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Genetics of non-conventional lipoprotein fractions

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Abstract

Lipoprotein subclass measures associate with cardiometabolic disease risk. Currently the information that lipoproteins convey on disease risk over that of traditional demographic and lipid measures is minimal, and so their use in clinics is limited. However, lipoprotein subclass perturbations represent some of the earliest manifestations of metabolic dysfunction, and their etiology is partially distinct from lipids, so information on the genetic etiology of lipoproteins offers promise for improved risk prediction, and unique mechanistic insights into IR and atherosclerosis. Here, I review the genetic variants validated as associating with lipoprotein measures to date, and show that the majority of identified variants have functionality that is best understood as related to lipid measures. Until we focus on the genes as they relate to lipoprotein subclass production, we are limiting our understanding of biological mechanisms underlying cardiometabolic disease.

Keywords

lipoprotein subfractions; genetics; small LDL; risk factors; HDL; review

Introduction

The recognition that the lipoprotein classes of high density (HDL), intermediate density (IDL), low density (LDL) and very-low density (VLDL) lipoproteins can be broken down into subclasses based on size, density and lipid and apolipoprotein content (Table 1) brought with it new markers of metabolic dysfunction. Whether these subclasses are useful predictors of disease over and above traditional demographic (age, gender), anthropometric (body mass index) and lipid (LDL- and HDL- cholesterol [LDL-C and HDL-C] and triglycerides [TGs]) remains controversial (1). Therefore, their clinical use has been limited to only specialist lipid clinics. Nonetheless, specific distributions of lipoprotein subclasses show clear associations with conditions of metabolic dysfunction such as insulin resistance (IR) and atherosclerosis (2–7), and are thus markers of cardiometabolic disease. Indeed,

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Conflict of Interest

Dr. Frazier-Wood has no conflict of interest.

Human and Animal Rights and Informed Consent

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hepatic IR in its earliest state is characterized by small LDL and large VLDL and HDL particles (8), and the atherosclerotic properties of small LDL are considered greater than those of the larger, more buoyant and “less sticky” LDL (9). Therefore, as the biology of lipoprotein subclass production, maintenance and catabolism is partially different to that of lipids (10)*, understanding their etiology offers the promise of unique insights into the mechanisms underlying the metabolic dysfunction in an IR/atherosclerotic state. In this paper I review the validated associations with lipoprotein measures, and discuss what they can – and cannot – tell us about pathways to disease via lipoprotein metabolism. However, as lipoprotein have been the focus of less genetic research than their lipid counterparts, after reviewing most compelling evidence for genetic associations, I will highlight newer questions and directions research needed, and how this will ultimately help us understand, and so prevent or treat IR and atherosclerotic states.

Selecting genes for inclusion in this review

While the genetics of lipoprotein subclasses is a newer area of research than for many other biomarkers of disease/disease risk, there are still valuable lessons to be learned from the history of genetics. It is often stated that the quest to identify the genetic variants that account for the estimated heritability of complex traits such as lipoprotein subclasses has not met expectations (11–13)**. The availability of sequencing analysis is a relatively recent addition to the geneticist’s toolbox, and typically genetic association studies designed to identify specific variants associated with disease have been conducted by one of two methodologies: candidate gene or a genome-wide association studies.

In the candidate gene approach a select number of variants within a pathway implicated in the trait of interest are selected a priori and analyzed for association with said trait. While these studies were often successful in identify variant-phenotype associations, the lack of replication in subsequent studies lead to the conclusions that candidate approaches on their own were not a suitable method for identifying genetic variants associated with complex traits. In addition, it became clear that the pathways between candidate genes and disease-related traits were more numerous, and more complex than previously thought. Therefore generating a priori lists of hypothesized putative gene regions was challenging.

Genome Wide Association Studies (GWAS) in conjunction with the International HapMap Project seemed like an ideal solution. With the potential to interrogate the majority of the genome without the need for a priori list of suspected variants, it was thought that GWAS could solve the methodological issues holding back genetic discovery with a candidate gene approach. However, still, for some of the most commonly studied traits, such as body mass index (BMI) more than 96% of the genetic variance remains unaccounted for (14). For identified variants, like with candidate gene studies, statistical difficulties with correcting for the sheer number of tests in a single GWAS have led to the majority of initial GWAS findings failed to replicate. Thus, many have called for a two-stage approach to gene-hunting: a discovery phase in which a genome-wide scan is conducted, and then a replication phase in which all associations from the first phase, reaching a given threshold (often $P < 1.0 \times 10^{-5}$; but can be between $P < 10^{-4}$ – 10^{-6}) are examined for associations in one or more independent samples to help identify true associations which may lie just under the

significance threshold when corrected for multiple testing, but also prevent the “winner’s curse” of initial associations that never replicate (15). In light of this history, this review will focus only on what are currently considered the most methodologically strong findings for variant-phenotype associations with lipoprotein subclasses. That is, findings from GWAS, where these findings have been validated through replication in at least one independent population.

Genetics of non-conventional lipoprotein fractions

Only two GWAS, with replication, have been conducted to date. The largest, by Chasman and colleagues was conducted in only women of European origin, but included replication across two further samples (16)^{***}. A later GWAS which included independent replication had a focus on ethnicity-specific differences in the genetics of lipoprotein subclasses, and was conducted on a smaller sample size (17). This latter GWAS replicated several of the findings in the larger GWAS; those findings that were not replicated may have been due to reduced power. Across these studies, several loci have been identified as associating with lipoprotein subclasses, however approximately three-quarters of the identified loci have also been associated with lipid metabolism (principally the cholesterol transport pathway). Only 10 loci appear to be lipoprotein subclass specific, and these give little additional mechanistic insight into lipoprotein metabolism, or the role this may play in metabolic dysfunction.

Genetic loci shared between lipids and lipoproteins

Several loci involved in the cholesterol transport pathway are associated with lipoprotein subclass concentrations or distributions. ATP-binding cassette, sub-family G, members 5 and 8 (*ABCG5/8*) regulates the absorption of cholesterol from food (18), mediates cholesterol secretion from macrophages (19), as well as sterol catabolism and elimination (20). 3-hydroxy-3-methylglutaryl-coA reductase (*HMGCR*; also commonly called *HMG-CoA*) is a rate-limiting enzyme in cholesterol synthesis and inhibited by statin use (21, 22). Proprotein convertase subtilisin/kexin type 9 (*PCSK9*) is expressed in liver, intestine and kidney tissues and encodes for the PCSK9 protein which degrades LDL liver receptors and so influences levels of LDL-C. The *PCSK9* gene plays a known role in cholesterol and fatty acid metabolism; nonsense mutations are associated with a reduction in LDL-C of approximately a third, and a significant reduction in the risk of coronary artery disease (23, 24), a finding supported by animal studies (25). Mutations in this gene have been repeatedly associated with autosomal dominant familial hypercholesterolemia (26).

Other loci identified as associating with lipoprotein subclasses, with clear mechanistic links to lipid metabolism include angiopoietin-like 3 (*ANGPTL3*), expressed in the liver which encodes a protein processed into an N-terminal coiled-coil domain-containing chain, important for lipid metabolism. *ANGPTL3* also reduces the activity of lipoprotein lipase (LPL) (27, 28), and endothelial lipase, increasing TGs and HDL-C in rats. The *ANGPTL3* protein is increased among diabetics (29), and exonic variants are associated with familial hypolipidemia (low LDL-C, HDL-C and TGs) in humans (30, 31). LPL exerts several effects on lipid and lipoprotein metabolism, most notably it hydrolyzes chylomicrons and VLDLs, with LDL being the final products in this step. LPL further facilitates the transport of lipids between lipoproteins, for example, transforming smaller HDL into larger, less

denser medium HDL subspecies (32), and replacing the cholesterol ester in HDL with TGs (33). Apolipoprotein A-5 is involved in the synthesis of VLDL from TGs (34, 35), the fatty acid desaturase gene cluster (*FADS1-3*) influences fatty acid levels by altering the sequence and enzymatic function of proteins arising from the *FADS* cluster. Hepatic lipase (LIPC) hydrolyzes TGs and phospholipids in chylomicrons and HDL and serves as a ligand that facilitates lipoprotein uptake, although some patients with LIPC deficiencies do not show perturbations in lipoproteins, suggesting that there may be secondary or compensatory mechanisms available (36). Cholesteryl ester transfer protein (CETP) facilitates the transfer of cholesteryl esters from HDLs to VLDLs, VLDL remnants, IDLs, and LDLs (37), and phospholipid transfer protein (PLTP) acts in concert with CETP to convert smaller HDLs to larger HDL through the removal of phospholipids (38). Endothelial lipase (LIPG) catabolizes larger HDL into medium HDL. The LDL-receptor (LDLR) is responsible for the uptake and catabolism of LDL by recognizing apolipoprotein B-100 on the LDL surface (apoE in chylomicrons, IDL and VLDL is also recognized).

A group of loci shared between the lipid and lipoprotein phenotypes may exert their association with lipoprotein subclasses by associating with individual differences in the components of lipoproteins; apolipoprotein-AII (ApoA-2) is the most prevalent protein in HDL particles; and apolipoprotein-B is a major constituent of LDL particles. Apolipoprotein A-1 accounts for almost 70% of the HDL mass and transports cholesterol from tissues to the liver, forming protection from atherosclerotic processes (39).

Other loci have been associated with lipid metabolism (broadly defined) but their role in lipoproteins is not clear. Kruppel-like factor 14 (*KLF14*), is a maternally expressed variant and nearby variants are strongly associated with type II diabetes (40) and HDL-C (41), and SNPs which regulate *KLF14* activity are additionally associated with body mass index, TGs, fasting insulin, glucose and adiponectin levels, and insulin sensitivity (42). Sortilin 1 (*SORT1*) is expressed in the liver and modulates hepatic VLDL secretion in mice, altering VLDL particle levels, although this has not been shown in human studies to date (43, 44). Glucokinase (hexokinase 4) regulator (*GCKR*) inhibits glucokinase in the liver, and is linked to lipid levels (45, 46), type II diabetes risk (47) and fatty liver in obesity (48). MLX-interacting protein-like (*MLXIPL*) encodes a protein which activates genes involved in triglyceride synthesis, thus is associated with plasma triglycerides (49, 50) and possibly due to the association with carbohydrate in this pathway, is also implicated in type II diabetes risk (51, 52), and Tribbles Pseudokinase 1 (*TRIB1*) is expressed in the liver and associated with lipid levels and cardiovascular diseases in humans (45, 53); in mice it is shown to reduce VLDL particle production, and so reduce plasma triglyceride levels (54). HNF1 homeobox (*HNF1A*) is responsible for a familial (albeit rare) form of diabetes, and regulates a number of genes expressed in the liver which synthesize apolipoprotein B (among other molecules) (55), the expression of *HNF1A* is partially controlled, in turn, by variants in the hepatocyte nuclear factor 4, alpha (*HNF4A*) gene. Preliminary evidence suggests that growth factor receptor-bound protein 14 (*GRB14*) may inhibit receptors in the insulin receptor class (56, 57). Thus the majority of loci associated with lipoproteins in GWAS have been either mechanistically, or statistically associated with lipids levels and cardiovascular diseases.

Their role in lipoprotein subclass differences is less well studied and so understood, but could give unique clues into the origins of disordered metabolism.

Loci specific to lipoproteins

Approximately one-third of loci associated with lipoprotein measures have not been previously associated with lipid levels of metabolism. In general, the role of variation in these genes with lipoprotein subclass differences, or with metabolic dysfunction is less clear. A locus across the propionyl o-enzyme A carboxylase, beta polypeptide (*PCCB*)/stromal antigen 1 (*STAG1*) gene, was associated with the concentration of small HDL particles. Defects in *PCCB* are associated with propionic acidemia which arises directly from body being unable to process proteins and lipids fully, affecting the liver, brain and heart. Variants proximal to the coiled-coil domain containing 6 (*CCDC62*), dynein, axonemal, heavy chain 10 (*DNAH10*) and zinc finger protein 664 (*ZNF664*), were associated with concentrations of large HDL and small LDL, HDL and LDL diameter, and total LDL particles. *DNAH10* is associated with familial elevated HDL (58) and may contribute to a genetic risk score for coronary artery disease (59). Other loci such as butyrophilin-like 2 (*BTNL2*), jumonji domain containing 1C (*JMJD1c*), and SET binding factor 2 (*SBF2*) have known roles in DNA repair (60) or conditions such as Charcot-Marie-Tooth neuropathy (61, 62), but their role in lipoprotein differences is not known and warrants both further validation and mechanistic investigation.

Synthesis

It's clear that pathways such as the cholesterol transport pathway, and organs such as the liver, are linked to both lipid and lipoprotein levels. It's not surprising that a number of genes associated with lipoproteins are also shared with lipids; an unanswered question is whether these are truly pleiotropic genetic effects (i.e. they associate lipids and lipoproteins independently) or whether the shared genetic associations can be attributed to the strong phenotypic associations between the phenotypes. It is clear that some loci, such as *CETP* and *SORT1*, have effects which would plausibly be seen to exert effects directly both on lipid levels and lipoprotein subclasses. However, for the majority of loci, their role in lipoproteins is poorly understood, if at all. Focusing on these mechanistic links is important however, and should be the subject of future research.

Future directions

Aside of more mechanistic studies, a better knowledge of the genetics of lipoprotein subfractions can help further our understanding of IR and atherosclerosis. While larger GWAS studies could help account for more of the heritability underlying lipoproteins, designs such as Mendelian randomization could answer questions about whether lipoprotein subclasses, and indeed, which lipoprotein subclass measures convey disease risk over and above lipids. Another current topic is whether the etiology of cardiovascular disease and its risk factors differs by ethnicity, and so can account for the increased prevalence of cardiovascular diseases among Hispanic- and African- ancestry Americans, compared to those of European ancestry. Lipoprotein diameters differ by ethnicity, and some evidence suggests that there are also different variants underlying lipoprotein diameters (17).

However, not enough evidence has accrued to be sure that these differential associations are not due to different SNPs within the same gene being strongly associated across ethnic groups likely due to known different linkage disequilibrium patterns) or, when the same SNP shows the strongest association, that the frequencies of the SNP minor allele may be different across groups affecting power.

Conclusions

Given that lipoprotein subclasses show strong, consistent and longitudinal associations with cardiometabolic diseases such as type II diabetes and cardiovascular diseases, and are sensitive and early indicators of their risk factors such as IR it stands to reason that they may give unique and valuable insights into the pathways of metabolic disturbance underlying these conditions. However, compared to lipids their etiology is less studied and poorly understood. Indeed, most of our mechanistic understanding of the genetic origins of lipoprotein subclasses is framed within our existing knowledge of the genetic origins of lipids. Moving away from this, and focusing on lipoproteins alone, will greatly enhance our ability to predict, prevent and treat cardiometabolic risk in its earliest stages.

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Table 1

Characteristics of lipoprotein subfractions

NMR Lipoprotein Parameter	Diameter Range (nm)	Density	Triglyceride content
VLDL			
Large VLDL	>60	highest	highest
Medium VLDL	35–60	↓	↓
Small VLDL	27–35		
LDL			
Large LDL	21.2–23	↓	↓
Small LDL	18–21.2		
HDL			
Large HDL	8.8–13	↓	↓
Medium HDL	8.2–8.8		
Small HDL	7.3–8.2		
Chylomicrons	80–500	lowest	lowest