Spotlight Issue: “What have we learned from GWAS in CVD research?”

Invited Spotlight Review: “Genetics of Lipids”

From lipid locus to drug target through human genomics

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Abstract

In the last decade over 175 genetic loci have robustly been associated to levels of major circulating blood lipids. Most loci are specific to one or two lipids, while some (*SUGP1*, *ZPR1*, *TRIB1*, *HERPUD1*, and *FADS1*) are associated to all. While exposing the polygenic architecture of circulating lipids and the underpinnings of dyslipidemia, these genome-wide association studies (GWAS) have provided further evidence of the critical role that lipids play in coronary heart disease (CHD) risk, as indicated by the 2.7-fold enrichment for macrophage gene expression in atherosclerotic plaques and the association of 25 loci (such as *PCSK9*, *APOB*, *ABCG5-G8*, *KCNK5*, *LPL*, *HMGCR*, *NPC1L1*, *CETP*, *TRIB1*, *ABO*, *PMAIP1-MC4R*, and *LDLR*) with CHD. These GWAS also confirmed known and commonly used therapeutic targets, including *HMGCR* (statins), *PCSK9* (antibodies), and *NPC1L1* (ezetimibe).

As we head into the post-GWAS era, we offer suggestions for how to move forward beyond genetic risk loci, towards refining the biology behind the associations and identifying causal genes and therapeutic targets. Deep phenotyping through lipidomics and metabolomics will refine and increase the resolution to find causal and druggable targets, and studies aimed at demonstrating gene transcriptional and regulatory effects of lipid associated loci will further aid in identifying these targets. Thus, we argue the need for deeply phenotyped, large genetic association studies to reduce costs and failures and increase the efficiency of the drug discovery pipeline. We conjecture that in the next decade a paradigm shift will tip the balance towards a data-driven approach to therapeutic target development and the application of precision medicine where human genomics takes center stage.

Introduction

Lipids are essential for life and have several important biological functions, including energy storage, formation of a phospholipid bilayer to protect the cell, signalling, and transport[1]. Given the important role that lipids play in the body, the “lipidome”, which is the totality of lipid molecules in cells and in circulation, can therefore reflect underlying metabolic processes that may be influenced by dietary, environmental, and genetic factors[2]. Indeed, in the last decade over 175 genetic loci have robustly been associated to levels of four major circulating blood lipids: total cholesterol (TC), low-density and high-density lipoprotein cholesterol (LDL-C and HDL-C, respectively), and triglycerides (TG)[3–12]. While exposing the polygenic architecture of circulating lipids and the underpinnings of dyslipidemia[6], these genome-wide association studies (GWAS) have provided further evidence of the critical role lipids play in influencing coronary heart disease (CHD) risk[3,13] and serving as markers for commonly used therapeutic targets[3].

As we head into the post-GWAS era[14,15], much of the focus has now turned towards determining how best to translate the enormous wealth of information gleaned through GWAS to inform clinical practice and therapeutics.

Here, we offer suggestions for how to move forward beyond genetic risk loci towards elucidating the biology underlying genetic associations and identifying causal genes. Ultimately, we hope these suggestions will help to increase the efficiency of the drug discovery pipeline[16].

The genetic architecture of circulating lipids

In order to better understand the role that genetics plays in affecting lipid levels, a number of association studies of major lipids have been conducted. One of the first large-scale meta-analyses of circulating lipids was published in 2010, which reported the discovery of 95 genetic loci significantly associated with plasma concentrations of total cholesterol, LDL-C, HDL-C, and triglycerides, of which 59 loci were novel at the time[3]. Currently, association studies have uncovered 175 genetic loci that affect lipid levels in the population (**Supplementary Table 1**)[3–12]. Most of these variants reside in non-coding portions of the genome, where the precise function is often not well known.

A graph summarising genetic associations with the four major circulating lipids is shown in **Figure 1**. This indicates that the majority of the genetic loci are only associated with a single lipid or two lipids, but there is a subset of genes that are associated with most or all of the lipids, such as *SUGP1*, *ZPR1*, *TRIB1*, *HERPUD1*, and *FADS1*. The diagram also annotates genes that exhibit tissue-specific mRNA expression in either human liver or adipose tissue relative to all other tissues assayed by the Human Protein Atlas (HPA) (for further details see: https://www.proteinatlas.org/humanproteome/tissue+specific)[17,18]. Specifically, 22 of the 175 genes appear to be liver-specific, with a further three genes showing specificity to adipose tissue. Furthermore, in analyses using the Functional Annotation Clustering tool at the Database for Annotation, Visualisation, and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/)[19], this list of 175 genes was found to be significantly enriched (enrichment score = 3.67 [p<0.05], mean fold enrichment = 2.66) with genes that are highly expressed (*i.e.*, in the 3rd quartile) in human plaque macrophages as measured by RNA-seq for the Cancer Genome Anatomy Project (CGAP)[20]. This is indicative of a link between many of these genes (**Figure 1**) and CHD[21].

Translational perspective on lipid GWAS

At present, 88 independent and replicated loci for CHD have been identified in European populations (**Supplementary Table 2**)[22–24], and from joint association analyses the total heritability of CHD is estimated to be 28%[25]. Of these 88 loci, 25 (such as *PCSK9*, *APOB*, *ABCG5-G8*, *KCNK5*, *LPL*, *HMGCR*, *NPC1L1*, *CETP*, *TRIB1*, *ABO*, *PMAIP1-MC4R*, and *LDLR*) are also associated with major circulating lipids (**Figure 2**). One example is *PCSK9*, which is involved in the regulation of LDL receptor recycling and therefore serves as a promising pharmacological target and has led to a major advance in cholesterol-lowering drug therapy[26]. Studies have shown that *PCSK9* inhibition results in a significant reduction in LDL-C in patients at high risk for cardiovascular disease (CVD)[26]. Common polymorphisms in the *HMGCR* and *NPC1L1* genes are also associated with attenuated LDL-C reduction and their gene products are targeted by successful lipid-lowering drugs (*i.e.* statins and ezetimibe, respectively)[3].

Moving beyond GWAS

Fine-mapping GWAS signals and finding causal genes

In less than ten years the number of published genome-wide association studies has grown at an approximately exponential rate[27]. By definition GWAS does not require any *a priori* information relating to the trait in question whilst enabling genome-wide coverage, *i.e.* a GWAS is hypothesis-free. GWAS therefore represents perhaps our most powerful tool for the robust and agnostic identification of novel genetic loci associated with complex diseases and traits. Thereby GWAS provide us with the enormous opportunity to extend our understanding of the underlying biology, and serve as an extensive knowledge-base for the development of therapies and the prevention of disease[28].

However, interpreting GWAS results has proven to be challenging as genetic associations do not necessarily point to the causal variant or gene directly.Likewise, functional annotation of the majority of associated variants is currently lacking[29]. To pinpoint causal variants and genes, various approaches, which are not mutually exclusive, have been developed and tested in practice.

Statistical models to fine-map the signal

Many functional and statistical fine-mapping approaches have been proposed and validated, which has led to the refinement of GWAS signals and prioritized the likely causal gene targets in associated loci. Custom-genotyping arrays have been designed by large consortia in collaboration with industry. The Metabochip, for instance, was developed to fine-map genetic regions associated with various metabolic traits and disease, such as blood lipids, glucose and insulin levels, body mass index, type 2 diabetes (T2D), and coronary artery disease[30]. Re-sequencing regions to gain a higher resolution of genetic variation in a region has identified a non-coding RNA, *ANRIL*, in the coronary artery disease (CAD)-associated 9p21 region[31]. Conditional analyses, by which other variants in an associated locus are analyzed conditional on the sentinel variant, have successfully identified secondary signals. Bayesian statistical methods, using prior knowledge to inform the statistical modeling, have also helped to identify “credible sets” of variants that are 95% likely to contain the causal variant(s), as demonstrated in several exemplary publications focused on type 2 diabetes, Graves’ disease, and CAD[29,32,33]. Additionally, leveraging ancestral genetic diversity through trans-ethnic meta-analyses of GWAS can help to refine signals[34,35].

Deep-phenotyping

The strength of an association with any genetic variant is partly determined by how, or how well, the phenotype was measured. Deep-phenotyping the genome, through refinement of

the phenotype, ultimately increases the resolution at which we search for causal and druggable pathways.

Due to technological limitations, up until recent years most studies of lipids have been relatively crude; despite the diversity of lipid species and the wide array of functions in which lipids are involved, the majority of studies have focused on major circulating lipids that can easily be measured by standard clinical chemistry assays. Although these standard lipid biomarkers at present remain a fundamental part of everyday clinical practice, lipidomics, the measurement of hundreds of individual lipid subfractions, takes a more global yet refined view of lipid metabolism and can provide a detailed picture of abnormalities in lipid levels[1]. For example, mass spectrometry analysis of circulating lipids in the Bruneck Study revealed 135 lipid species across 8 different lipid classes[36]. Lipid species from the cholesterol ester (CE16:1) and triacylglycerol (TAG54:2) classes showed the strongest predictive value for 10-year CHD risk. Adding these to a model including traditional risk factors improved the risk prediction and classification for CHD[36].

In contrast, metabolomics, the measurement of small metabolic markers, can provide a more direct reflection of the physiological state, making them an ideal method of tracking changes induced by the environment, disease or treatment. Metabolites can also facilitate deeper phenotyping since they are closer in proximity to clinical outcomes than proteins or genes and contain more information on the health status of individuals compared to other “-omics" technologies[37]. Identification of metabolites that are implicated in the onset of atherosclerotic-related diseases can lead to directed screening, early-detection of at-risk individuals, and better treatment of high-risk individuals[38].

A metabolite-based genome-wide association study (mGWAS) is defined as a GWAS where metabolic traits are used as the phenotypic traits[39]. The metabolites include all lipid-related traits as well as apolipoproteins, and in addition other metabolic classes such as amino acids, ketone bodies, glycolysis-related metabolites, carbohydrates, cofactors and vitamins,

energy-related metabolites, nucleotides, peptides, and xenobiotics. The first-ever mGWAS, published in 2008, involved quantitative measurement of 363 metabolites in serum samples from 284 male participants in the KORA study[40]. Although four loci associated with metabolites were discovered (*FADS1*, *LIPC*, *SCAD*, and *MCAD*), the sample size was quite limited and there was no replication data. However, a follow-up study was published in 2010 using a much larger sample from the same population (1,809 participants) along with a replication cohort of 422 participants from TwinsUK, which resulted in the discovery of eight replicated loci associated with metabolites[41]. The number of published mGWAS has been steadily rising in recent years. Building on a 2015 literature review that identified 21 mGWAS publications, we conducted a more up-to-date and comprehensive literature review of mGWAS with a focus on studies of high-dimensional metabolomics (*i.e.* studies that measured a wide variety of metabolic traits that are involved in human metabolism), excluding studies that measured only a handful of metabolites and were therefore not high-dimensional. Our review identified 31 published mGWAS and one metabolite-based exome-wide association study which collectively report associations of metabolites with 885 genetic loci (**Supplementary Table 3**). The integrated data of two of these studies are freely accessible through the *Metabolomics GWAS Server* at http://mips.helmholtz-muenchen.de/proj/GWAS/gwas/[42–44].

Metabolomics can be used to assess the contribution of specific genes to disease onset, of for instance CHD, and to identify specific metabolic phenotypes that are associated with these genetic modifications[45]. Given that many cardiovascular pathologies have an underlying metabolic basis, metabolomics can reasonably be used to estimate the relative risk of patients, understand pathophysiological mechanisms, and monitor treatment progress[45]. Metabolomics and lipidomics are also expected to play an important role in identifying and characterising disease states and in cardiometabolic drug development[46]. A number of studies have examined the association of metabolites with risk of chronic diseases. These studies have provided evidence that metabolites are associated with risk of

a range of diseases, including diabetes[47–50], impaired fasting glucose[48], hypertension[51], and CVD[36,52,53].

"Genetics: an expression of interest" regulating causal pathways

As gene expression is both heritable and associated with disease, genetic analyses of gene expression patterns across tissues and populations has been a focus of many studies[54–58]. Variants that modulate the expression of genes, expression quantitative trait loci (eQTL), can affect disease by changing the transcription of genes[55,59]. For instance, common genetic variation at the 1p13 locus is robustly associated to circulating LDL-C[3] and susceptibility to myocardial infarction (MI)[60,61]. Individuals of European descent and homozygous for the major alleles of these genetic variants have a 16 mg/dL higher LDL-C as well as ~40% increased risk of MI[62]. Deep-phenotyping of LDL-C subclasses in two independent population cohorts showed a strong association of these genetic variants with very small LDL[62]. These variants mapped to a region between two genes, *CELSR2* and *PSRC1*, with unknown function, and tissue-specific eQTL analyses revealed a third gene, *SORT1*, that showed the largest change in expression[62,63]. Fine-mapping in other cohorts identified a 6.1-Kb region which harboured a haploblock, that showed the largest luciferase activity upon transfection into human hepatocellular carcinoma cells with a luciferase construct, consistent with the gene expression results[62]. Subsequent analyses of a smaller region within this haploblock using electrophoretic mobility shift assays, identified a single variant (rs12740374) that disrupts a C/EBP (CCAAT/enhancer binding protein) transcription factor binding site and alters the hepatic expression of the *SORT1* gene[62]. Thus, integrating GWAS with transcriptome-wide association studies in relevant tissues or cell-types can further assist in the prioritization of causal genes in proximity of the GWAS signal[64], and thereby identify causal gene networks related to a disease. These networks may be linked through the associative signals of genetic variation, expression, and other experimental evidence[65].

Indeed, many trait-associated variants act on regulatory elements that impact gene expression, and studying epigenetic chromatin marks, such as trimethylation of histone H3 at lysine 4 (H3K4me3), has informed fine-mapping efforts by identifying cell-type specific chromatin marks pointing to active regulatory regions[66]. Moreover, recent epigenome-wide association studies (EWASs) have linked DNA methylation (DNAm) at cytosine-phosphate-guanine dinucleotides (CpGs) in whole blood with HDL functionality[67], and levels of circulating lipids in Europeans[68–71] and Afro-Americans[72]. To date the most replicated CpGs lie near *ABCG1*, *CPT1A*, *TNNT1*, *MIR33B*, *SREBF1*, and *TNIP*[73]. One EWAS reported the discovery of 193 CpGs associated to blood lipids; 25 were not previously associated to blood lipids, one of which was cg27243685 that associated to HDL-C and TG[69]. The same CpG also affected the expression of a nearby gene (*ABCG1*, a key regulator of lipid metabolism), and was associated with an increased of incident CHD[69]. The same study reported *cis*-acting methylation quantitative trait loci (meQTL) at 64% of the 193 reported CpGs which were enriched for known GWAS loci associated to circulating lipids and CHD risk[69], adding support to the hypothesis that GWAS loci act on regulatory gene networks. Another approach to fine-map the signal involves analyzing the spatial organization of chromosomes through circular chromosome conformation capture (4C), which can uncover networks of interacting genetic loci[74,75]. Indeed, combining 4C with eQTL analyses in relevant cell types was shown to identify novel candidate genes for known cardiovascular risk loci[76].

Such approaches are not limited to the transcriptome and epigenome, rather they extend into any “-omics”-field. Of specific interest might be the identification of protein quantitative trait loci (pQTL), as these may dampen or amplify the genetic effects and contribute to the phenotypic variation in traits, independently of mRNA expression[77,78]. Thus, integrating data on DNA sequence variation, the epigenome, transcriptome, proteome, lipidome, metabolome[79], and chromosomal organization identifies cell-type specific regulatory gene networks and refines the initial signal uncovered through GWAS[80].

Beyond the common: human knockouts

As complex diseases are part of a quantitative spectrum of conditions[81], not all of the genetic determinants will be captured by common variation and so of late, focus has also shifted towards rare variants[15,82].Studying rare variation is not trivial, as such variants will only be present once or twice in a population of thousands[83,84]. Methods collapsing rare variation into gene-based scores alleviate some of the analytical issues[83,84]. Sequencing efforts have revealed rare variation associated with both lipid-traits and risk for cardiovascular disease[85] for example, a deletion in the asialoglycoprotein receptor (*ASGR1*) gene is associated with reduced LDL-C, triglycerides, and risk of CVD[85].

Recent effort by Lek *et al.* shows the existence of human knockouts without apparent phenotypic abnormalities by examining exomes sequences from thousands of individuals[86]. They show that on average, each individual carries 85 heterozygous and 35 homozygous protein-truncating variants (PTVs)[86]. A total of 3,230 loss-of-function (LoF) intolerant genes were identified, including the majority of known severe haploinsufficiency disease genes[86]. These genes are under extreme selective constraint, and 72% of these LoF genes have yet no known phenotype[86]. Moreover, these genes are depleted of eQTLs, but enriched in genome-wide associated trait loci[86]. A similar earlier effort, by deCODE genetics, revealed that genes are knocked out differentially between tissues, with the lowest percentage in the brain[87]. These studies are useful as they allow for the identification of “naturalistic” human knockouts and provide a means to study the effects on metabolic processes analogous to the knockout of orthologues in mice. In addition, they shed light on the potential genetic redundancy that exists and the effects on human traits and diseases.

A prominent exemplar for studying knock-outs to identify therapeutic targets is represented by studies of *PCSK9*. Some individuals carry rare variants leading to dysfunctional or low-

expressing *PCSK9* and subsequently extremely low LDL-C levels and markedly reduced risk of cardiovascular disease[88]. At present, antibodies against PCSK9 are on the market and are the subject of investigation in clinical trials[88–91]. While clinical trials are still ongoing, these genetic analyses and first reports from clinical trials indicate that human genetic studies have successfully identified a therapeutic target[92]. Since the Food and Drug Administration (FDA) has approved the use of *PCSK9* inhibitors (PCSK9i) in patients with clinical atherosclerotic CVD (ASCVD), but has permitted a broad range of LDL-C thresholds and statin usage intensities to determine eligibility, approximately 8.4 million Americans are eligible for PCSK9i[93]. To reduce the need for costly PCSK9i therapy, a range of other options such as lifestyle modifications and targeting a subset of patients with ASCVD at higher risk of CVD events should be considered[93].

Inferring causality to identify valid drug targets

Traditional epidemiological studies can suffer from measurement error, confounding and reverse causality, which often renders the inference of causality impossible. However, in recent years human genetics has been used to deduce causal effects of presumed biomarkers or drug targets[94]. Indeed Mendelian randomization (MR) studies make use of the intrinsic properties of the human genome; specifically, the random assortment of (risk-conferring) alleles from parent to offspring at conception imply that the genetic information is not influenced by disease status (reverse causality) and necessarily free from confounding by risk factors[95].

This concept of MR was first coined by Martijn Katan[96] and has since gained traction. For instance, the genetic variation in the *CETP* locus is robustly associated with increased HDL-C levels – considered the “good” cholesterol and considered protective against CHD. Cholesterol ester transfer proteins (encoded by *CETP*) in exchange with triglycerides are known to facilitate the off-loading of cholesterol from HDL particles to particles rich in

apolipoprotein B (encoded by *APOB*)[97]. Thus, therapeutic modalities directed at decreasing CETP and thereby increasing HDL-C are thought to be beneficial. However, the same locus did not show evidence for an association with CHD[3,22] Indeed, all but one clinical trial for CETP inhibitors (CETPi) failed by lack of efficacy (dalcetrapib[98–103], by Roche Inc. and evacetrapib[104–106], by Eli Lilly & Co.), or because of side-effects (torcetrapib from Pfizer, Inc.)[107–110]. Interestingly, the REVEAL trial showed that treatment with anacetrapib (Merck, Inc.)[111] on top of atorvastatin significantly reduced LDL-C, while substantially increasing HDL-C and decreasing composite cardiovascular endpoints[112]. Some argue that this was entirely expected as, like the other CETPi, anacetrapib alone did not have a large effect on the levels of atherogenic lipoproteins, as measured by LDL-C or APOB[97]. The decrease in CHD risk is reminiscent of the effects of statins and ezetimibe and likely due to the reduction in non-HDL-C particles such as APOB[97,113,114]. Further, a recent MR study showed that a genetic score comprising *CETP* variants associated with increases in HDL-C does not show a protective causal effect for CHD[113–115]. Consequently, in October 2017 Merck issued a statement stating that "the clinical profile for anacetrapib does not support regulatory filings"[116]. Amgen, Inc. followed suit soon thereafter dropping their CETP inhibitor (AMG899) owing in part to the limited genetic evidence[117], while DalCor Pharmaceuticals continues the development of dalcetrapib for CHD reduction in *ADCY9* mutation carriers[118]. Similarly, epidemiological studies showed an inverse association of apolipoprotein A1 (ApoA1) with CHD. Intriguingly, the ApoA-1 Milano genetic variant is associated with a protection against atherosclerosis development and low ApoA1 levels, despite low HDL-C levels. This formed the basis of the development of an ApoA1 inhibitor, MDCO-216 which showed profound ABCA1-mediated cholesterol efflux and was generally well-tolerated[119,120]. However, in 2016 The Medicines Company (MDCO) announced that MDCO-216 failed to meet the primary end point of change in intracoronary atherosclerotic plaque volume as measured by intravascular ultrasound in the MILANO-PILOT[121], and MDCO subsequently abandoned further development of the compound[122].

In retrospect the enormous costs associated with running a clinical trial for CETP inhibitors or others could have been prevented had MR analyses been performed sooner.

Lastly, clinical trials of Darapladib – an inhibitor of lipoprotein-associated phospholipase A2 (Lp-PLA_2) involved in inflammation (a hallmark of atherosclerosis) – have stopped due to a failure to reduce major adverse cardiovascular events[123–127]. Indeed, recent studies showed that inhibiting Lp-PLA_2 is unlikely to be beneficial[128,129]. This was hinted early on in a GWAS of Lp-PLA_2 activity and mass where eight associated loci were discovered that showed no effect for CAD susceptibility[123–126,130], although this did not involve a formal MR study. Human genetics may also point to early unwanted on-target side-effects or may also highlight associations with seemingly distinct diseases[94]. Indeed, variants in *CETP* associated with increased HDL-C levels are also associated with systolic blood pressure as well as increased risk for age-related macular degeneration[114]. Information such as this will of course enable a more comprehensive assessment of the safety and cost-benefit ratio of drug targets prior to embarking on clinical trials and other costly research endeavours.

Past the inflection point: back to the future

Moving beyond GWAS of lipids, or any other complex trait, requires creative approaches and the analysis, and perhaps more importantly, synthesis, of the ever-expanding wealth of multi "-omics" data now available. It will also require re-discovering 'old' models, implementing new methodology,and an open-mind towards conceivably futuristic technologies. We touch upon a few here.

Re-discovering the 'classical' preclinical experimental model

Lipids play a pivotal role in atherosclerosis, an inflammatory disease involving the deposition of oxidized lipid-particles in the arteries contributing to their hardening[131]. The resulting

restriction in blood flow to vital organs can lead to a heart attack or stroke and other forms of cardiovascular disease. Experimental animal models of atherosclerosis have brought about valuable insights with respect to CVD development and progression, as well as putative molecular and cellular processes involved. They remain, however, experimental. The poor track record of drug development[132] is evident of a caveat in translating putative cardiovascular drug targets (or indeed any potential drug target) identified in animal models to the human species.

The most widely used models of atherosclerosis, and by extension CVD, are mice lacking the ApoE or LDL-receptor gene, two genes critical in lipid metabolism. Yet, the majority of murine-derived atherosclerotic genes show no human genetic evidence for association with—let alone causality to—cardiovascular disease[133]. In contrast, when studying those genes identified in GWAS of CVD mechanistically in murine models of atherosclerosis the concordance is much higher[134]. Results from genomic analyses comparing murine models to the human condition in studying immune response have been conflicting[135–137]. Likewise, murine models of atherosclerosis frequently lack a fibrous cap in coronary lesions, making them less suitable as a model for fibrous cap rupture[138]. Such studies raise the question to what extent the hypotheses driving animal research bares relevance for human CVD—sound as they may be both scientifically and biologically[136,139,140]. Preclinical research is still the cornerstone of many drug development programs, but the mounting evidence from human genetic studies may challenge this paradigm[16,141]. The goal of human genetics is to agnostically identify strong footings for the development of therapies and preventive strategies. It seems only sensible to align our scientific methods with the knowledge coming out of human genetic analyses, before entering into expensive preclinical research programs. In other words, a scientific process going from humans to mice to humans seems more meaningful, logical, and timely (**Figure 3**)[140].

Aside from GWAS and MR studies, the recent Mouse and Human ENCODE projects[142–146] provide another starting point to prioritize putative atherosclerotic genes. As phenotypic effects of orthologous genes frequently differ between humans and mice, a "sensible approach would be to group genes based on mouse-human orthology to improve the translational power"[147]. Many GWAS signals are found in noncoding regions[148], and Stergachis *et al.*[144] show that even though 95% of the regulatory transcription network is identical between humans and mice, most conservation is found in promoters, rather than distal elements. Taking such information into account seems relevant when designing experiments involving murine knockouts.

A crisp future for precision medicine

Experimentally generating knock-outs in humans *in vivo* will clearly never become legally or ethically acceptable. Nevertheless, enormous progress has been made in recent years in the field of gene editing, which has enabled the routine knock-down of human genes *in vitro* with unprecedented efficiency and cost-effectiveness. The CRISPR-Cas9 system has emerged as the preferred method, largely due to its high levels of precision relative to other methods[149]. Indeed, Cas9 endonucleases are found in microbial organisms and contain repetitive elements named CRISPR; the CRISPR-Cas9 system has the natural ability to insert any form of mutation (single nucleotide changes, insertion, or deletions) with precision at any given location[149]. The technology can aid in the creation of knockouts or the introduction of synthetic variants to study the effect on gene regulation and transcription[150]. When integrative "-omics" studies have identified potential gene targets, effects of "credible" causal variation (*e.g.* identified through fine-mapping, analyses of knock-outs, or MR studies)—as surrogate of therapeutics—can be systematically analyzed in high-throughput setups.

In conclusion, GWAS identified 175 loci robustly associated to major blood lipids. To move forward in the post-GWAS era we argue the need for deeply phenotyped, large genetic association studies to reduce costs and failures and increase the efficiency of the drug discovery pipeline. We concur that the field of biomedicine is at an inflection point[151]: as the fields of human genetics, bioinformatics, and epidemiology have matured, they collide into an age of unparalleled 'cloud' computing power, and 'big data'[152]. We surmise that in the next decade a paradigm shift will tip the balance towards a data-driven approach to therapeutic target development and the application of precision medicine where human genomics takes center stage (**Figure 3**).

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Figures

Figure 1: Lipid-loci and their cellular location.

Figure 2: Circos-plot showing co-localization of lipids-associated loci and CAD-associated loci. The outer ring is a circular ideogram of the human genome (b37) annotated with chromosome number, gene name, karyotype. Each SNP is marked green, red, yellow or blue in the graph; represented by a line on the inner side of the karyotype, or a dot in the colored rings. *Green* dots in the green shaded ring represent SNPs associated to **HDL-C**; *Red* dots in the red shaded ring represent SNPs associated to **LDL-C**; *Yellow* dots in the yellow shaded ring represent SNPs associated to **TC**; *Blue* dots in the blue shaded ring represent SNPs associated to **TG**. Different formats reveal if the SNP is firstly (circle),

secondly (triangle), thirdly (rectangle) or fourthly (small dot) associated to the trait. In the innermost ring are lipid associations located in CAD-associated loci, with colours highlighting their first associations as described previously. The three genes currently targeted by drugs are marked bold in bright pink: *PCSK9* by monoclonal antibodies, *HMGCR* by statins, *NPC1L1* by ezetimide. In blue is the *CETP* gene that was targeted by inhibitors, but failed in three clinical trials.

Figure 3: Shifting the paradigm of lipid drug discovery: moving beyond GWAS. Traditionally drug discovery is hypothesis driven, that is it is based on basic research followed by pre-clinical research. In a precision medicine discovery framework human genomics takes center stage by starting off with a genome-wide association study of the trait or disease of interest, and then followed by various fine-mapping efforts. When a handful of likely causal variants or genes are identified, basic and preclinical research are starting. We envision that such an approach will likely yield more successful candidates that end up being valid therapeutic targets.

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