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Hypertriglyceridemia: phenomics and genomics

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Abstract Hypertriglyceridemia is a common complex metabolic trait that is associated with increased atherosclerosis risk, presence of the metabolic syndrome and, with extreme elevation, increased risk of pancreatitis. Hierarchical cluster analysis using clinical and biochemical features of the Frederickson hyperlipoproteinemia types can generate hypotheses for molecular genetic studies. High throughput resequencing of individuals at the extremes of plasma triglyceride concentration has shown that both rare genetic variants with large effects and common genetic variants with moderate effects explain a relatively large proportion of variation. Very recent progress using high-density sets of genome-wide markers have identified additional genetic determinants of plasma triglyceride concentrations, albeit within largely normolipidemic subjects and with small effect sizes. Phenomic evaluation of patients with hypertriglyceridemia might help to clarify genotype–phenotype correlations and responses to interventions.

Keywords Lipid · Lipoprotein · Monogenic · Complex trait · Quantitative trait · Chylomicron · DNA mutation · Single nucleotide polymorphism

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Hypertriglyceridemia refers to fasting plasma triglyceride (or triacylglycerol; TG) concentration that is increased typically >95th percentile for age and sex, although additional quantitative or qualitative lipoprotein abnormalities can be present [1]. Patients can fluctuate between degrees of hypertriglyceridemia; mild or moderate hypertriglyceridemia can deteriorate into severe hypertriglyceridemia given an appropriate metabolic stress. Elevated plasma TG contributes to increased cardiovascular disease (CVD) risk both directly and because elevated TG is associated with such risk factors as obesity, metabolic syndrome, pro-inflammatory, and pro-thrombotic biomarkers, and type 2 diabetes mellitus (DM) [1]. The patient with TG > 10 mmol/l has increased pancreatitis risk.

Basics of TG metabolism

A brief overview of TG metabolism is shown in Fig. 1. The main sources of plasma TG are exogenous, from dietary fat, and endogenous, from the liver. Dietary fats are packaged into TG-rich chylomicron particles in the intestine [2]. Chylomicrons are initially secreted into the lymphatics but reach the systemic circulation via the thoracic duct. In the systemic circulation, chylomicrons acquire additional C apolipoproteins (apos) and apo E from high density lipoproteins (HDL) [3]. Apo C-II is important because it is a key cofactor for LPL [4]. More recently, apo A-V has been shown to enhance hydrolysis of TG-rich lipoproteins [5], through an incompletely defined mechanism, while a newly characterized protein, namely glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1), appears to facilitate lipolysis by anchoring chylomicrons to endothelium, providing stability for LPL activity [6]. In the liver, TG is

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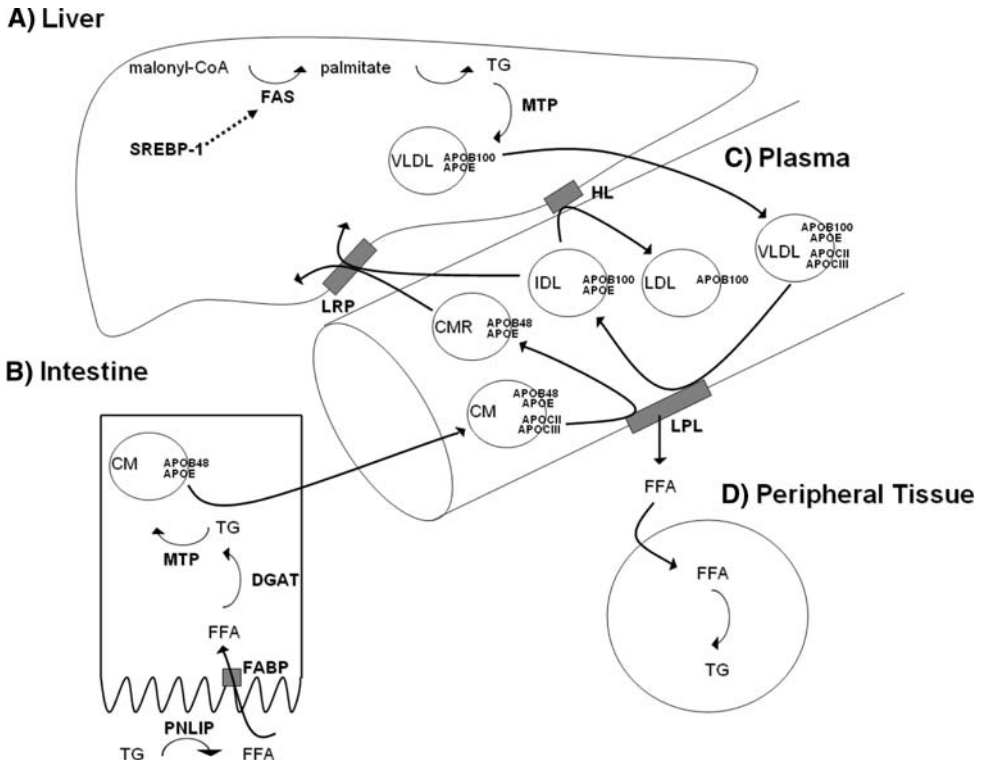

Fig. 1 Schematic overview of triglyceride (TG)-rich lipoprotein metabolism. The main sources of plasma TG are exogenous, from dietary fat, and endogenous, from the liver. In the intestine, dietary TG is hydrolyzed by pancreatic lipase (PNLIP) into monoglyceride and FFAs, forming micelles. Fatty acid binding protein (FABP) transports FFA from the intestinal lumen into enterocytes, and TG is re-synthesized through the sequential acyltransferase reactions, of which microsomal diacylglycerol acyltransferase (DGAT) catalyzes the terminal committed step. Microsomal triglyceride-transfer protein (MTP) mediates assembly of TG with apolipoprotein (apo) B-48 and apo E into chylomicrons. After secretion into lymph, chylomicrons acquire apo C-II and C-III, which modulate the plasma metabolism of TG-rich lipoproteins. Apo C-II is an obligatory cofactor for lipoprotein lipase (LPL), while apo C-III may interfere with LPL. Apo A-V has been shown to enhance hydrolysis of TG-rich lipoproteins, through an incompletely defined mechanism, while a newly characterized protein, namely glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1), appears to facilitate lipolysis by anchoring chylomicrons to endothelium, providing stability for LPL activity. Apo B-48 is the signature protein of intestinally derived TG-rich lipoproteins. In the liver, TG is synthesized from FFA extracted from plasma and from fatty acids synthesized de novo. A central enzyme for de novo synthesis is fatty acid synthase (FAS), which catalyzes the conversion of malonyl-CoA to palmitate. FAS is induced by the membrane-bound transcription factor sterol regulatory element-binding protein-1 (SREBP-1), which is itself regulated by polyunsaturated fatty acids, glucose, and insulin. Hepatic MTP mediates TG assembly with cholesterol esters, apo B-100 and apo E to form VLDL, which is released into the space of Disse by exocytosis. Apo B-100 is the signature protein of intestinally derived TG-rich lipoproteins. In adipose and muscle capillaries, TG in chylomicrons and VLDL are hydrolyzed into FFA by endothelial-bound LPL. FFA is then re-esterified and stored in adipocytes, or oxidized for energy in myocytes. Chylomicrons and VLDL are remodeled, respectively, into the short-lived, smaller, denser, cholesterol ester (CE)-enriched chylomicron remnants (CMR) and VLDL remnants (also called intermediate-density lipoprotein [IDL]). CMR and some IDL are cleared by apo E-mediated endocytosis through hepatic remnant receptors (LRP). IDL can also be hydrolyzed by hepatic lipase (HL), making smaller, CE-rich LDL particles

synthesized from free fatty acids (FFAs) extracted from plasma and from fatty acids synthesized de novo. In adipose and muscle capillaries, TG in chylomicrons and very low-density lipoprotein (VLDL) is hydrolyzed into FFA by endothelial-bound lipoprotein lipase (LPL), with contributions from apo C-II, apo A-V, and GPIHBP1. The mechanisms that increase the plasma TG-rich lipoproteins include increased production from the liver and intestine, through pathological upregulation of synthetic and secretory pathways, and/or decreased catabolism in the periphery, predominantly through reduction of LPL activity. Apo E directs the hepatic clearance of chylomicron remnants by the low-density lipoprotein (LDL) receptor and the LDL receptor-related protein LRP1 [7].

Plasma TG and atherosclerosis risk

Moderate hypertriglyceridemia is an independent risk factor for atherosclerotic disease, particularly coronary heart disease (CHD) [8]. Meta-analyses of thousands of patients followed for >10 years showed that a 1 mmol/l TG

elevation increased CHD risk by 32% and 76%, respectively, even after adjustment for HDL cholesterol [9]. More recently, plasma concentrations of non-fasting TG have been strongly associated with CHD risk [10, 11]. Complex mechanisms underlie the TG-atherosclerosis association and this complexity obscures ascertainment of a direct causal relationship. Pro-atherogenic metabolic abnormalities, such as obesity, type 2 DM, low HDL cholesterol, increased small-dense LDL, increased FFA, dysglycemia, hyperinsulinemia, increased plasma viscosity, increased inflammatory molecules, impaired fibrinolysis, and prothrombosis, are often associated with elevated TG [1]. Furthermore, TG-rich lipoproteins and their remnants may directly contribute to arterial wall foam cell formation. There are rare reports of atherosclerosis in hyperchylomicronemic patients [12], while chylomicrons remnants, VLDL, and IDL appear to be atherogenic [13].

Hypertriglyceridemia and pancreatitis

Very high TG resulting from elevated chylomicrons is associated with increased risk of acute pancreatitis [14]. The mechanism underlying this association is uncertain, but the relatively unique capacity of the pancreas to produce an exocrine lipase might play a role [1]. Many patients with pancreatitis from high TG probably have pre-existing abnormal lipoprotein metabolism, and milder hypertriglyceridemia will frequently persist after recovery [1]. Pancreatitis risk is accentuated with type 2 DM, pregnancy, estrogen use, alcohol consumption, or any factor that can abruptly increase plasma TG >10.0 mmol/l. Serum amylase may not exceed common diagnostic cut points. Associated clinical clues include eruptive xanthomata and/or lipemia retinalis. Treatment involves hemodynamic stabilization, cessation of oral intake, and control of primary metabolic disturbances. Plasmapheresis may be considered in extreme cases, although the benefit is transient [1].

Clinical phenomics of hypertriglyceridemia

Since accurate phenotyping is required to facilitate the analysis of complex traits such as hypertriglyceridemia, the discipline of clinical "phenomics" has emerged as a helpful tool for phenotype definition. Clinical phenomics involves the systematic acquisition of several levels of objective data, using clinical, biochemical, and imaging methods to provide accurate quantification of the degree to which individuals are affected with the trait of interest [15, 16]. Clinicians have been trained in this type of detailed phenotyping for characterization of many different types of genetic disorders for many decades, and the use of this technique has aided in differentiating the clinical severity of subcategories of numerous disorders that were perceived to have a genetic basis. A prime example of this was the systematic characterization in the 1970s of clinical and biochemical phenotypes that produced the so-called Fredrickson or World Health Organization classification scheme of hyperlipoproteinemia (HLP) [17].

Classification of hypertriglyceridemia

Hypertriglyceridemia can be divided into primary and secondary types. A classification system for TG disorders might be based upon a molecular diagnosis, however, genetic research has uncovered the molecular basis of <10% of primary hypertriglyceridemia and no replicable genetic susceptibility for secondary hypertriglyceridemia [1]. However, in clinical practice, therapeutic decisions can be made based on the degree of elevation of TG and the context of associated lipid abnormalities. We recently suggested four TG strata for CVD risk assessment: normal < 1.99 mmol/l, moderately elevated 2.0–4.99 mmol/l, high 5.0–9.99 mmol/l, and very high >10.0 mmol/l [1]. Nonetheless, the Fredrickson system of HLP phenotypes continues to hold value for lipidologists and lipoprotein researchers [17]. Five of the six Fredrickson types contain elevated TG as an essential diagnostic feature (Table 1), with additional qualitative differences that suggest etiological differences. These will be discussed in more depth below.

Fredrickson HLP type 1

HLP type 1, familial chylomicronemia, is characterized by the pathological presence of chylomicrons after a 12–14 h period of fasting and typically presents during the pediatric or adolescent age range. Clinical features in HLP type 1 include eruptive xanthomata, lipemia retinalis, hepatosplenomegaly, focal neurological symptoms—such as irritability in an infant—and recurrent epigastric pain with strong predisposition to pancreatitis. Overnight refrigerated plasma develops a creamy supernatant and a clear infranatant; fasting TG is typically >10 mmol/l. Biochemical diagnosis of LPL deficiency is established by the absence of LPL activity in plasma collected after intravenous heparin injection, although the expertise to perform this diagnostic test has dwindled to a few laboratories attached to academic centers. In HLP type 1, functional LPL deficiency most often results from familial LPL deficiency or familial apo C-II deficiency—both rare autosomal recessive diseases. Frameshift, missense, and nonsense mutations within the *LPL* gene have been reported to

Table 1 Classification of primary hypertriglyceridemia

Name (Type; MIM number)	Population prevalence	Lipid profile	Primary lipoprotein disturbance	Biochemical genetics
Familial chylomicronemia (HLP type 1; MIM 238600)	~1:10^6	Lipemic serum ↑TC ↑↑↑TG	↑chylomicrons with ↓LDL and ↓HDL	LPL deficiency; *LPL* mutations (95% of cases) apo CII deficiency; *APOC2* mutations (very rare)
Familial combined hyperlipidemia (HLP type 2B; MIM 144250)	~5%	↑↑TC ↑↑TG	↑VLDL and ↑LDL	Basis unknown in the majority; some have mutations in *USF1*, *APOB*, *APOC3*
Familial dysbetalipoproteinemia (HLP type 3; MIM 107741)	~1:10^4	↑↑TC ↑↑TG	↑IDL	Homozygous *APOE* E2 mutation plus secondary genetic or medical factors
Familial hypertriglyceridemia (HLP type 4; MIM 145750)	~5–10%	↑TC ↑↑TG	↑VLDL	No replicated causative or susceptibility gene
Primary mixed hyperlipidemia (HLP type 5; MIM 144650)	~1:10^3	Lipemic serum ↑↑↑TC ↑↑↑TG	↑chylomicrons with ↑VLDL	LPL deficiency; heterozygous *LPL* mutations (5–10% of cases)

MIM Mendelian inheritance in man (http://www.ncbi.nlm.nih.gov/Omim/); *HLP* hyperlipoproteinemia (Frederickson/World Health Organization classification); *TC* total cholesterol; *TG* triglycerides; *LDL* low-density lipoprotein; *HDL* high-density lipoprotein; *VLDL* very low-density lipoprotein; *LPL* lipoprotein lipase; *apo* apolipoprotein; *LPL* gene encoding LPL; *APOC2* gene encoding apo CII; *USF1* gene encoding upstream stimulatory factor 1; *APOB* gene encoding apo B; *APOC3* gene encoding apo CIII; ↑↑↑ very highly elevated (>99th percentile for age and sex); ↑↑ highly elevated (>95th percentile for age and sex); ↑ moderately elevated (>90th percentile for age and sex); ↓ low

cause chylomicronemia, with plasma TG between 10 and 200 mmol/l [14]. Frameshift, missense, nonsense, and splicing mutations have also been described in the *APOC2* gene, leading to chylomicronemia with plasma TG between 5 and 100 mmol/l [4]. Affected individuals have lifelong hypertriglyceridemia and must consume a low-fat diet. HLP type 1 is distinct from the other Fredrickson HLP types that are characterized by elevated TG because HLP type 1 is clearly a monogenic trait, whose molecular basis and biochemical consequences have been well defined. Only HLP 2A (familial hypercholesterolemia) has been defined to a similar degree at the molecular genetic level [18]. The remaining HLP types that each feature hypertriglyceridemia, namely HLP 2B, 3, 4, and 5, are not yet as completely defined at the molecular level; each likely represents a complex metabolic phenotype with numerous genetic determinants.

Fredrickson HLP type 2B

HLP type 2B, also called familial combined hyperlipoproteinemia (FCH), shows autosomal dominant (AD) inheritance with variable penetrance, and is a relatively common lipoprotein phenotype [18]. The main lipoprotein abnormalities are increased VLDL and LDL, with depressed HDL. An abnormal lipoprotein profile in at least one-first-degree relative is a formal diagnostic criterion. Occasional affected subjects are obligate heterozygotes for mutations of *LPL* or *APOC3* genes, but the molecular basis underlying FCHL is unknown in most instances. A recently defined gene that may be causative for FCHL is *USF1*, encoding an upstream stimulatory factor [19].

Fredrickson HLP type 3

Familial dysbetalipoproteinemia, HLP type 3, is a rare autosomal recessive phenotype. The main lipoprotein abnormality is the increase in TG-rich lipoprotein remnants, also called intermediate density lipoproteins (IDL) or "beta-VLDL," which produce an equimolar elevation of plasma total cholesterol and TG [20]. An increased VLDL-cholesterol to TG ratio and homozygosity for the apo E2 isoform are pathognomonic. Affected subjects frequently have tuberous or tuberoeruptive xanthomata on extensor surfaces of extremities, planar and/or palmar crease xanthomata, and predisposition to early CVD. HLP type 3 is almost always found in individuals homozygous for the binding defective *APOE* E2 isoform, but phenotypic expression usually requires other factors such as obesity, type 2 DM or hypothyroidism [20]. Secondary genetic factors are also considered to play a role in HLP type 3, but few have been defined.

Fredrickson HLP type 4

HLP type 4, familial hypertriglyceridemia, is defined by an isolated elevation of VLDL, which are less TG-rich than chylomicrons. HLP type 4 has high population prevalence and is a frequent cause of mild to moderate hypertriglyceridemia. The molecular basis of the phenotype is unknown in most cases, but it likely has a polygenic basis requiring a secondary factor for clinical expression [17, 18]. Typically, HLP type 4 patients have moderately elevated plasma TG that range between 2.3 and 5.7 mmol/l, usually with low HDL. HLP type 4 is associated with increased CHD risk,

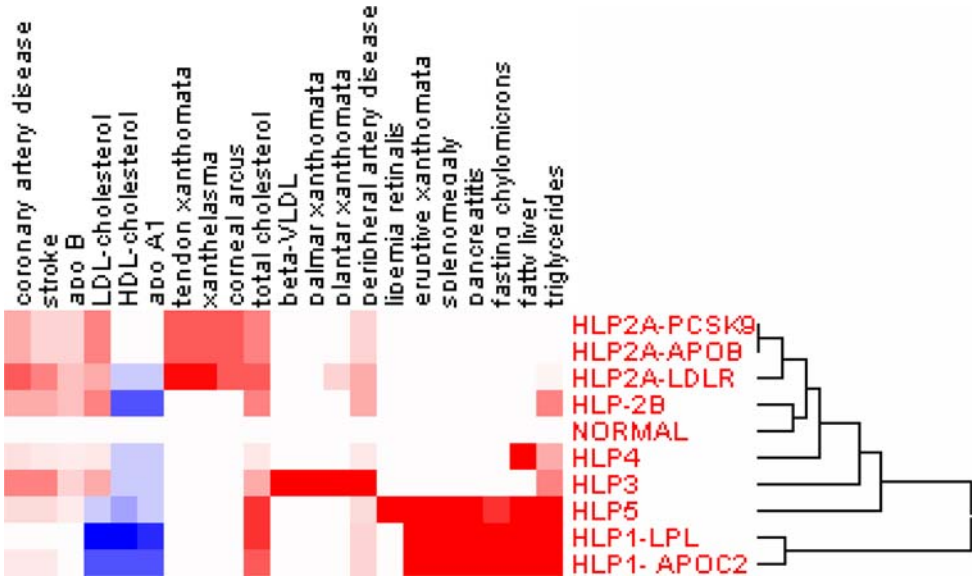

Fig. 2 Clinical phenomics for Fredrickson hyperlipoproteinemia (HLP) phenotypes. Hierarchical Clustering Explorer 3.5 (available at http://www.cs.umd.edu/hcil/hce/)was used to evaluate whether clinical phenomics could detect phenotypic similarities between Fredrickson HLP phenotypes. In this figure, the vertical axis lists nine HLP phenotypes and the normal control or reference phenotype. The disease phenotypes are: HLP2A-PCSK9, HLP2A-APOB, and HLP2A-LDLR, which are the three molecular subtypes of familial hypercholesterolemia (HLP type 2A) caused by heterozygous mutations in the genes encoding proprotein convertase subtilisin-like kexin type 9 (PCSK9), apolipoprotein B (APOB) and low-density lipoprotein receptor (LDLR), respectively; HLP2B, also called "familial combined hyperlipoproteinemia," NORMAL, which represents reference values for quantitative and qualitative phenotypes in a healthy individual without any HLP type, HLP4, also called "familial hypertriglyceridemia," HLP3, also called "familial dysbetalipoproteinemia," HLP5, also called "primary mixed hyperlipidemia" and HLP1-LPL and HLP1-APOC2, which are two molecular subtypes of familial chylomicronemia. The horizontal axis lists clinical features, such as susceptibility to coronary artery disease, stroke, or peripheral arterial disease, presence of lipoprotein-specific physical findings such as tendon, palmar, planar and eruptive xanthomata, xanthelasma, lipemia retinalis, splenomegaly, and fatty liver, and biochemical features such as quantitative deviations from normal of plasma total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, total triglycerides, apolipoproteins (apo) B and A1, and presence of fasting chylomicrons and beta-VLDL particles. The fold-change for each quantitative variable was compared to the normal reference range. The fold-changes were converted to colors using conventional "heat mapping" of quantitative traits, where expression of a phenotypic feature is depicted as shades of red or blue. The deeper the blue color, the lower the expression of this phenotypic feature in the affected individuals compared to normal healthy (unaffected) controls. Similarly, the deeper the red color, the greater the expression of this phenotypic feature in the affected individuals compared to controls. Areas that are white represent no difference from normal controls. The clustering algorithm sorted the phenotypes according to similarity of expression of the quantitative traits. The dendrogram to the right of the sorted phenotypes indicates the Euclidean distance (hierarchical relationships) between them based on phenotypic similarity. There are three clusters for these phenotypic data: the first cluster is the three HLP2A subtypes and HLP2B. The second cluster is normal. The third cluster is HLP4, HLP3, HLP5, and HLP1 subtypes. A hypothesis arising is that causative genes for HLP type 2A might also be causative for some patients with HLP type 2B, while causative genes for HLP type 1 might also be causative for some patients with HLP types 3, 4, or 5

obesity, insulin resistance, hyperglycemia, hypertension, and hyperuricemia.

Fredrickson HLP type 5

HLP type 5, primary mixed hyperlipidemia, is characterized by the pathological presence of chylomicrons after a 12–14 h fasting period [1]. Clinical features seen in HLP type 5 include eruptive xanthomata, lipemia retinalis, hepatosplenomegaly, focal neurological symptoms—such as inability to concentrate in an adult—and recurrent epigastric pain with strong predisposition to pancreatitis. Fasting TG is typically >10.0 mmol/l in both HLP type 1 and 5. Key features that distinguish HLP type 5 from type 1 include: (1) childhood and adulthood presentation for HLP types 1 and 5, respectively (2) biochemically proven deficiency of LPL or apo C-II activity and/or homozygous gene mutations for HLP type 1; (3) higher population prevalence of HLP type 5 compared with HLP type 1; (4) presence of secondary factors such as alcohol consumption, poor diet, obesity, type 2 DM, or hypothyroidism in HLP type 5; and (5) elevation of chylomicrons alone with low concentrations of total cholesterol and other lipoproteins in HLP type 1, while total cholesterol and other lipoproteins (particularly VLDL) are elevated in "mixed" HLP type 5.

Hierarchical cluster analysis of Fredrickson HLP types

To illustrate how deep phenotyping of hypertriglyceridemia can suggest new scientific hypotheses, a preliminary hierarchical cluster analysis of Fredrickson HLP types was performed. Hierarchical cluster analysis is used extensively in molecular biology to search for shared patterns of fold-changes in gene expression from normal under different experimental conditions, typically from expression microarray experiments. Here, the same algorithm (Hierarchical Clustering Explorer 3.5 http://www.cs.umd.edu/hcil/hce/) was used to cluster clinical and biochemical components of the Fredrickson HLP phenotypes according to the analogous fold-changes in clinical and biochemical variables compared to normal reference ranges (Fig. 2). The analysis indicates that HLP type 2A most closely resembles HLP type 2B, while HLP types 3, 4, and 5 most closely resemble HLP type 1. Thus, causative genes for HLP type 2A might also be causative for some patients with HLP type 2B, while causative genes for HLP type 1 might also be causative for some patients with HLP types 3, 4 or 5, although these hypotheses remain to be proven.

Secondary hypertriglyceridemia

Some metabolic conditions are frequently but not universally associated with high TG, suggesting that subjects who develop secondary hypertriglyceridemia might have a subtle inherited metabolic defect that confers susceptibility. Obesity—elevated TG is part of the definition of the metabolic syndrome—is probably the most commonly associated metabolic stressor in hypertriglyceridemic patients [1]. Other secondary factors include poorly controlled type 2 DM, excessive alcohol intake, renal disease, pregnancy, hypothyroidism, non-alcoholic hepatosteatosis, and medications (e.g. anti-retrovirals and second generation anti-psychotics) [1]. It is likely that these secondary factors exacerbate the TG-elevation in an individual who is genetically predisposed to high TG, although this hypothesis remains to be proven.

Genetic determinants of plasma triglyceride concentration

Investigators have spent the last 20 years searching for large genetic effects on plasma lipoproteins; this search has proved relatively fruitless [21]. In the general population, most candidate genes have small to undetectable effects. Genetic predisposition probably comprised multiple, relatively common genetic variants, each with small to modest effects that alone or in combination with modifier genes or environmental factors modulate risk of disease [22]. This has been called the "common-disease-common-variant" model [23]. An alternative model, called the "heterogeneity" model, maintains that genetic predisposition to common diseases in some patients is caused by rare genetic variants [24]. Such rare variants explain extreme versions of quantitative traits related to the disease. Since carriers of such mutations are found at the extremes of the distribution of the trait, this model would explain risk for a relatively small proportion of patients. In contrast, the "common-disease-common-variant" model invokes common genetic variants that are found at a higher frequency and could affect a larger proportion of patients. Each individual variant has a small effect, but cumulatively variants could exert a large effect and thus might explain susceptibility among individuals clustered around the center of Gaussian distribution of a quantitative trait [25]. In reality, a blend of both models likely explains the genetic basis of hypertriglyceridemia [22].

Common candidate gene polymorphisms with detectable genetic effects

Certain common variants in *LPL*, such as the D9N and N291S variants, appear to be fairly consistently associated with elevated plasma TG, while the S447X variant appears to be fairly consistently associated with depressed TG [26]. In addition, common variants at nucleotides −482 and −455 relative to the start site of transcription within the promoter region of the *APOC3* gene as well as another common variant in the *APOC3* 3'-untranslated region have shown consistent associations with plasma TG concentrations [26]. Finally, common variants in *APOA5*, designated −1131 T > C and S19W, have each shown independent strong associations with plasma TG [27]. The S19W variant further appears to have a consistent relationship with several Fredrickson HLP phenotypes (Fig. 3). Meta-analysis shows a summary of odds ratio (OR) for *APOA5* S19W carriers of 2.67 (95% confidence interval [CI] 2.24–3.18) for these HLP phenotypes.

Common alleles identified by large scale genome wide association studies

Recent genome-wide association (GWA) studies suggest the existence of some consistently associated genetic markers for plasma TG in the general population. In addition to replicating such known loci as *APOA1/C3/A4/A5* and *LPL*, these studies found promising associations of plasma TG in the population with common single nucleotide polymorphisms of genes encoding the glucokinase

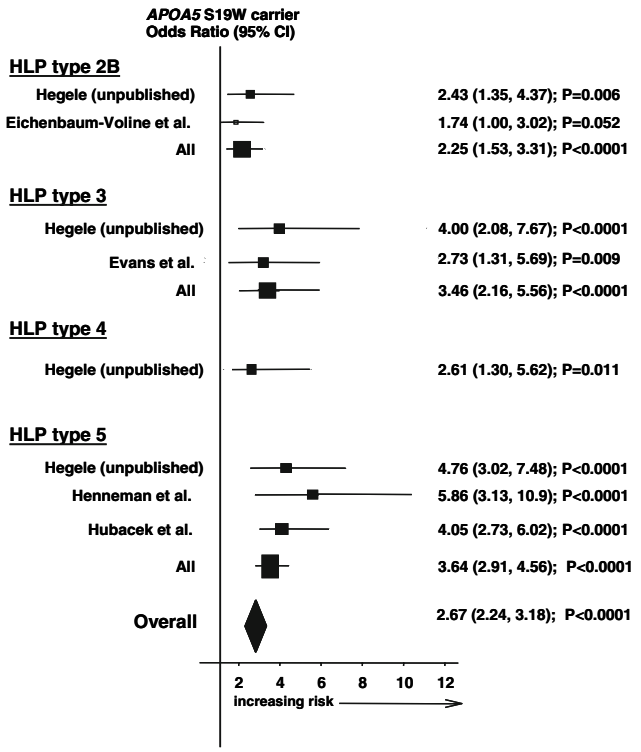

Fig. 3 Tree-plot of meta-analysis of odds ratios for classical primary hyperlipoproteinemia (HLP) phenotypes for observations from the author's clinical samples (Hegele, unpubl.), Eichenbaum-Voline et al. [41], Evans et al. [42], Henneman et al. [43], Hubacek et al. [44]. Odds ratios (OR) and 95% confidence limits are shown graphically and textually. Vertical line indicates OR of 1 (line of identity). The summary OR is based on a total of 893 HLP cases and 3,572 normolipidemic control subjects

regulatory protein (*GCKR*), homolog of Drosophila Tribbles 1 (*TRIB1*), MLX-interacting like protein (*MLXIPL*), angiopoietin-like protein 3 (*ANGPTL3*), UDP-*N*-acetyl-alpha-D-galactosamine:polypeptide *N*-acetylgalactosaminyltransferase 2 (*GALNT2*) and pre-B-cell leukemia transcription factor 4 (*PBX4/CILP2* locus) [28–31]. Since these associations were found in samples ascertained from the general population, it is not clear whether they will also be important determinants of pathological hypertriglyceridemia phenotypes. Furthermore, these markers all showed relatively modest associations with these traits: in order to detect the association signal, tens of thousands of individuals had to be studied. While important new biological insights might arise from these analyses, the potential for clinical utility, such as diagnosis or risk prediction is questionable.

While an effect-size, risk ratio, of 1.4 appears large at the population level, the chances that this type of marker will be clinically meaningful for the individual patient are slim, especially when the knowledge of all contributing factors, and their interactions, is incomplete. Furthermore, genetic effects and the interactions between them and environmental factors may vary across substrata of the specific populations, not to mention across geographical ancestries in which background unmeasured genetic variability and environmental, social and cultural factors might attenuate the signal-to-noise ratio for any particular genetic marker of risk.

Rare alleles with large effects

Within certain families and communities, the effect of a single gene on atherosclerosis susceptibility may be profound, e.g. mutations in *LPL* which cause familial chylomicronemia [1]. However, this paradigm also appears to hold true at the population level – a key question is whether cumulatively these rare alleles affect a clinically relevant proportion of individuals. For instance, we sequenced candidate genes in Oji-Cree individuals with extreme diabetes phenotypes (young and lean with diabetes versus old and overweight without diabetes) successfully to identify a private diabetes-causing loss-of-function mutation in hepatic nuclear factor-1-alpha, namely *HNF1A* G319S [32, 33]. We next showed how sequencing of genomic DNA of individuals with a diagnosis of common "garden variety" type 2 DM indicated that ~ 5% carry mutations in genes that cause a rare monogenic subtype of diabetes called MODY [34]. A similar strategy was used to examine patients with subgroups of quantitative lipoprotein phenotypes defined by threshold [35, 36]. This approach was also used to show that nonsynonymous variants in angiopoietin-like protein 4 (ANGPTL4) were more prevalent in individuals with TG levels in the lowest quartile than in individuals with levels in the highest quartile, although these levels were all well within the normal range [37].

Recently, we resequenced >2 million base pairs of genomic DNA from 110 non-diabetic patients with severe hypertriglyceridemia and determined the prevalence of coding sequence variants compared to 472 age- and sex-matched normolipidemic controls [38]. The candidate genes studied were *LPL*, *APOC2*, and *APOA5*. We found: (1) heterozygous loss-of-function mutations in *LPL* or *APOC2* in 10.0% of HLP type 5 patients compared to 0.2% of controls (carrier OR 52, 95% CI 8.6 to 319) and (2) an association of the *APOA5* S19W missense variant with HLP type 5 (carrier OR 5.5 95% CI 3.3 to 9.1). Furthermore, either rare loss-of-function mutations or the *APOA5* S19W variant was found in 41.8% of HLP type 5 subjects compared to 8.9% of controls (carrier OR 7.4, 95% CI 4.5 to 12.0). The findings indicated how both common and rare DNA variants in candidate genes were found in a substantial proportion of patients with severe hypertriglyceridemia [38].

We used the same strategy to sequence the *GPIHBP1* gene, whose protein product plays a critical role in lipolytic

processing of chylomicrons in mice [6]. We screened the coding regions of the human *GPIHBP1* from the genomic DNA of 160 unrelated adults with HLP type 5 [39]. Only 1 out of 160 HLP type 5 patients had a coding sequence variant, in this case homozygosity for the G56R missense mutation, which was absent from the genomes of 600 control subjects and 610 patients with hyperlipidemia [39]. A subsequent biochemical study showed no apparent loss of in vitro function for the mutant protein [40]. The findings together indicate that mutations in *GPIHBP1* are neither an important nor frequent contributor to clinically ascertained human hypertriglyceridemia [39].

Potential clinical use of genotyping for hypertriglyceridemia

The TG-associated loci detected to date might represent the "low hanging fruit" for genetic determinants of plasma TG. Future studies, perhaps, requiring hundreds of thousands of patients might find additional significantly associated markers with even more marginal relative risk ratios (say $\sim$1.10 or less); the clinical relevance of such markers would be even less obvious than those so far detected. Further research—to understand the architecture of genetic susceptibility, to characterize the pathophysiological mechanisms underlying genetic associations, to define interactions between genetic variants and the environment, to discover new forms of variation and to validate genetic associations—are all required before routine genotyping for hypertriglyceridemia.

An established biomarker such as plasma TG represents the integration of many genetic and environmental effects—both known and measurable, but also unknown and unmeasurable factors. Even with "complete" genomic information, confounders such as genetic pleiotropy (causation by different genes), epistasis (interactions between genes underlying a trait), influence of genetic background, or gene-environment interactions, could complicate a direct relationship between genotype and phenotype. Thus, any attempt to account for all inputs into an individual's plasma TG concentration will be incomplete at best. Finally, biochemically measured TG concentration has already been proven to be a valid risk predictor and therapeutic target, replicated over millions of patient-years of clinical use. The possible clinical value of an adjunctive genetic test is unclear at best.

Conclusion

Hypertriglyceridemia represents a complex phenotypic trait that demonstrates considerable quantitative and qualitative heterogeneity. Earlier studies implicated variants of a few biochemical candidate genes as contributing to a portion of the genetic component of hypertriglyceridemia. High throughput resequencing of individuals at the extremes of the Gaussian distributions of plasma TG have shown that both rare variants with large effects and common variants with more moderate effects are present in a high proportion of affected individuals. Very recent progress using high-density sets of genome-wide markers have identified additional genetic determinants of plasma TG, albeit within largely normolipidemic subjects and with relatively small effect sizes. The objective and systematic acquisition of phenotypic data through clinical phenomics of hypertriglyceridemia might be a tool to help to identify and to clarify genotype–phenotype correlations and responses to interventions. Traditional genetic approaches and genome-wide association studies together with the use of "deep phenotyping" through phenomics will provide a complementary path to identify novel pathways, mechanisms, and genes responsible for hypertriglyceridemia subphenotypes. With reduced barriers to automated DNA sequencing, clinical phenomics may provide a path to improved genomic interrogation and reproducible genetic results, particularly in the context of the complex trait of hypertriglyceridemia. However, the clinical application of DNA analysis in hypertriglyceridemia is not imminent; more research is required.

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