Title

Pharmacogenomics of blood lipid regulation

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Abstract

Blood lipids are important modifiable risk factors for coronary heart disease and drugs target different lipid fractions. Considerable efforts have been made to identify genetic variants that modulate responses to drugs in the hope of optimizing their use. Pharmacogenomics and new biotechnologies now allow for meaningful integration of human genetic findings and therapeutic development for increased efficiency and precision of lipid-lowering drugs. Polygenic predictors of disease risk are also changing how patient populations can be stratified, enabling targeted therapeutic interventions to patients more likely to derive the highest benefit, marking a shift from single variant to genomic approaches in pharmacogenomics.

Introduction

Coronary artery disease (CAD) is a major cause of mortality and morbidity in industrialized countries with a growing global prevalence [1]. Even though the etiology of CAD is multifactorial, blood lipids are important modifiable risk factors and different lipid fractions have been identified as independent risk factors for CAD [2-4]. Notably, the traditional blood lipids (LDL-c, HDL-c and triglycerides) as well as other lipoproteins like lipoprotein(a) [Lp(a)] all contribute to CAD risk and multiple current and future drugs aim at modulating them.

Genomic approaches have provided a most valuable tool to help unravel the complexity of blood lipid regulation. Both family-based rare variant studies and large-scale genome-wide scans have contributed to the identification of more than 250 loci confidently associated with lipid phenotypes [5-11]. These findings have helped guide and interpret functional studies which elucidated multiple pathways for lipid regulation that informed the design of modern lipid-modifying agents.

In this review, we will take the broad definition of pharmacogenomics including the use of genetic variation to better understand drugs and, in the more classical sense, the inter-individual variation in drug response. We will discuss how genetic associations and Mendelian randomization studies can help predict the safety and efficacy of drug targets and how genetic variants can affect drug response and be used as predictive biomarkers. Finally, we will consider a recent shift from approaches based on single variants to methods focusing on polygenic risk which can offer useful prognostic biomarkers informing clinical treatment decisions in targeted populations.

Current approaches for lipid regulation

Statins

In 1994, the Scandinavian Simvastatin Survival Study (4S) marked a turning point in the management of CAD by demonstrating a 42% risk reduction in coronary death for individuals treated with simvastatin over a 5.4 years median follow-up [12,13]. Since then, the use of statins has grown to an estimated prevalence of 17% in 2011-2012 in the United States in a nationally representative sample of the National Health and Nutrition Examination Survey (NHANES) [14]. Statins inhibit the 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG CoA) reductase responsible for a rate-limiting step of cholesterol biosynthesis in the mevalonate pathway [15]. Despite possible pleiotropic effects, the primary benefit of statins is achieved through their LDL lowering effect [16,17].

The genetic determinants of statin response are amongst the most widely studied subjects in cardiovascular pharmacogenomics. A 2015 systematic review identified 8 GWAS of statin response and 166 candidate gene studies [18]. Among the confidently associated genes are transporters (*SLCO1B1, ABCG2*), metabolizing enzymes (*CYP2D6, CYP3A4, CYP7A1*), lipoprotein or metabolism genes (*APOE, LDLR, COQ2*) and the drug target itself (*HMGCR*) [18-20]. Variants in these genes have been associated with both the LDL-c response to statins and the development of adverse effects such as statin-induced myopathy. For instance, the *ABCG2* gene encodes the ATP-binding cassette subfamily G member 2, a multidrug transporter contributing to the intestinal absorption and the hepatic elimination of multiple statins [21]. In rosuvastatin users, a common SNP in this gene (rs2231142) was associated with increased LDL-c reduction of 6.9% which is equivalent to the effect of doubling the dose of the drug [22].

Despite their positive risk-benefit ratio, statins are associated with an increased risk of muscle-related adverse reactions ranging from non-specific myalgias to rhabdomyolisis [23]. These symptoms can lead to statin discontinuation and consequently to an increased risk of coronary events which prompted research efforts aimed at identifying genetic predictors of statin-induced myopathy [24]. Genetic studies have been hampered by small sample sizes and

heterogeneous diagnostic criteria for statin-induced myopathy for which definitions range from reversible on-statin muscle pain to 10 times the upper limit of normal levels for creatine kinase (CK), a biomarker of muscle damage. Additionally, in a recently published unblinded and unrandomized extension of the Anglo-Scandinavian Cardiac Outcomes Trial, the rate of adjudicated muscle-related adverse effects was reported to be increased in unblinded statin users compared to blinded users suggesting a nocebo effect [25]. This effect, compounded by the relatively small sample size of genetic studies of statin induced myopathy, could explain the limited success and reproducibility of these approaches. Nonetheless, a gene has been consistently associated with statin-induced myopathy in genetic studies, *SLCO1B1*, encoding the organic anion transporting polypeptide 1B1 responsible for hepatic statin uptake [26]. Clinical guidelines from the Clinical Pharmacogenetics Implementation Consortium (CPIP) suggest avoiding simvastatin for individuals with low SLCO1B1 function based on haplotypes containing the loss-of-function rs4149056T>C mutation [27]. One promising venue to further improve our understanding of statin-induced myopathy is to gain a better knowledge of disease biomarkers to achieve more sensitive diagnoses. Reference CK ranges are notoriously wide and predicting an individual's normal values using genomic data could lead to improved detection of statin-induced myopathy. Our group has conducted a GWAS of CK levels and identified variants in two possibly relevant genes, *CKM* and *LILRB5* but the derivation of a diagnostic biomarker based on these findings remains ongoing work [28].

Multiple high-quality reviews provide a more thorough description of the genetic determinants of statin response and here we will focus on recent developments and other lipid-regulating drugs [20,29]. The latest large-scale effort to identify genetic determinants of statin response was a meta-analysis of GWAS including over 40,000 individuals. This study confirmed previously known associations with *APOE* and *LPA* while identifying *SLCO1B1* and *SORT1* as novel determinants of the baseline adjusted LDL-c response to statins. The *SORT1* gene encodes sortilin 1 which is responsible for LDL receptor (LDLR)-independent uptake of apoB-containing lipoproteins including LDL [30]. The identified variants are predicted to increase *SORT1* expression depleting LDL subtypes with increased SORT1 affinity in favor of subtypes with greater LDLR affinity. This relative enrichment then favors statin response as statins increase the activity of the SREBP2 transcription factor leading to increased LDLR expression [31].

Ezetimibe

Another commonly used drug to lower LDL-c levels is ezetimibe, which blocks the Niemann-Pick C1-like protein 1 (encoded by the *NPC1L1* gene) intestinal transporter of dietary sterols. The Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) comparing ezetimibe to placebo on a background of simvastatin therapy showed a HR = 0.94 and an absolute risk reduction in cardiovascular outcomes of 2% in acute coronary syndrome patients, with a difference in LDL-c medians between both arms of 15.8 mg/dl [32]. Few pharmacogenomic studies of ezetimibe have been conducted, but polymorphisms in the *SLCO1B1* (also known as *OATP1B1*) gene could modulate ezetimibe excretion and drug response [33]. Variants in the drug target itself (*NPC1L1*) have also been associated with improved reduction in LDL-c levels in patients treated with ezetimibe [34].

For both statins and ezetimibe, genetic variants in the genes encoding their drug targets have effects concordant with those of the drugs. In a 2x2 Mendelian randomization study, Ference *et al.* used variants in the drug targets to derive gene-based scores predictive of protein activity to predict the effect of statin monotherapy (*HMGCR* mutations), ezetimibe monotherapy (*NPC1L1* mutations) or ezetimibe+statin (mutations in both genes) on CAD risk [35]. Individuals with *NPC1L1* mutations but no *HMGCR* mutation, when compared to the control group, had lower LDL-c levels by 2.4 mg/dl and a reduced risk for CAD of 5%. Individuals with *HMGCR* mutations and no *NPC1L1* mutations had almost identical effects. Interestingly, in the group of individuals carrying mutations in both genes, the LDL-c reduction was of 5.8 mg/dl and the risk reduction for CAD was of nearly 11%. This finding is in line with the IMPROVE-IT trial results showing the added benefit of further reducing LDL-c levels using ezetimibe in addition to statins [32].

Fibrates

Fibrates are agonists of the peroxisome proliferator-activated receptor alpha (PPARα) that reduce triglyceride levels and total cholesterol and increase HDL-c levels [36]. In a large meta-analysis of randomized controlled trials, fibrate therapy was associated with a 10% risk reduction of major cardiovascular events [37]. The results from the ACCORDION trial, a post-trial follow-up of The Action to Control Cardiovascular Risk in Diabetes study group (ACCORD), found that diabetic patients with dyslipidemia, defined as triglyceride levels above 204 mg/dl and HDL-c under 34 mg/dl, benefited from the addition of fenofibrate to statin therapy (HR 0.73, 95% CI 0.56 – 0.95) [38]. The Pemafibrate to Reduce Cardiovascular OutcoMes by Reducing Triglycerides IN patiENts With diabeTes trial (PROMINENT, NCT03071692) is currently testing pemafibrate, a new and potent PPAR agonist, in a similar patient population. Most pharmacogenetic studies of fibrate response have focused on candidate gene approaches and variants in the drug target (*PPARA*) have been associated with modulation of the effect of fenofibrate on triglyceride levels [39]. A first GWAS of fibrate response in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) and Action to Control Cardiovascular Risk in Diabetes (ACCORD) studies identified the gene *PBX4* encoding the pre-B-cell leukemia homeobox 4 gene as a modulator of the LDL-c response to fenofibrate [40].

Niacin

Niacin is a ligand for the G-protein coupled receptor niacin receptor 1 (also known as GPR109A or NIACR1 and encoded by the *HCAR2* gene). After binding with niacin, the G_i subunit inhibits the adenylate cyclase and the protein kinase A signaling pathway leading to a decrease of lipolysis and consequently free fatty acid levels [41]. Niacin increases HDL-c and reduces all apoB-containing lipoproteins including Lp(a) which is unaffected by most other lipid-lowering drugs (proprotein convertase subtilisin/kexin type 9 [PCSK9] inhibitors being a notable exception) [42-45]. Regardless of its interesting effects on the lipid profile, niacin use is hampered by flushing adverse reactions and lack of beneficial effects when added to background statin therapy in large clinical trials [46,47]. To date, no large-scale studies have evaluated the genetic determinants of niacin response but a small candidate gene pharmacogenetic study with 196 participants reported an association between niacin-induced flushing and a polymorphism in the liver X receptor α (LXRα) [48]. In a targeted genotyping

study, a missense variant in the drug target (*HCAR2*) was also associated with a decreased reduction in Lp(a) by niacin [49].

Recent developments in lipid-lowering drugs and a look at the future

Genomics for drug target identification and validation: PCSK9 inhibitors

A hallmark of recent developments in cardiovascular drugs is the first successful phase 3 clinical trial of a PCSK9 antibody. In the Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) trial, evolocumab added to background statin therapy further reduced LDL-c to a median of 30 mg/dl in patients with atherosclerotic vascular disease and reduced cardiovascular events with a hazard ratio of 0.85 as compared to statin therapy alone [50]. The primary endpoint, a composite of cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina or coronary revascularization, occurred in 9.8% of the participants in the evolocumab arm compared to 11.3% in the placebo arm. Clinically, PCSK9 inhibitors could be used in statin intolerant patients or when LDL-c targets are not achieved by statins alone such as for familial hypercholesterolemia patients.

The development of this new drug is deeply rooted in modern human genetics and is an excellent example of genomics-driven drug development. *PCSK9* was discovered in 2003 by Seidah NH *et al.* who also underlined its role in cholesterol regulation [51] (reviewed in [52]). Two missense gain of function *PCSK9* mutations causing autosomal dominant hypercholesterolemia were discovered in French families which validated its role in lipid disorders [53]. A sequencing study in 128 African American subjects with low plasma LDL-c levels identified two nonsense loss of function *PCSK9* mutations associated with a 40% reduction in plasma LDL-c [54]. A follow-up of the effect of these mutations in 3,363 participants of the Atherosclerotic Risk in Communities (ARIC) study demonstrated that the 85 carriers of the loss-of-function mutations had an 88% reduction in risk of coronary heart disease when compared to non-carriers [55]. This study also identified rs11591147 (R46L), a loss-of-function mutation that is more frequent in individuals of European descent (MAF ≈ 3%) with concordant yet weaker effects on heart disease risk and LDL-c levels. In a larger meta-analysis of 66,698 individuals, individuals with PCSK9 protein variant R46L had reductions of LDL-c levels by 0.43 mmol/l (13%) and of ischemic heart disease by 30% compared to non-carriers (95% CI for the OR: 0.58 to 0.86) [56]. As the authors noted, the observed reduction in ischemic heart disease is larger than expected based on LDL-c reduction alone which could be attributed to pleiotropic effects of the mutation, but the lifelong effect of reduced LDL-c levels certainly plays a role in this difference. Taken together, these studies have shown how inhibition of PCSK9 lowers LDL-c levels and the risk for CAD without other obvious side effects, prompting the development of therapeutic PCSK9 inhibitors for the treatment of CAD.

In the Open-Label Study of Long-Term Evaluation against LDL Cholesterol (OSLER) and Long-term Safety and Tolerability of Alirocumab in High Cardiovascular Risk Patients with Hypercholesterolemia Not Adequately Controlled with Their Lipid Modifying Therapy (ODYSSEY LONG TERM) outcome trials of PCSK9 inhibitors, it was noted that neurocognitive events occurred more frequently in the treatment arm than the placebo arm (meta-analysis OR=2.81 [1.32, 5.99]) implying possible adverse effects [57-59]. In contrast, there was no significant

deleterious effect of evolocumab on cognition in the EBBINGHAUS substudy of FOURIER [60]. Among the other possible consequences of low LDL-c levels achieved by PCSK9 inhibitors is the development of type II diabetes, a side effect shared in common with statins [61]. Predicting these adverse events can be achieved by looking at the phenotype of individuals carrying *PCSK9* loss-of-function mutations as it was done to predict efficacy. In a Mendelian randomization study based on 111,194 Danish individuals, there was no causal association between variants in *PCSK9* or *HMGCR* and the risk of Alzheimer's disease, vascular dementia, all dementia or Parkinson's disease despite increased observational risk for the latter in participants with LDL-c levels <1.80 mmol/l when compared to individuals with levels ≥4.0 mmol/l [62]. This finding is also supported by prior observational results showing no association between PCSK9 R46L and cognitive performance, activities of daily living and non-cardiovascular events in 5,777 participants of the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) [63]. For type II diabetes, the genetic evidence is revealing. Two large instrument variable studies based on *PCSK9* gene scores showed increases in type II diabetes risk in individuals with genetically lowered LDL-c levels [64,65]. This observation is consistent with LDL-c reduction attributable to genetic variants in the *HMGCR* gene [65] and through its pharmacological inhibition by statins [61]. Analyses based on all LDL-c-associated variants revealed a negative and significant correlation between LDL-c levels and type II diabetes (r=-0.21; P=0.025), but there was heterogeneity in the relationship suggesting that not all LDL-c lowering pathways may lead to increased type 2 diabetes risk [5].

Another interesting approach to predict the consequences of modulating a drug target is to test the association between functional variants in the drug target and all the diseases recorded in patients' medical records using a phenome-wide association study (PheWAS). This method was first developed by Denny *et al.* in 2010 and its value is now recognized in the context of predicting adverse drug reactions or finding drug repurposing opportunities [66-68]. A recent unpublished study tested for the association between the PCSK9 R46L genetic variant and 11 selected phenotypes in a targeted analysis as well as with 278 phenotypes in an exploratory scan using data from the UK Biobank [69]. A protective effect with ischemic stroke (replication OR 0.58 (0.36, 0.89), p=0.02) was detected which is consistent with the known atheroprotective effect of PCSK9 inhibitors and the results from recent clinical trials of evolocumab [50]. The phenome-wide scan did not show an association between the R46L genetic variant and type II diabetes. This may be explained by insufficient statistical power to detect such an association or by the phenotyping in the UK Biobank where diagnoses are only available for in-patient hospitalizations (and only after 1997-04-01) along with self-reported data.

Human genetics for precision medicine: CETP inhibitors

The cholesteryl ester transfer protein (CETP) is responsible for the exchange of cholesteryl esters and triglycerides between HDL and very low-density lipoproteins and LDL [70].

Interest for CETP as a drug target stems from human genetic evidence that some CETP mutations increase HDL-c while slightly lowering LDL-c and apolipoprotein B leading to an atheroprotective lipid profile [71]. Multiple polymorphisms affecting plasma CETP

concentrations or CETP activity have been discovered. Among the most widely studied are TaqIB (rs708272) located in intron 1 and -629C>A (rs1800775 also identified in HGVS as NM_000078.2:c.-656C>A and NM_001286085.1:c.-656C>A) located in the promoter. These two variants are in high linkage disequilibrium (LD) (R^2=0.77 in 1000 genomes Europeans) and haplotype and statistical fine-mapping suggested that the association of TaqIB is likely mediated by the functional -629C>A variant [72]. Other CETP promoter variants explain additional variance in CETP concentration and HDL-c levels which suggests a spectrum of regulatory alleles. When considered alone, the -629C>A variant had the strongest association with CETP concentration explaining 7.9% of the variance and 4.6% of the variance in HDL-c levels. Functional studies have also shown that the 'A' allele reduces CETP levels potentially because of increased affinity with the Sp1 and Sp3 transcription factors that are repressors of promoter activity [73]. In a meta-analysis of CETP phenotypes and coronary disease, both TaqIB and -629C>A had a significant effect on CETP activity, HDL-c levels and apolipoprotein A-I concentrations. The HDL-c increasing allele for these variants also had a protective effect against coronary disease (-629C>A OR=0.95 (0.91, 1.00)) [74]. These results are consistent with the latest results from the Myocardial Infarction Genetics and CARDIoGRAM Exome Consortia projects presenting results from an exome-wide association meta-analysis of 42,335 coronary artery disease cases and 78,240 controls genotyped on the exome array. In this study, CETP -629C>A was the lead SNP at the locus and the OR for the HDL-c increasing allele was 0.96 (0.94, 0.97) [75]. The protective allele was also associated with decreased LDL-c and triglyceride levels suggesting pleiotropic effects of CETP modulation.

Recently, targeted *CETP* sequencing in 58,469 participants identified protein-truncating variants (PTV). PTVs are more likely to yield proteins with lower activity or reduced expression through nonsense-mediated decay and are more likely to have effects similar to pharmacologic antagonists. In this study, individuals carrying *CETP* PTV had increased HDL-c levels (by 22.6 mg/dl), lower LDL-c levels (by 12.2 mg/dl) and lower triglyceride levels (by 6.3%) as well as a reduced risk for coronary heart disease (OR=0.70, 95% CI 0.54 to 0.90) as compared to non-carrier individuals [76]. This effect is far greater than the one observed with common promoter variants and the greater reduction in risk for CAD is concordant with a dose-response effect of CETP modulation. The authors also highlight that the effect of *CETP* PTV is concordant with the lifelong benefits of LDL-c reduction as observed in carriers of *PCSK9, APOB* or *NPC1L1* PTV.

Despite the genetic evidence supporting CETP as a useful drug target, previous trials of CETP inhibitors have failed due to safety concerns (for torcetrapib) or lack of efficacy alone (evacetrapib) [77]. Nonetheless, in 2017 the Randomized EValuation of the Effects of Anacetrapib Through Lipid-modification (REVEAL) trial compared anacetrapib to placebo in 30,449 atherosclerotic vascular disease patients receiving atorvastatin, and showed a statistically significant reduction in the primary composite outcome of coronary death, myocardial infarction or coronary revascularization in the anacetrapib group [78]. The observed rate ratio between both arms of 0.91 (95% CI, 0.85 – 0.97) represents a relatively modest risk reduction given the background statin therapy. The exact mechanism behind the observed protective effects is still debated, but the results from this clinical trial are in line with the observations from genetic variants reducing CETP activity and provides a first validation of the drug target from a randomized controlled trial.

After the dal-OUTCOMES trial of dalcetrapib in acute coronary syndrome patients failed to show efficacy in reducing cardiovascular event rates globally, our group performed a pharmacogenomic GWAS to identify genetic modulators of dalcetrapib response. This scan revealed an association at the *ADCY9* locus where homozygotes for the 'A' allele at the lead SNP, rs1967309, which represents approximately 17% of Europeans, had a 39% (HR=0.61, 95% CI, 0.41 – 0.92) relative risk reduction for a composite of cardiovascular events as compared to placebo [79]. This risk reduction was also supported by results from an imaging study, dal-PLAQUE-2, where several variants in the locus and in linkage disequilibrium with rs1967309 were associated with a reduction of intima-media thickness in individuals with the protective alleles treated with dalcetrapib, but not with a placebo. Individuals with the rs1967309 "GG" genotype, representing approximately 35% of Europeans, had a 27% increase in risk of cardiovascular events when treated with dalcetrapib compared to placebo (HR: 1.27, 95% CI, 1.02 – 1.58) and heterozygotes had a neutral effect. In "G" allele carriers, there was also an increase in C-reactive protein in individuals treated with dalcetrapib but this systemic inflammation marker was unchanged in "AA" individuals. The capacity of the participant's serum to efflux cholesterol from J774 macrophages was also measured. Cholesterol efflux is an important function of HDL where peripheral cholesterol is ultimately transported back to the liver through the reverse cholesterol transport pathway, potentially reducing atherosclerosis and the risk for CAD independently from HDL-c levels [80,81]. Individuals with the "AA" genotype treated with dalcetrapib had a 22.3% increase in cholesterol efflux from baseline vs 3.5% in the placebo arm, in contrast to patients with the "GG" genotype who had no benefit of dalcetrapib [82]. Interestingly, rs1967309 genotypes were also associated with changes in weight in response to dalcetrapib treatment but not with placebo. The underlying mechanisms responsible for the interaction between *ADCY9* and CETP have not yet been entirely elucidated. *ADCY9* encodes adenylate cyclase type 9, an enzyme producing the ubiquitous second messenger cyclic AMP. Studies in genetically-modified mice have shown that the removal of *ADCY9* expression results in a large reduction in atherosclerosis, but only in the absence of CETP (results pending publication). In a GWAS meta-analysis, an *ADCY9* SNP known to influence gene expression has been linked with class I obesity in over 39,000 cases [83]. Another recent report also identified familial mutations in another adenylate cyclase isoform encoded by *ADCY3* causing monogenic obesity [84].

Currently, the dal-GenE trial (NCT02525939) is testing dalcetrapib compared to placebo in acute coronary syndrome patients on the reduction of cardiovascular events in a targeted genetic population defined by the rs1967309 "AA" genotype. This phase III trial could be the first to lead to the approval of a cardiovascular drug specifically in individuals with a genetically-determined responder profile and co-developed with a companion diagnostic test. A positive trial would go a long way to validate the hypothesis-free approach used by our group to identify predictive biomarkers of drug efficacy, representing an important milestone toward the personalization of novel therapies [85].

Drugs in the pipeline

Multiple novel small molecules for lipid-lowering therapies are currently in the drug development pipeline. The review by Gryn and Hegele provides a very interesting overview of

developments in orally administered lipid-lowering therapies [86]. Here, we will focus primarily on the development of novel drugs that are supported by strong human genetics evidence.

Triglyceride levels are consistently associated with increased CAD in prospective studies [87]. Mendelian randomization studies where genetic variants are used to compare the effect of genetically-determined triglyceride levels on CAD support the causality of the observed association, suggesting that interventions aimed at lowering triglyceride levels could be used to reduce the risk for CAD [88]. The effect size of variants associated with triglyceride levels also correlates with that on CAD, which supports the hypothesis of causality (Pearson's R=0.46, p=0.02) [6].

In a sequencing study of Amish participants of the Heredity and Phenotype Intervention (HAPI) Heart Study, a rare nonsense mutation in the gene encoding apolipoprotein CIII (*APOC3*) was identified [89]. Apolipoprotein CIII is an important modulator of triglyceride levels. It inhibits lipoprotein lipase (LPL) which catalyzes the hydrolysis of triglyceride-rich lipoproteins and modulates the hepatic uptake of their remnants [90]. APOC3 is also thought to have other pleiotropic effects including the modulation of cholesterol efflux [91,92]. In heterozygous *APOC3* mutation carriers, the median fasting triglyceride level was 31 mg/dl vs 57 mg/dl for non-carriers. Individuals carrying the mutation also had significantly higher HDL-c and lower levels of LDL-c, VLDL-c, IDL-c and remnant lipoprotein cholesterol. The development of CAD was not evaluated in these individuals, but the heterozygotes did have less detectable coronary artery calcification, a marker of subclinical atherosclerosis [89]. Subsequent sequencing studies have confirmed this nonsense mutation (R19X) and identified three other loss-of-function *APOC3* mutations associated with reduced triglyceride levels (R19X, IVS2+1G>A, A43T and IVS3+1G>T) [93,94]. Participants carrying these mutations were protected against ischemic vascular disease, emphasizing the benefit of lifelong low nonfasting triglyceride levels and established APOC3 as an interesting drug target. Furthermore, the authors found no association between the *APOC3* mutations and inflammation, dementia, cancer or total mortality supporting the lack of serious on-target adverse effects [94]. An antisense inhibitor of APOC3, volanesorsen (ISIS 304801), was proven to reduce triglyceride levels and is currently being evaluated in phase III trials. The successful development of this drug would, as for PCSK9 inhibitors, be an example of genomics-driven drug development.

Another therapeutic target modulating triglyceride levels is angiopoietin-like 3 encoded by the *ANGPTL3* gene which inhibits the lipoprotein lipase (LPL) and endothelial lipase (LIPG) responsible for the hydrolysis of circulating triglycerides and HDL phospholipids, respectively [95]. This gene was first associated with lipid metabolism in mice where a 4 bp insertion leads to a frameshift and a premature termination of the protein along with decreased triglyceride levels [96]. In a 2008 GWAS, a common *ANGPTL3* variant rs1748195 (MAF=30%) was associated with a 7.12 mg/dl reduction in triglyceride levels [10,11]. In a later GWAS with increased sample size, statistically significant effects of *ANGPTL3* variants on total cholesterol, LDL-c, triglycerides but not HDL-c were detected [6]. Similar results were obtained in a large-scale study of the deCODE project where the *ANGPTL3* variants were associated with triglyceride levels and non-HDL cholesterol despite having no effect on HDL-c [97]. To date, the association between common *ANGPTL3* polymorphisms and CAD has been conflicting. Two large studies found no

association between common *ANGPTL3* variants and CAD in CARDIoGRAM (22,233 cases) and deCODE (33,090 cases) and a meta-analysis of two case-control studies, three cross-sectional and seven prospective studies including 5,794 CAD cases found a counterintuitive association where the total cholesterol, LDL-c and triglyceride lowering allele increased risk for CAD [97-99]. These results could be attributable to the milder effect of common polymorphisms as recent large-scale studies of rare loss-of-function *ANGPTL3* variants have consistently shown a risk reduction for CAD of around 40% [100,101]. To date, both evinacumab, a monoclonal antibody against ANGPTL3 and IONIS-ANGPTL3-L_{Rx}, an antisense targeting *ANGPTL3* mRNA, have shown promising results in phase II trials and a phase III trial is underway (NCT03399786) [101,102].

ANGPTL4 is another interesting angiopoietin-like protein that also inhibits LPL. The strongest association signal with common variants in *ANGPTL4* was originally found with HDL-c levels, but associations with other lipid fractions including triglycerides were later shown [6,97]. Common triglyceride-lowering variants in *ANGPTL4* are protective against CAD in case-control studies [97]. As for *ANGPTL3*, exome sequencing and genotyping studies found a mutation, *ANGPTL4* p.E40K, which reduces triglyceride levels and risk of CAD [103,104]. This variant was also found to be protective for type 2 diabetes and to have no effect on fatty liver, a frequent consequence of hypobetalipoproteinemia [5,105]. Mutations in *LPL* have also been found to increase triglyceride levels and the risk for CAD [106]. Using monoclonal ANGPTL4 antibodies in mice and monkeys resulted in lowered triglyceride levels, but also resulted in the accumulation of lipids in mesenteric lymph nodes, possibly decreasing the interest in pursuing ANGPTL4 as a drug target in humans [103].

Finally, lipoprotein(a) [Lp(a)] is an atherogenic lipoprotein similar to LDL with an added apolipoprotein(a) covalently bound to the apolipoprotein B100 molecule [107]. Lp(a) has been linked to increased risk for CAD in epidemiological studies and in Mendelian randomization analyses [108,109]. In addition to its atherogenic effect, the similarity between apolipoprotein(a) and plasminogen interferes with fibrinolysis leading to a prothrombotic effect. In addition, Lp(a) carries proinflammatory oxidized phospholipids [110,111]. Two genetic variants, rs10455872 and rs3798220, explain 36% of the total variance in Lp(a) levels and carriers of two or more variant alleles have a 2.57 (95% CI, 1.80 – 3.67) odds ratio for coronary disease as compared to non-carriers [112]. Niacin and PCSK9 inhibitors are the only drugs currently known to significantly reduce Lp(a) levels. Recently, phase II trials of LPA antisense oligonucleotides have demonstrated reductions in Lp(a) levels of 67-72% [113]. Although the results are promising, whether this reduction in Lp(a) levels will translate into improvements of cardiovascular outcomes will need to be assessed in larger phase III trials.

This section provided multiple examples of drug targets discovered or supported by human genetic studies. The ongoing development of the next generation of lipid-regulating drugs has been heavily influenced by the observable consequences of mutations in human populations. This genomics-driven approach, catalyzed by the emergence of monoclonal antibodies and antisense drugs, could improve the performance of the drug discovery pipeline for cardiovascular diseases and reduce the current high attrition rates.

The polygenic shift in precision medicine

The main goal of pharmacogenetics has long been to identify predictive biomarkers associated with drug response. Such predictive genetic variants can then serve to optimize treatment by allowing to select the appropriate medication or adjust dosage. The Clinical Pharmacogenetics Implementation Consortium (CPIP) regularly publishes clinical guidelines based on the pharmacogenetics literature to guide clinical implementation [114]. Guidelines for many drugs including clopidogrel, warfarin and simvastatin have been published, but there are still only few real-world clinical cases of implementation. In these pharmacogenetic approaches, variants are used as predictive biomarkers of the treatment safety or efficacy [115]. Recently, the possibility of using prognostic pharmacogenetic markers has emerged. The goal of prognostic biomarkers is not to directly predict drug response but rather to predict disease incidence and severity which in turn can be used to guide pharmacological treatment. For example, in a recent analysis of primary and secondary prevention statin trials, it has been shown that individuals in the high genetic risk group for CAD derived increased relative and absolute benefit from statins [116]. Using a genetic risk score based on 27 SNPs confidently associated with CAD, the authors have observed a relative risk reduction of cardiovascular events in the primary prevention setting of 34% with statin therapy compared to placebo for individuals in the low genetic risk group, 32% in the intermediate group and 50% in the high genetic risk group. These differences are potentially clinically relevant as the number of individuals needed to treat with statins to prevent one cardiovascular event in 10 years greatly differed from 66 in the low group to 25 in the high-risk group in the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial. Another group observed a similar risk reduction in cardiovascular events of 44% with pravastatin versus placebo in the high genetic risk group based on the West of Scotland Coronary Prevention Study (WOSCOPS) statin primary prevention trial [117]. In this study, participants in the high genetic risk had also increased coronary artery calcification and a higher burden of carotid plaque. The findings from these studies could tilt the controversial risk-benefit balance of statin pharmacotherapy in the primary prevention setting for patients at high genetic risk of CAD [118].

As GWAS of common diseases become larger, it becomes possible to derive meaningful polygenic measures that account for a large proportion of the total genetic risk of disease. Compounded with methodological improvements in genomic prediction models, we expect these tools to help identify high benefit subpopulations that can streamline drug development and help guide clinical decisions. In a recent study, individuals with a high polygenic risk score for CAD were shown to have a cardiovascular risk profile similar to heterozygous familial hypercholesterolemia mutation carriers [119,120]. For the same level of risk, 1 in every 53 individuals was at high polygenic risk compared to 1 in 256 individuals for the familial genetic variants. This recent evidence reinforces the relevance of polygenic measures of risk as prognostic biomarkers and marks a shift in complex trait genomics away from single-variant approaches that could help fulfill the promise of genomic medicine.

Future perspective

In this review, we have illustrated the different ways by which genomic approaches can be used to inform on the use and development of lipid-regulating medication. A useful drug development model has emerged which builds on the single causal variant discoveries from rare familial diseases followed by the rapid development of antisense oligonucleotides or monoclonal antibodies. These innovations are in line with the initial expectations of the Human Genome Project and can be considered a success of genomic medicine.

At the population level, the importance of polygenic risk scores is becoming evident. Thanks to GWAS of impressive sizes, it is now possible to build strong genomic predictors of disease incidence that also predict an individual's response to environmental factors or drugs. Because these genetic scores are constant throughout life, they offer interesting practical and economic benefits which could change the way preventive cardiovascular medicine is done. Nonetheless, additional challenges will need to be addressed for a true personalized medicine to emerge. For instance, polygenic scores have heterogeneous performance across ethnicity and sex and are strongly biased towards European males who are overrepresented in the GWAS from which polygenic scores are typically based. We expect further methodological developments to partially account for these biases, but increasing diversity in large genetic trials will be paramount to truly improve the clinical relevance of these promising novel methods.

Executive summary

Pharmacogenetics of current blood lipid drugs

- Statins are the most widely studied blood lipid drugs in pharmacogenetics. Variants in different transporters, metabolizing enzymes and lipid metabolism genes are associated with adverse effects or statin response.
- Other important drugs are ezetimibe, fibrates and niacin.

PCSK9 inhibitors as an example of genomics-driven drug development

- Both gain-of-function and loss-of-function PCSK9 mutations have been identified in families and at the population scale. Loss-of-function of PCSK9 is protective for coronary artery disease.
- Monoclonal antibodies against PCSK9 have now been demonstrated to reduce cardiovascular events in a phase III trial.

CETP as a drug target is supported by human genetics evidence and common variants in *ADCY9* determine dalcetrapib efficacy

- Common functional variants and rare protein truncating variants in *CETP* are associated with increased HDL-c and lower risk for coronary artery disease.
- The recent phase III REVEAL trial demonstrated a reduction in coronary events with anacetrapib, a CETP inhibitor.
- A common *ADCY9* variant determines dalcetrapib efficacy and is currently being used as an entry criterion in the dal-GenE phase III trial.

Novel blood-lipid drugs

- Many recent blood-lipid drugs are antisense or monoclonal antibodies for genes implicated in lipid regulation such as *APOC3* and *ANGPTL3*.
- Lipoprotein(a) is another important risk factor supported by recent genetics evidence. Antisense oligonucleotides are being developed to reduce Lp(a) levels further than current drugs such as PCSK9 inhibitors and niacin.

The polygenic shift in precision medicine

- Identifying variants that modulate drug response is important for the development of predictive biomarkers of drug efficacy.
- New approaches focus on the use of prognostic biomarkers of disease risk such as polygenic risk scores to identify patients with high potential benefit from pharmacological treatment.

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A patent pertaining to pharmacogenomics-guided CETP inhibition was submitted and Drs. Tardif and Dubé are mentioned as authors. Drs. Tardif and Dubé have a minor equity interest in DalCor. Dr. Tardif has received research support from Amarin, AstraZeneca, DalCor, Eli-Lilly, Ionis, Merck, Pfizer, Sanofi and Servier, and honoraria from DalCor, Pfizer, Sanofi and Servier. Dr Dubé has received honoraria from Dalcor.

Reference annotations

** [35]: A 2x2 mendelian randomization study of genes encoding drug targets for statins and ezetimibe

** [78]: First successful phase III trial of a CETP inhibitor

** [50]: First successful phase III trial of a PCSK9 inhibitor

** [116]: The authors used a genetic risk score for CAD to identify patients that derive a greater benefit from statins. This paper is among the first to suggest using a polygenic approach to guide pharmacotherapy.

** [5]: Well powered study summarizing most of the current knowledge about how exonic variants influence blood lipid regulation at the population level.

** [82]: Discovery of a genetic variant with a large effect on dalcetrapib response which motivated the dal-GenE trial.

** [65]: Study of gene activity scores to predict the effects of statins and PCSK9 inhibitors.

* [89]: Paper who fine-mapped a genetic association effectively identifying an important null mutation in APOC3 and confirmed its effect on triglyceride levels and atherosclerosis.

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