Curr Genet Med Rep (2016) 4:130–141
DOI 10.1007/s40142-016-0097-y

Genetics of Lipid and Lipoprotein Disorders and Traits

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Published online: 7 June 2016

Abstract
Purpose of Review Plasma lipids, namely cholesterol and triglyceride, and lipoproteins, such as low-density lipoprotein (LDL) and high-density lipoprotein, serve numerous physiological roles. Perturbed levels of these traits underlie monogenic dyslipidemias, a diverse group of multisystem disorders. We are on the verge of having a relatively complete picture of the human dyslipidemias and their components.
Recent Findings Recent advances in genetics of plasma lipids and lipoproteins include the following: (1) expanding the range of genes causing monogenic dyslipidemias, particularly elevated LDL cholesterol; (2) appreciating the role of polygenic effects in such traits as familial hypercholesterolemia and combined hyperlipidemia; (3) accumulating a list of common variants that determine plasma lipids and lipoproteins; (4) applying exome sequencing to identify collections of rare variants determining plasma lipids and lipoproteins that via Mendelian randomization have also implicated gene products such as *NPC1L1*, *APOC3*, *LDLR*, *APOA5*, and *ANGPTL4* as causal for atherosclerotic cardiovascular disease; and (5) using naturally occurring genetic variation to identify new drug targets, including inhibitors of apolipoprotein (apo) C-III, apo(a), ANGPTL3, and ANGPTL4.
Summary Here, we compile this disparate range of data linking human genetic variation to plasma lipids and lipoproteins, providing a "one stop shop" for the interested reader.

Keywords Dyslipidemia · DNA variants · Monogenic · Polygenic · Atherosclerosis

Introduction

Plasma lipids, namely cholesterol and triglyceride (TG), are carried within complex lipoprotein particles, such as low-density lipoprotein (LDL) and high-density lipoprotein (HDL) [1]. Lipids and lipoproteins serve numerous physiological roles. Although the terms "lipids" and "lipoproteins" are often used interchangeably, especially by clinicians for convenience, they are different biochemical entities [1]. Plasma lipid levels represent the integrated lipid component of various lipoprotein species. For instance, plasma TG is the sum of TG carried within chylomicrons and very-low-density lipoprotein (VLDL) particles and their metabolic remnants, while plasma total cholesterol is the sum of cholesterol carried within these particles and also within LDL and HDL. In contrast, lipoproteins are discrete molecular entities that are likewise subject to manifold genetic and environmental influences and show complex metabolic interrelationships with one another [1]. Thus, observations that genetic determinants of lipids and lipoproteins often overlap between phenotypes were predictable a priori based on understanding the metabolism of these complex traits. Here, we attempt to respect the biochemical distinction between "lipid" and "lipoprotein."

Recent technological advances have helped identify numerous genetic variants, ranging from ultra-rare to

This article is part of the Topical collection on *Cardiovascular Genetics*.

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common, which have significant effects, ranging from large to small, on inter-individual differences in plasma lipid and lipoprotein levels. Over the past 3 years, the range of causative genes and mutations underlying rare familial dyslipidemia syndromes has expanded, while new insights have emerged from genotyping and next-generation sequencing (NGS) studies in unrelated individuals. Here we summarize recent findings of (1) rare genetic variants underlying monogenic dyslipidemias in clinically ascertained patients; (2) common variants contributing to a polygenic component of clinical dyslipidemias; (3) common and rare variants contributing to variations of plasma lipids and lipoproteins in epidemiologic samples; (4) common and rare variants from (3) that have been implicated as causative for atherosclerosis; and (5) targets for drug development to treat dyslipidemia and possibly to prevent atherosclerosis. We also summarize various lines of evidence supporting the biological and clinical significance of genetic variants and loci underlying perturbed lipid and lipoprotein metabolism.

Expanding the Genetic Etiologies in Monogenic Dyslipidemias: Focus on Familial Hypercholesterolemia

Monogenic dyslipidemias are classified according to the primary lipid or lipoprotein disturbance: elevated or depressed concentrations of LDL cholesterol (LDL-C) or HDL cholesterol (HDL-C), or elevated TG [1]. Currently, 27 monogenic dyslipidemias are defined by extreme deviations of plasma lipid or lipoprotein values typically with discrete clinical signs and symptoms caused by numerous rare mutations affecting a total of 25 genes (Table 1) [2]. Over the past 3 years, no new monogenic dyslipidemias have been added to this list, although some new genes for known dyslipidemias have been identified. For instance, recent high throughput NGS of 213 selected family members from 41 kindreds with suspected Mendelian inheritance of extreme levels of LDL-C and no previously detected mutation, only revealed mutations in known causative genes [3]. However, other NGS efforts have identified a few rare large-effect variants in new genes underlying some of these disorders, particularly familial hypercholesterolemia (FH) [4].

Previous estimates that heterozygous FH (HeFH) occurs in 1 in 500 individuals underestimated the actual prevalence of 1 in ~217 individuals as determined from carrier status of pathogenic variants in the LDL receptor gene (*LDLR*) [5•]. However, FH mutation carriers show a relatively wide range of LDL-C levels; while a cut-point of 4.4 mmol/L (169.9 mg/dL) was proposed to discriminate between carriers and non-carriers, there was considerable overlap of carrier status at lower levels of LDL-C [5•]. A specific association with mutation status was superior at higher levels, i.e., LDL-C >7 mmol/L (270.3 mg/dL) [5•]. Others have suggested a diagnostic cut-point for HeFH of total cholesterol >8.6 mmol/L (332.1 mg/dL) [6]. Higher numbers of mutation carriers are captured at a lower LDL-C cut-point—i.e., higher sensitivity—at the expense of lower specificity of association with true causative mutations. The goals of implementing particular cut-points in clinical practice need to be carefully considered. The revised population prevalence may affect prevention strategies for atherosclerotic cardiovascular disease (CVD) risk in HeFH patients and their families [7].

Currently, nine genes underlie FH or FH-like phenotypes (Table 1). In addition to canonical causative genes, namely *LDLR*, *APOB*, and *PSCK9* for co-dominant forms of FH, and *LDLRAP1* (alias *ARH*) for purely recessive FH [8], NGS revealed that the *APOE* p.Leu167del variant causes a dominant presentation of FH [9, 10], while recessive mutations in *ABCG5* (and likely *ABCG8*) [11] and *LIPA* can cause an FH-like phenotype [12]. Exome sequencing in uncharacterized FH families also showed that ultra-rare mutations in *STAP1* encoding signal transducing adaptor family member 1, likely causes autosomal dominant FH [13]. The heterozygous *APOE* p.Leu167del mutation was previously associated with combined hyperlipidemia, splenomegaly and sea-blue histiocytosis [14]; its association now with simple hypercholesterolemia suggests that secondary genetic or environmental factors modulate phenotypic expression. Similarly, *ABCG5* and *LIPA* cause the discrete syndromes sitosterolemia (or phytosterolemia) and lysosomal acid lipase deficiency (also known as Wolman disease in its more severe form), respectively [15]. So among genes that have been implicated in FH, the primarily causative genes, in order of importance and prevalence, remain *LDLR*, *APOB*, and *PCSK9*, while causative mutations in the rest are quite rare [2].

Exome sequencing has also identified potential new FH loci. For instance, whole exome sequencing (WES) of 554 individuals with extreme LDL-C levels found a high burden of rare or low-frequency variants in *PNPLA5* encoding a phospholipase-domain-containing protein implicated in fatty liver disease [16]. In particular, 3.1 % of individuals with LDL-C >98th percentile carried a rare missense variant in *PNPLA5* compared to 1.2 % of non-extreme individuals and only 0.5 % of individuals with LDL-C <2nd percentile; each *PNPLA5* variant allele increased LDL-C by ~1 mmol/L (38.6 mg/dL). The association was replicated in an independent sample, although neither vertical transmission in families nor mechanistic impairment was demonstrated [16]. Exome sequencing in other unrelated FH subjects identified other possible new genes, namely *CH25H* and *INSIG2*, which have yet to be validated [17].

Table 1 Monogenic dyslipidemias and dyslipoproteinemias

Phenotype	Disorder	Alternative name	Gene symbol	Chr
High LDL-C	Familial hypercholesterolemia	Hyperlipoproteinemia type 2A	*LDLR*	19p13.3
	Familial defective apolipoprotein B	Autosomal dominant hypercholesterolemia type 2 (binding-defective apo B)	*APOB*	2p24-p23
	Autosomal dominant hypercholesterolemia	Autosomal dominant hypercholesterolemia type 3 (*PCSK9* gain-of-function)	*PCSK9*	1p32.3
	Autosomal dominant hypercholesterolemia	Autosomal dominant hypercholesterolemia type 4	*STAP1*	4q13.2
	Autosomal dominant hypercholesterolemia	Autosomal dominant hypercholesterolemia type 5	*APOE*	19q13
	Autosomal recessive hypercholesterolemia		*LDLRAP1 (ARH)*	1p36-p35
	Cholesterol ester storage disease	Includes Wolman disease	*LIPA*	10q21.31
	Sitosterolemia	Phytosterolemia	*ABCG5/ ABCG8*	2p21
Low LDL-C	Abetalipoproteinemia	Bassen-Kornzweig syndrome	*MTTP*	4q24
	Hypobetalipoproteinemia		*APOB*	2p24-p23
	PCSK9 deficiency with low LDL-C	Hypobetalipoproteinemia (*PCSK9* loss-of-function)	*PCSK9*	1p32.3
	Familial combined hypolipidemia	ANGPTL3 deficiency	*ANGPTL3*	1p31.1-p22.3
	Chylomicron retention disease	Anderson disease	*SAR1B*	5p31.1
High HDL-C	Cholesteryl ester transfer protein deficiency	Hyperalphalipoproteinemia	*CETP*	16q21
	Hepatic lipase deficiency		*LIPC*	15q21-q23
	Scavenger receptor B1 deficiency		*SCARB1*	12q23.31
	Endothelial lipase deficiency		*LIPG*	18q21.1
Low HDL-C	Tangier disease		*ABCA1*	9q31
	Apolipoprotein A-I deficiency		*APOA1*	11q23
	Familial LCAT deficiency (complete or partial)	Includes Fish-eye disease	*LCAT*	16q22
High TG	Lipoprotein lipase deficiency	Familial chylomicronemia	*LPL*	8p22
	Apolipoprotein C-II deficiency	Familial chylomicronemia	*APOC2*	19q13
	Apolipoprotein A-V deficiency	Severe hypertriglyceridemia	*APOA5*	11q23
	Lipase maturation factor deficiency	Severe hypertriglyceridemia	*LMF1*	16p13.3
	Glycosylphosphatidylinositol anchored HDL binding protein 1	Severe hypertriglyceridemia	*GPIHBP1*	8q23
	Glycerol-3-phosphate dehydrogenase-1	Infantile hypertriglyceridemia	*GPD1*	12q13.12
	Dysbetalipoproteinemia	Hyperlipoproteinemia type 3	*APOE*	19q13

Chr chromosome, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *TG* triglyceride

The role of polygenic determinants in FH was recently clarified [18]. Up to 40 % of HeFH individuals diagnosed clinically have no monogenic mutation identified through sequencing, but instead have accumulated a burden of small-effect, LDL-C-raising single-nucleotide polymorphism (SNP) alleles that cumulatively raise LDL-C levels into the HeFH range [19]. Polygenic effects explain the high LDL-C levels in many but not all patients with clinically diagnosed FH who lack a monogenic mutation [18]. Thus, polygenic determinants should be included when screening for molecular causes of FH [2]. The contribution of polygenic factors to familial combined hyperlipidemia is much greater than in FH [20].

Common and Low-Frequency Variants Associated with Lipid and Lipoprotein Traits

The Global Lipids Genetics Consortium (GLGC) genome-wide association studies (GWASs) identified common genetic variants governing plasma lipids and lipoproteins in essentially normolipidemic populations [21, 22]. The

157 loci identified by GLGC explain 10–20 % of the total variation in total, LDL-C, HDL-C, and TG, and a higher proportion of variation attributable to genetic factors [22]. Evaluating the polygenic determinants of plasma lipids typically starts with the lead SNP genotypes identified in these landmark publications [23].

Many of the genetic loci identified by GWAS—perhaps one-third—harbor genes whose products were already implicated in plasma lipoprotein metabolism [23]. Other associated loci have generated hypotheses to evaluate new pathways and mechanisms, as exemplified by molecular and biochemical studies of such GWAS specified genes as *SORT1* [24], *TRIB1* [25], and *GCKR* [26]. Another new mechanistic lead was the association of lower total cholesterol and LDL-C levels with a SNP at the haptoglobin locus (*HP*) [21], which marks haplotypes with exonic deletions that likely affect expression of haptoglobin and possibly interaction with apolipoprotein (apo) E-containing lipoproteins [27]. Each new locus identified from GLGC could lead to comparable lines of investigation.

The microarrays used in the first GLGC studies surveyed primarily common variants, defined as those with minor allele frequencies >5 % in the general population. A few low-frequency variants, defined as those with minor allele frequencies between 0.5 and 5 %, were represented on earlier microarrays. However, newer platforms, such as the "exome array," allowed for a more systematic evaluation of low-frequency variants, some of which had larger phenotypic effects [28]. For instance, using the exome array to screen >200,000 low-frequency and rare coding sequence variants across the genome in 56,538 individuals of varied ancestries identified four low-frequency (frequencies between 0.1 and 2 %) variants, namely *ANGPTL8* rs145464906, *PAFAH1B2* rs186808413, *COL18A1* rs114139997, and *PCSK7* rs142953140, with relatively large effects on HDL-C and TG, although none of these was associated with atherosclerotic CVD [28].

Another recent insight is that frequencies of genetic variants differ across human populations: a variant considered as low frequency or ultra-rare in certain populations can be common in others. For instance, the *LDLR* p.G116S missense variant is absent from virtually all populations except Inuit from the circumpolar north [29]. This variant is absent from microarrays and was identified by candidate gene sequencing of Greenland Inuit with high LDL-C levels. Genotyping p.G116S in 3324 Inuit from Alaska, Canada, and Greenland showed an allele frequency of ~10 % [29]. Each allele raised LDL-C by 0.54 mmol/L (20.9 mg/dL) and carriers had a 3.0-fold increased risk of hypercholesterolemia [29]. In vitro, p.G116S showed 60 % reduced ligand-binding compared with wild-type receptor, indicating causality for elevated LDL-C [29]. These findings suggest that such terminology as "common," "low frequency," and "rare" is context-dependent, often reflecting ascertainment bias and the sampling strategy that led to the initial detection of the variant.

Rare Variants Associated with Lipid and Lipoprotein Traits

In contrast to common and low-frequency variants, rare variants are defined as having a population prevalence of <1 % and sometimes much lower [30, 31]. Their low frequency might result from a higher probability of biochemical dysfunction, reflecting effects of recent population explosion or of negative (purifying) selection [32]. However, in the lipid field, WES has revealed numerous examples of rare—even personal—variants with no obvious functional consequences upon health or survival [33]. Rare variants present logistic barriers to investigation of genetic determinants of plasma lipids and lipoproteins. The first issue is technical: rare variants need to be detected through high throughput sequencing technology, since they are generally not represented on microarrays. The second issue is statistical: any individual rare variant may not be statistically associated with the trait of interest, even in large samples. Solutions include (1) increasing the sample size to tens or hundreds of thousands of subjects, with its attendant burden on resources; and (2) bundling together likely causative rare variants within a particular gene or rationally grouped genes according to mechanisms or pathways, and testing the association of the bundle of variants with the trait of interest.

An early rare variant association study demonstrated an 8-fold increased frequency of a bundle of heterozygous rare nonsynonymous sequence variants in *ABCA1*, *LCAT*, and *APOA1* genes in individuals with low HDL-C compared to those with high HDL-C levels [34]. Another study showed a 2-fold increased frequency of a bundle of rare variants in *LPL*, *APOA5*, *GCKR*, and *APOB* genes in individuals with severe hypertriglyceridemia compared to normal controls [35]. A wrinkle complicating this approach was seen when *PCSK9* was sequenced in patients with extremes of LDL-C [36]. While several variants were found at each extreme, it soon became clear that uncommon and rare loss-of-function variants (premature truncations) were cumulatively more prevalent in individuals with low LDL-C, while rare missense mutations shown to result in a gain-of-function were enriched in individuals with high LDL-C. Testing the association of bundled uncommon and rare loss-of-function variants in *PCSK9* with atherosclerosis end-points is a now classic example of the Mendelian randomization (MR) approach [37], which

identified PSCK9 as a drug target to reduce LDL-C and CVD risk.

Association of Lipid and Lipoprotein Variants with Atherosclerotic Cardiovascular Disease

Many genetic determinants of plasma lipoproteins are also significant determinants of atherosclerotic CVD. A recent synthesis of coronary heart disease (CHD) GWAS results indicated 58 significantly associated loci [38], of which about one-quarter overlapped with GWAS loci for lipids, outnumbering the contributions of loci associated with blood pressure or diabetes [30]. Because lipid-associated GWAS loci were determinants of either LDL-C, TG, or lipoprotein(a) (Lp[a]), the complexity of the biology seemed reducible to the common presence of apo B in these particles; genetic determinants of apo B-containing lipoproteins seemed to be the unifying element underlying these observations [30]. However, discordance between levels of apo B and LDL-C or TG is well-known [39]; furthermore, apo B and Lp(a) levels are uncorrelated. Until a GWAS of apo B (or non-HDL-C) concentration is performed, invoking apo B as the unifying intermediate phenotype for genetic determinants of CHD seems premature.

Genetic Evidence for a Causal role for LDL-C in Coronary Heart Disease Susceptibility

The causal relationship between LDL-C and CHD has been supported by early observations in families and cohorts with FH due to rare variants in *LDLR* [40]. More recently, an evaluation of 164 heterozygous carriers of 16 different rare gain-of-function variants in *PCSK9* [41] showed a high prevalence of early onset CHD, with 33 % of carriers expressing symptoms and hard end-points at mean age of 49 years. But other types of genetic evidence have superseded observational studies in families and cohorts as arbiters of causality.

For instance, MR experiments using common or low-frequency variants of candidate genes have both supported and refuted causative roles for various lipids and lipoproteins in atherosclerosis. Theoretical underpinnings of the MR approach, and its strengths and limitations are discussed elsewhere [42, 43]. In contrast to other criteria for causality, such as Koch's postulates [44] or Bradford Hill's criteria [45], Mendel likely never envisioned that laws of inheritance would provide a litmus test for a causal relationship between a genetically determined intermediate trait and a temporally remote disease outcome. Nonetheless, MR now routinely arbitrates causal relationships between markers and disease. Numerous examples of MR experiments examine either common or rare variants, individually or bundled together, affecting lipid and lipoprotein metabolic pathways. In the case of rare variants, probable loss-of-function or "inactivating" variants (i.e., truncation, nonsense or splicing) are preferred when testing concurrently for association with lipids and atherosclerosis end-points, since functional compromise is more easily inferred without the need for mechanistic confirmation. The preferred MR study design concentrates on favorable outcomes, such as protection from CHD; positive results are more easily translated since a drug or biological intervention can pharmacologically mimic the genetic deficiency linked to the favorable outcome.

As mentioned, the MR design proved that protection from CHD was associated with uncommon loss-of-function variants in *PCSK9* [37, 46]. This motivated development of PCSK9 inhibitors, which recently became available for prescription to reduce LDL-C, proving that MR can identify causal relationships and drug targets [47]. Interestingly, extending the MR approach in Copenhagen cohorts showed no causal relationship between low LDL-C and either cancer or gallstones [48, 49]. Another MR experiment assembled 13 common genetic determinants of LDL-C into a polygenic score and found that an $\sim$0.8 mmol/L (30.9 mg/dL) genetically determined increase in LDL-C was associated with a 2.1-fold increased risk of CHD [50]. MR evaluation of 10,464 CHD events occurring in 108,376 individuals from 14 studies showed that common polymorphisms in *NPC1L1* or *HMGCR* both reduced LDL-C and CHD risk [51•]. Furthermore, WES showed that heterozygotes for very rare *NPC1L1* inactivating mutations had LDL-C that was 0.31 mmol/L (12.0 mg/dL) lower than non-carriers and a 53 % reduced CHD risk [52]. These observations supported the contemporaneous publication of randomized clinical trial results showing that ezetimibe, which targets the NPC1L1 transporter, reduced both LDL-C and CHD risk [53]. A study of common *NPC1L1* variants genotyped in 67,385 individuals not only replicated reductions in LDL-C and CHD risk, but also documented increased risk of gallstone disease [54].

A final illustration of how MR can illuminate causal pathways was seen in a meta-analysis of genotypes of *HMGCR*, which encodes the target of statin drugs. Statins slightly increase the risk of type 2 diabetes [55]. To address a possible causal relationship, *HMGCR* genotyping in 223,463 individuals from 43 studies showed associations with reduced LDL-C and increased body weight, waist circumference, plasma insulin and glucose [55]. This supported a causal relationship between altered HMG coenzyme A reductase activity and worsened glycaemia and increased diabetes risk, perhaps mediated by slightly increased body weight.

Genetic Evidence for a Diminished Causal Role for HDL-C in Coronary Heart Disease Susceptibility

MR data for a causal relationship between CHD and HDL-C are much weaker than for LDL-C. The Copenhagen group first showed that lower HDL-C in carriers of rare loss-of-function variants in *ABCA1* were not associated with increased CHD risk [56]. They showed similar neutral results for low-frequency variants in *LIPC* encoding hepatic lipase [57] and *LCAT* encoding lecithin cholesterol acyl transferase [57]. A meta-analysis using several independent approaches showed that a polygenic score that raised HDL-C by one standard deviation had no effect on CHD risk, while a comparable polygenic score for LDL-C was strongly associated [50]. The same study reported borderline association of variants in *CETP* encoding cholesteryl ester transfer protein that raised HDL-C and reduced CHD risk [50]. Furthermore, the Copenhagen group showed that common variants associated with reduced CETP activity were associated with increased HDL-C and reduced LDL-C, together with reduced risk of CHD, other CVD end-points and increased longevity [58], which sustains hope for the strategy of CETP inhibition. However, the termination of three drug development programs for CETP inhibitors supports the idea that simply increasing HDL-C quantity is not associated with CHD risk, although results of the Randomized Evaluation of the Effects of Anacetrapib Through Lipid-Modification (REVEAL) trial with the CETP inhibitor anacetrapib are pending [59]. In observational studies in families with monogenic extreme HDL-C deviations, where association with CVD was also inconsistent [60]. The complexities of HDL-C levels were further highlighted following the discovery of the loss-of-function variant, p.P367L, in *SCARB1* encoding scavenger receptor BI [61]. This variant not only was identified in individuals with significantly elevated levels of HDL-C, but carriers of this poorly functioning variant also demonstrated a significantly increased risk for CHD [61]. Perhaps genetic determinants of HDL function, such as efflux capacity, are more mechanistically related to atherosclerosis susceptibility than determinants of simple HDL-C quantity [62, 63•].

Genetic Evidence for a Causal Role for TG in Coronary Heart Disease Susceptibility

The ying-yang relationship between concentrations of TG and HDL-C and underlying mechanisms have been appreciated for decades [64•]. Before the MR era, elevated TG levels in this joint phenotype were considered to be an innocent bystander, with depressed HDL-C levels thought to underlie disease susceptibility. Concurrent with diminished recent enthusiasm for HDL-C, MR studies suggest that high TG plays a proximal causal role in atherosclerosis, although disentangling the association with low HDL-C is challenging. GLGC version 1.0 indicated that common TG-raising variants were associated with atherosclerosis risk; however almost all of these had joint effects, particularly depressed HDL-C [21]. Furthermore, common *APOA5* variants associated with higher TG and lower HDL-C were also associated with increased CHD risk [65]. Also, the common *LPL* p.S447X gain-of-function variant (also known as p.S474X) has long been associated with reduced TG, increased HDL-C, and reduced CHD risk in small cohorts [66], while the relatively common *LPL* p.D9N loss-of-function variant (also known as p.D36N) has been associated with increased TG, reduced HDL-C, and increased CHD risk [67]. Associations of these two *LPL* variants with the high TG/low HDL-C atherogenic dyslipidemia complex and with CHD risk were recently confirmed in a large case-control sample [68•]. Using statistical models, genetic determinants with predominantly TG-related effects were correlated with increased CHD risk, while genetic determinants with predominantly HDL-C-related effects were not [69]. These associations might be related in part to the cholesterol content of TG-rich lipoprotein remnant particles [70].

Exome sequencing showed that rare heterozygous loss-of-function *APOC3* mutations are primarily associated with reduced plasma TG levels: mutation carriers had significantly reduced CHD risk, again supporting the idea that TG might contribute directly to atherosclerosis [71, 72]. However, these rare variants were almost always associated with reduced LDL-C and increased HDL-C [71]. In addition, carriers of heterozygous rare loss-of-function mutations in *APOA5* that increased plasma TG levels had a 2-fold increased risk of early CHD [73], but these variants were associated with increased LDL-C and decreased HDL-C. Furthermore, inactivating variants in *ANGPTL4* [74] were associated with reduced TG and reduced CHD risk [68•, 75•]. This clarified earlier inconsistent observations of ANGPTL4 E40K variant association with CHD in small cohorts [76, 77].

While recent genetic studies support longstanding prior biological knowledge of the importance of the joint high TG/low HDL-C phenotype as an integrated read-out of a complex network of underlying processes [70, 71], some questions remain. For instance, why are patients with severely elevated TG and depressed HDL-C due to familial chylomicronemia from homozygous *LPL* variants not at increased risk of atherosclerosis [78, 79]? Also, why are common or rare variants in some other LPL-associated genes, namely *APOC2*, *LMF1*, and *GPIHBP1* (Table 1),

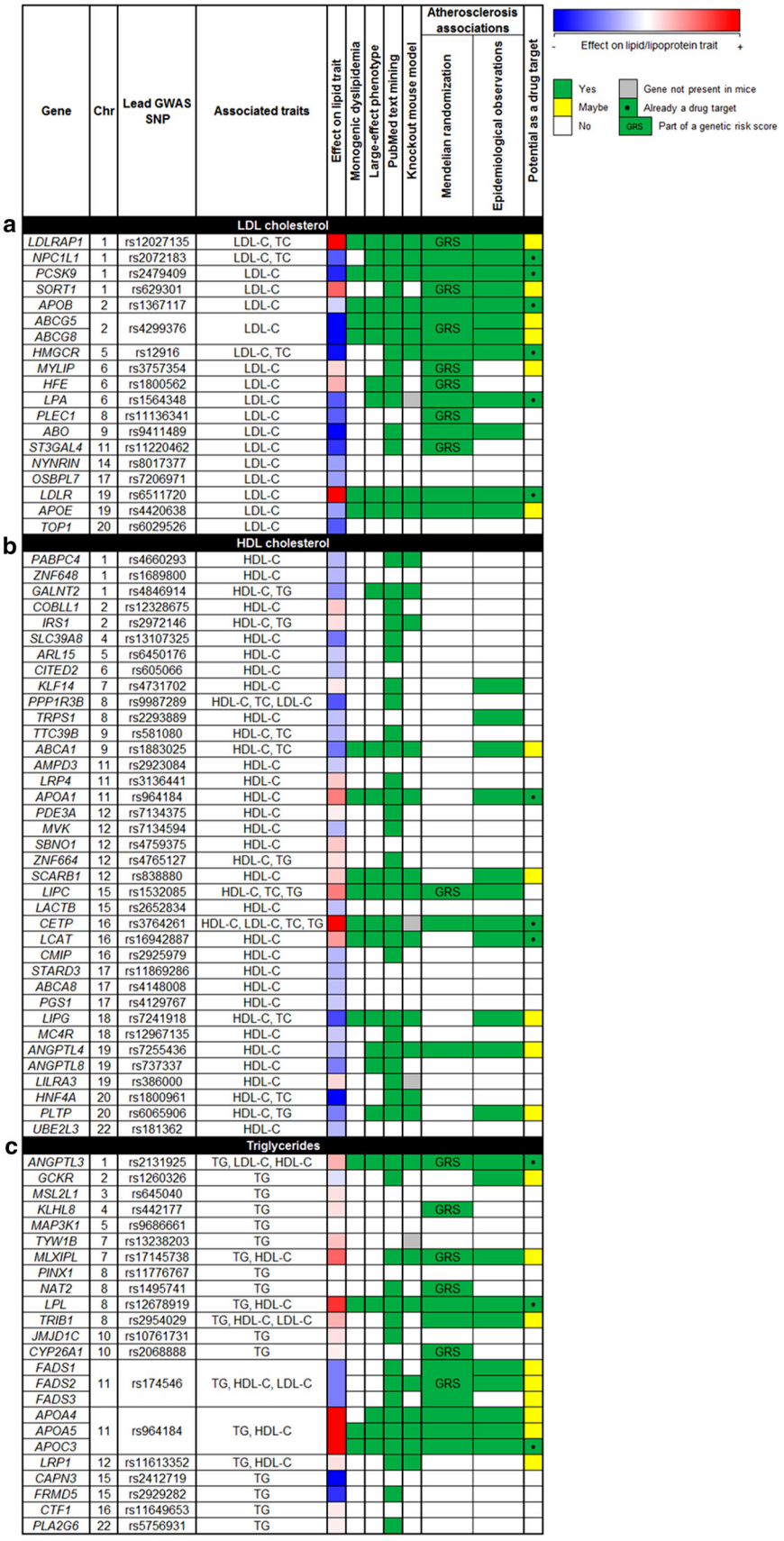

Fig. 1 Summary of current information for genes associated with: *a* LDL cholesterol (LDL-C), *b* HDL cholesterol (HDL-C), *c* Triglyceride (TG)

which all cause severe hypertriglyceridemia and have well established roles in lipolysis [70, 71], not associated with increased CHD risk in GWAS or WES studies?

Renaissance of Lipoprotein(a)

The relationship between Lp(a) and atherosclerosis has been appreciated for decades [80]. Recent meta-analyses in case-control studies using SNPs at the *LPA* locus on chromosome 6q25-26 confirm association with CHD [81]. GWAS also shows that *LPA* genotype is significantly associated with CHD [82•]. The range of associated phenotypes has recently been broadened to include calcific aortic stenosis [83, 84] and heart failure [85]. This convergence of genetic data suggesting that isolated reduction of Lp(a) can protect against CHD and other adverse phenotypes has prompted development of antisense therapy targeting Lp(a), which in a recent phase 1 study showed reductions of up to 78 % with no apparent adverse effects [86]. This agent should help advance evaluation of Lp(a)'s role in pathogenesis and of possible benefits of targeted reduction. It is also interesting that PCSK9 monoclonal antibodies reduce plasma Lp(a) levels by ~30 % [87], which may explain part of their clinical benefits [88].

Translation to Other Therapies

PCSK9 inhibitors, whose development could be traced to human genetic observations, are now in widespread clinical use and appear to have beneficial effects on outcomes [88, 89], as well as potential adverse effects [90] and economic implications [91]. Analogous advances include development of an anti-sense inhibitor of *APOC3* (volanesorsen) as a treatment for familial chylomicronemia due to homozygous mutations in *LPL* and other genetically undefined forms of severe hypertriglyceridemia [64•, 65]. While volanesorsen may prove to be useful clinically for severe hypertriglyceridemia with its attendant risk of life-threatening pancreatitis, suggestions that it might also be used to prevent CHD need to be carefully considered. The specific targeting of *APOC3* has pleiotropic effects on the lipid profile, including reductions of apo C-III-containing subfractions of LDL, HDL, and Lp(a) [92]. Also, *ANGPTL3* and *ANGPTL4* are active drug targets as they modulate LPL activity, and because of the apparently favorable phenotypes observed in carriers of inactivating or loss-of-function variants [68•, 75•, 93].

As mentioned above, some patients with mutations in *LIPA* that otherwise cause cholesterol ester storage disease or Wolman syndrome can present clinically with a phenotype that resembles recessive FH (Table 1) [12]. Patients with lysosomal acid lipase deficiency (LALD) classically express some combination of hepatomegaly, elevated transaminases, and dyslipidemia (usually elevated LDL-C) [94]. Liver biopsy shows hepatosteatosis that can progress to fibrosis, cirrhosis, and liver failure [94]. A recent multicentre randomized, double-blind, placebo-controlled study in LALD patients showed that enzyme-replacement therapy with sebelipase alfa was associated with improved plasma lipids and transaminases and reduced hepatic fat, together with only mild adverse effects [95]. The long-term benefits, risks, and costs of this interesting new treatment remain to be determined.

Integrated Overview of Genetic Determinants of Plasma Lipids and Lipoproteins

A PubMed search on March 5, 2016 using the terms "human genetics" and "lipoproteins" yielded 17,660 hits. Has this investment in resources and effort been worth it? In a first attempt to summarize and integrate some of the accumulated knowledge of the genetics of lipid and lipoprotein traits, we have compiled results from various types of studies for LDL-C (Fig. 1a), HDL-C (Fig. 1b), and TG (Fig. 1c). Because GWAS have been the largest single source of trait-associated variants, the rows of each figure are populated by GWAS loci identified from the GLGC meta-analyses [22], sorted by chromosomal location. The lead SNP at each locus is shown, although some loci had more than one lead SNP or significant association signal. Because most loci had joint associations with other traits, these are also shown. The relative effect size and direction of association of the lead SNP with the trait are indicated. Association of each locus with a monogenic disorder or syndrome, or with an extreme biochemical deviation in the absence of other systemic involvement, are indicated. The extent to which the non-genetic literature (i.e., biochemical, cell biological, pathological, epidemiological) supported the candidacy of the gene locus was gaged and noted. Of particular importance was the recapitulation of the representative phenotype in knock-out mouse models. Because of the importance of lipids and lipoproteins in atherosclerosis risk, evidence of association with these endpoints from MR and observational studies in affected kindreds and cohorts of variant carriers is highlighted. Finally, rapid prioritization of loci that have led to drug development projects or could represent promising targets for such development are shown.

Conclusions and Future Directions

The scope of genetic determinants of inter-individual phenotypic variation both in dyslipidemia syndromes and the general population as revealed by GWAS and NGS is

staggering. While a few more rare monogenic dyslipidemias may still be identified and characterized, by and large the knowledge of these disorders is complete. Furthermore, we have a good start on understanding genomic variants contributing to lipid and lipoprotein levels, although the proportion of variation unexplained remains large. While rare variants underlying lipid phenotypes are proving to be informative, they only explain a small amount of variation; factors such as gene-gene, gene-environment, epigenetic modifications, and perhaps new aspects of biology may fill in our knowledge gaps. Human genetics has also inspired development of new interventions to improve the quality and quantity of life for patients with dyslipidemia, but also more generally for those at risk of the devastating end-points of atherosclerotic CVD.

Acknowledgments RAH is supported by the Jacob J. Wolfe Distinguished Medical Research Chair, the Martha G. Blackburn Chair in Cardiovascular Research, and operating grants from the Canadian Institutes of Health Research (Foundation Grant), the Heart and Stroke Foundation of Ontario (T-000353) and Genome Canada through Genome Quebec (award 4530).

Compliance with Ethical Standards

Disclosure Jacqueline S. Dron declares that she has no conflict of interest. Robert A. Hegele is a consultant and speaker's bureau member for Aegerion, Amgen, Sanofi, Pfizer, and Valeant.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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