant to CDV have been and continue to be reported, including recent additions on lipids [172], metabolic syndrome (including racial differences) [173, 174], ischemic stroke [175], high blood pressure [176], too numerous to feature. Relevant pioneering examples include an EWAS identifying modifications likely driving cardiomyopathies independent of obvious GWAS association [177], the studies on beta-cell adaptation in type 2 diabetes (for a recent review [178]), and differentially methylated CpG island subsets in association with the smoker status of patients located in the *KLHL35*, *CNN2*, *SERPINB9*, and *ADCY10P1* loci [179].

In the context of this review, EWAS are likely to add a rich layer to our understanding of the findings from GWAS. In fact, in recent years, numerous genetic variants have been

identified as located at sites of DNA methylation and siRNA seed sequences, underscoring the interplay between genetic and epigenetic risk factors and allele-specific gene expression. For instance, Agha, G. et al. [180] uncovered 52 CpG (cytosine-phosphate-guanine) sites associated with incident coronary heart disease (CHD) or myocardial infarction from nine population-based cohorts that included profiled epigenome-wide blood leukocyte DNA methylation levels. They were associated with genes involved in calcium regulation (*ATP2B2*, *CASR*, *GUCA1B*, *HPCAL1*), serum calcium–related risk of CHD (*CASR*), coronary artery calcified plaque (*PTPRN2*), and kidney function (*CDH23*, *HPCAL1*), among others, while also mapping close to active regulatory regions proximal to lncRNAs. Beyond the mechanistic insights, this study also underscores the diagnostic value of epigenetic approaches. In the opposite direction, the EWAS study by Jones, G.T. et al. [168] identified a relatively uncommon variant (rs76735376) at the CpG site, cg17028067, in intron 1 of the *LPA* gene associated with plasma Lp(a) levels by altering enhancer activity. Finally, recently Wang, B. et al. [181] addressed the question of why the hundreds of genetic loci associated through GWAS with atrial fibrillation explain only a small proportion (3.5%) of heritability in this disease. They combined GWAS, EWAS, and transcriptomics (TWAS) and uncovered 1931 genes (compared to the 206 identified by GWAS alone) that explained 10.4% of heritability. These examples make a compelling case for routine integration of epigenomics in multiple platforms for the advancement of GWAS findings.

Limitations and Opportunities In summary, in spite of some limitations for sound validation across independent studies, epigenomics has earned its place as a rapidly emerging technology that will contribute significantly to unravel the impact of environmental signals in the metabolome as well as in the manifestation of genetic variation in human diseases when used in combination with GWAS.

Summary and Perspectives

The expansion of GWAS in the last 15 years has afforded the scientific community great progress in understanding the genetic basis of disease at large, and cardiovascular disease in particular, and has paved the way to uncover novel pathways and mechanisms through functional studies in multidisciplinary settings. Yet, the relative contribution of genes identified from GWAS to chronic diseases appears more modest than first anticipated, accounting for 15–50% increase in disease risk [182]. New and ever-improving technologies have provided methods for collection and analysis of curated data spanning gene expression, changes in metabolites, protein abundance and post-translational modification, and the epigenetic regulation of gene expression, while improved gene editing facilitates a more efficient and expedited creation of highly specific in vitro and in vivo models. As these new layers became incorporated with GWAS in multi-omics, multi-platform integrated approaches, we saw considerable improvement in the identification of genomic predictors and the dissection of their functional consequences. Currently, some barriers still remain which, in themselves, constitute opportunities for progress. On one side, the improvement of the different platforms and the new promise of "organ-on-a-chip" approaches will continue to expand the repertoire of enabling technologies for broadening the impact of GWAS findings. On the other side, incorporating acquired susceptibility (for instance, through environmental exposures, acute or chronic), evidenced in epigenetic modifications and operating on genetic predisposition, can help identify modifiable risk factors or inform pharmacogenomics, including drug design [183]. How the microbiome interacts with the host genetics is a relatively new area of research in metabolomics and may hold promise for clinical intervention. Finally, the routine integration of these different platforms could have an invaluable impact on the field of pharmacogenomics. It is incumbent upon us, our institutions and patient advocates to engage the "customers" (the public at large and the patients in particular), the funding agencies and health care systems, including clinicians and social epidemiologists, and other stakeholders globally, into multidisciplinary consortia and "moon-shot" initiatives that stem from the potential for a high return on investment derived from supporting research of multidisciplinary teams systematically integrating GWAS, multi-omics, drug development, and models of disease in order to accelerate the realization of the still elusive dream of personalized medicine.

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Declarations

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