Triglyceride-Rich Lipoproteins and Coronary Artery Disease Risk

New Insights From Human Genetics

Sumeet A. Khetarpal, Daniel J. Rader

Despite ample success in reducing coronary artery disease (CAD) risk through reduction of low-density lipoprotein cholesterol (LDL-C), there remains substantial residual risk.[1–4] Recent prospective studies have demonstrated that elevated triglycerides (TGs) are independent predictors of CAD risk.[5–9] Furthermore, TGs are strongly associated with incident CAD events in patients with low LDL-C levels treated with statin.[10] Thus, triglyceride-rich lipoproteins (TRLs) offer a potentially orthogonal risk factor to LDL-C for lowering CAD risk, but only if TRLs are causally associated with atherosclerotic disease.[11]

Human genetics has the potential to reveal the causal relationships of biomarkers found to be associated with disease outcomes.[12–15] For example, genetic variants associated with plasma LDL-C levels are consistently associated with CAD risk in the right direction,[15–18] consistent with a causal relationship. Importantly, similar studies have causally implicated the key TG-regulating enzyme lipoprotein lipase (LPL) in CAD risk. A common gain-of-function *LPL* variant, S447X, confers an antiatherogenic lipid profile characterized by low levels of TGs, and in several studies, it has been associated with lower incidence of vascular disease or myocardial infarction (MI).[19–25] Conversely, several loss-of-function (LOF) *LPL* variants associated with elevated TG levels have been reported to be associated with increased CAD risk.[21,26] Furthermore, multiple genome-wide association studies in the last 5 years have identified common noncoding variants at the *LPL* gene locus associated with both TG and CAD risk in the same direction.[27–29]

Beyond *LPL* itself, common variants that influence TG levels are significantly associated with CAD risk even after adjusting for their effects on other lipid traits.[30] Do et al[30] surveyed 185 single-nucleotide polymorphisms (SNPs) that were genome-wide significantly associated with ≥1 plasma lipid trait and identified a subset of 44 SNPs with large effects on TG levels but minimal effects on LDL-C. They tested the association of these SNPs with CAD in >86 000 individuals. The strength of association of the SNPs with TG levels predicted the magnitude of association with CAD risk. Among the common variants with strong associations with both TG and CAD were those at a gene locus containing the genes *APOC3* and *APOA5*, which encode apolipoproteins (apoC-III and apoA-V, respectively), found on TRLs and known to be the regulators of LPL activity and TG levels.

ApoC-III is a key regulator of fasting and postprandial plasma TG levels and is thought to act at multiple nodes influencing TG homeostasis. A small (8.8 kDa) secreted apolipoprotein, apoC-III, is expressed in the liver and intestine and circulates on and exchanges between TRLs and high-density lipoprotein (HDL).[31,32] Several studies have suggested that apoC-III negatively regulates LPL activity.[33] Further insight gained from transgenic mice overexpressing *APOC3* and *Apoc3* knockout mice has shown that apoC-III delays very LDL (VLDL)–TG hydrolysis in vivo and may delay the catabolism of TRL remnants by the liver and other tissues.[34–37] In addition, 1 human coding variant in *APOC3*, K78E, is associated with low TG and high HDL-C levels and was shown to reduce VLDL secretion in vivo.[38] This suggests that *APOC3* may contribute to plasma lipids at least in part through influencing hepatic VLDL assembly and secretion.

Like apoC-III, apoA-V is also an exchangeable apolipoprotein between HDLs and TRLs, which is primarily secreted from the liver. It is a 39-kDa protein and has a low concentration in human plasma (≈150 ng/mL)[39] compared with the major apolipoproteins, including apoC-III. Despite its low abundance, apoA-V is thought to play a crucial role in TG metabolism. *Apoa5* knockout mice demonstrate profound hypertriglyceridemia, whereas human *APOA5* transgenic mice have significantly lower plasma TG than controls.[40] ApoA-V has been shown to enhance LPL activity on VLDL particles. Recent work has suggested that it may do so by facilitating proximity between TRLs and LPL in part through apoA-V's interaction with glycosylphosphatidylinositol-anchored HDL binding protein 1, a chaperone for LPL. Like apoC-III, apoA-V may have a critical intracellular role in regulating TG metabolism as well. Numerous studies in cultured hepatocyte-like cell lines have suggested that apoA-V accumulates in the endoplasmic reticulum after translation and remains associated with hepatic lipid droplets.[39]

Sequencing Reveals *APOC3* and *APOA5* as Causal Mediators of CAD Risk

Two recent reports, published concurrently in the *New England Journal of Medicine*, used complementary approaches to demonstrate that LOF mutations in *APOC3* are

From the Departments of Genetics and Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia.

Correspondence to Daniel J. Rader, Perelman School of Medicine at the University of Pennsylvania, 11-125 Translational Research Center, 3400 Civic Center Blvd, Philadelphia, PA 19104. E-mail rader@mail.med.upenn.edu

(*Arterioscler Thromb Vasc Biol*. 2015;35:e3-e9.
DOI: 10.1161/ATVBAHA.114.305172.)

***Arterioscler Thromb Vasc Biol* is available at http://atvb.ahajournals.org**
DOI: 10.1161/ATVBAHA.114.305172

robustly associated with lower TG and decreased incidence of CAD.[41,42] One of these studies, a large collaboration of the Exome Sequencing Project of the National Heart, Lung, and Blood Institute, sequenced the exomes of 3734 subjects and tested the association of identified mutations, either individually or in aggregate within a gene, that were associated with plasma TG.[41] They identified 7 coding variants in *APOC3* in a total of 33 individuals. Of these variants, all were rare in frequency, with 3 missense, 1 nonsense, and 3 splice-site variants identified. When tested in aggregate, the *APOC3* variants were robustly associated with lower TG (by 39 mg/dL) relative to noncarriers. Four of the 7 variants were found in heterozygosity at an aggregate frequency of 1 in 150 in individuals of European descent. These variants were associated with approximately half of the circulating apoC-III concentrations of noncarriers, supporting the notion that these variants conferred the loss of apoC-III function. The authors tested the association of these 4 variants with the presence of CAD in >110 000 subjects and found 40% lower CAD risk in mutation carriers. Notably, 1 of the 4 variants studied by Crosby et al,[41] a nonsense mutation R19X (rs76353203) was previously shown in an Amish population to reduce TG and improve the clearance of dietary fat in an oral fat challenge and was associated with reduced coronary artery calcium scores, a surrogate measurement of atherosclerosis.[43]

Working independently in Denmark, Jørgensen et al[42] tested the association of plasma TG with the presence of ischemic vascular disease (CAD or cerebrovascular disease) in 2 prospective cohorts comprising 75 725 subjects.[21] They found that the subjects with TG <90 mg/dL had significantly lower risk of ischemic vascular disease compared with the subjects with TG >350 mg/dL. Initial deep medical resequencing of the exons of *APOC3* and subsequent genotyping in the larger cohort identified 260 heterozygous carriers for 1 of 3 *APOC3* mutations, which were associated with lower fasting TG. The 3 variants identified by this approach were among the 4 SNPs that drove the association of *APOC3* variants with TG described by the Exome Sequencing Project of National Heart, Lung, and Blood Institute. Of the 75 725 subjects studied, 10 797 subjects developed ischemic vascular disease, of which 7557 had ischemic heart disease. When separated by *APOC3* genotype, they noted a 41% reduction in risk of vascular disease among mutation carriers. The association of the variants with lower incidence of vascular disease was attenuated when comparisons were adjusted for nonfasting TG levels in the participants, implying that the effect of apoC-III on TG levels is at least partially responsible for the protection from disease conferred by the variants.

These 2 recent studies present the argument that apoC-III's influence on plasma TG is responsible for the relationship of apoC-III with CAD risk. However, given apoC-III's pleiotropic influence on lipoprotein metabolism and additional contributions to vascular risk, others have suggested that this interpretation may be incomplete. Cohen et al[44] recently commented on the possibility that the reduced LDL-C levels in *APOC3* mutation carriers may account for the observed protection from vascular disease. ApoC-III on intermediate-density lipoproteins and LDLs is thought to delay hepatic clearance of these particles by lipoprotein receptors, and LDL-containing apoC-III was shown to be positively associated with development of coronary heart disease.[34,35,45] In addition, LDL-bound apoC-III was shown to be associated with levels of the proatherogenic small dense LDL independently of plasma TG.[46] For these reasons, deeper mechanistic studies in humans carrying these variants are warranted to explore the exact contribution(s) attributable to *APOC3* LOF that confers protection from vascular risk. These studies will undoubtedly require an isolated study of the specific candidate processes influenced by apoC-III in carriers versus noncarriers of the identified mutations.

In contrast to these studies identifying disease-protective *APOC3* LOF coding variants, studies of *APOA5* have revealed several risk-conferring LOF coding variants. Several coding variants have been implicated in severe hypertriglyceridemia or hyperchylomicronemia through case-control and family-based sequencing studies.[47] Many of these studies identified rare variants in *APOA5* but demonstrated that they were robustly associated with hypertriglyceridemic phenotypes when considered in aggregate.[47,48] In addition, some common coding variants in *APOA5* associated with increased TG have also been attributed to increased CAD risk.[49,50]

In December 2014, investigators from the Broad Institute reported in *Nature* a large exome sequencing experiment in early-onset MI cases compared with older healthy controls that implicated *APOA5*.[51] To test the hypothesis that rare alleles may contribute to the extreme phenotype of early-onset MI, Do et al[51] performed exome sequencing in a discovery cohort of 1027 early MI cases (men, ≤50 years old and women, ≤ 60 years old) and 946 older controls without MI (men, ≥60 years old and women, ≥70 years old) through participation in the Exome Sequencing Project of National Heart, Lung, and Blood Institute, selecting subjects from a total of 11 studies. In assessing the results of this sequencing effort, the authors collapsed rare variants in the same gene and tested their aggregate frequency within a given gene between cases and controls (gene-burden testing).[52,53] They compared the collective variants within each gene between cases and controls by 3 metrics of variant annotation: nonsynonymous variants without functional annotation, deleterious variants as identified by the prediction tool PolyPhen2-HumDiv, and disruptive (indel, frameshift, nonsense, and splice-site) variants only. This preliminary effort did not identify any variants studied collectively that were associated with MI when using a significance threshold of $P=8\times10^{-7}$, a conservative limit to account for testing ≈20 000 genes by 3 different variant classification schemes.

On expansion of the exome sequencing effort from 1973 to 9793 participants (4703 MI cases and 5090 controls), the investigators performed gene-burden testing again and found that rare alleles in the *LDLR* were significantly associated with the risk of MI. In total, they identified 285 *LDLR* variants in cases compared with 208 in controls, resulting in an effect size of 1.5 fold ($P=4\times10^{-6}$). To comprehensively filter the identified variants to yield the mostly likely functional ones for association testing with MI, the authors developed 5 sets of criteria based on combinations of existing coding variant prediction

tools and applied each set of criteria to the identified variants. After applying the more stringent annotation criteria sets, the authors found an even greater enrichment of rare *LDLR* variants in MI cases, with an effect size of 13 fold when only variants considered to be most disruptive were included (9×10^{-5}). A total of 156 unique nonsynonymous coding, splice-site, and frameshift variants within LDLR were identified, of which 77 were previously reported as underlying causes of familial hypercholesterolemia, suggesting that the identified variants could cause MI through disruption of LDLR function and subsequent LDL-C elevation. This study provides hypothesis-free support for the well-established observation that genetically elevated LDL-C levels are frequently associated with increased risk of early MI.[54,55] Further functional study of the remaining 79 novel, rare *LDLR* variants will be required to determine whether and how they disrupt *LDLR* gene function and cause familial hypercholesterolemia.

The second major finding of this study was borne from additional targeted sequencing of 6 candidate genes (*APOA5*, *CHRM5*, *SMG7*, *LYRM1*, *APOC3*, and *NBEAL1*) that were identified as nominally significant ($P<0.005$) in the initial exome sequencing discovery phase. The coding regions of these 6 candidate genes were initially resequenced in 2 independent cohorts, 1 Italian cohort comprising 1716 early MI cases and 1519 controls, and another cohort from Ottawa consisting of 552 early MI cases and 586 controls. These initial efforts revealed an enrichment of rare *APOA5* mutations in early MI cases, prompting further sequencing of this gene in additional cohorts. Overall, sequencing of *APOA5* in 6721 early MI cases and 6711 MI-free controls identified 46 individual rare single-nucleotide variations. These variants were identified in 93 MI cases versus 42 healthy controls, and the >2-fold risk of MI in mutation carriers was primarily driven by variants found in only 1 or 2 study participants (private or near-private variants). Application of each of the 5 sets of variant annotation criteria for deleteriousness demonstrated a significant enrichment of rare *APOA5* alleles in MI cases, with greater relative risk of MI in individuals harboring variants deemed more deleterious by stricter criteria sets (strict and disruptive criteria).

The plasma lipids of harmful *APOA5* mutation carriers are important especially in light of the findings from the authors' previous study of CAD-protective *APOC3* variants associated with plasma TG. *APOA5* mutation carriers in the exome sequencing study had 63 mg/dL higher fasting TG and 14 mg/dL lower HDL-C than noncarriers, but notably, plasma LDL-C was comparable between carriers and noncarriers. These findings suggest that disruption of *APOA5* gene function increases the risk of CAD/MI through a mechanism that increases TRLs but does not involve alteration of LDL levels and provides further support to the previous evidence implicating genetically elevated TRLs in the risk of CAD/MI.

Targeting the LPL Pathway to Reduce CAD Risk

These recent studies have provided powerful evidence that plasma levels of TRLs are causally related to the development of CAD and specifically that apoC-III promotes and apoA-V protects against CAD. The results of Do et al[51] implicating *APOA5* LOF with increased TGs, no elevation in LDL-C, but increased MI risk also adds credence to the concept that it is the reduction in TRLs that primarily drives the association of the *APOC3* variants with reduced CAD/vascular disease incidence. Collectively, these 3 studies thus offer strong support to the hypothesis that intervention to lower TRL levels may decrease the risk of CAD. Taken together with previous investigations, they implicate the LPL pathway as a potential target for reducing the risk of CAD through modulation of TRL metabolism. Translating these findings to tangible therapeutic strategies will undoubtedly necessitate a better understanding of how the LPL pathway and its regulators, such as apoC-III and apoA-V, actually work in concert to regulate this metabolism.

In the case of apoC-III, 1 therapy to reduce its production is already in clinical development. ISIS Pharmaceuticals has developed an antisense oligonucleotide that silences *APOC3* expression in the liver.[56] This small chemically modified oligonucleotide is delivered subcutaneously and is internalized in the liver where it inhibits the translation of *APOC3* mRNA and promotes mRNA degradation through activation of RNase H. This anti-*APOC3* antisense oligonucleotide has been reported to significantly reduce plasma apoC-III and TG levels and blunt postprandial TG elevations on treatment of rodent models and a nonhuman primate model with anti-*APOC3* antisense oligonucleotide and in healthy human volunteers.[56] In December 2014, this anti-*APOC3* antisense oligonucleotide was reported to reduce TG levels in 3 patients with familial chylomicronemia.[57] Based on the human genetics, the expectation is that intervention to reduce plasma apoC-III levels will not only reduce TG levels but also decrease the risk of CAD.

Alternative approaches to targeting apoC-III will benefit from better structural elucidation of the apoC-III protein and mechanistic insights into the effects of the disease-protective variants identified. Although 3 of the 4 *APOC3* variants mainly responsible for the robust association with lower TG and CAD risk putatively function through affecting the production of full-length apoC-III protein (2 splice-site and 1 nonsense variant), the fourth variant, A43T (rs147210663), is a missense variant. This suggests that the variant may alter apoC-III function in a manner to render it less effective in maintaining plasma TG. A previous study of the biochemical properties of this variant suggested that it may alter lipid binding and thus may influence the exchangeability of apoC-III among lipoproteins or stability in circulation.[58] Further insight into apoC-III structure and lipid-binding properties, and the specific effects of such missense variants on these properties, may aid the development of small molecules or other structure-guided therapeutics that target a defined property of apoC-III function.

Development of treatments focused on enhancing the activity of apoA-V is conceptually more difficult to envision. Nevertheless, any efforts to do this will also benefit from careful structure–function studies of the lipid- and lipoprotein-binding properties of this protein. Given the low plasma concentration of apoA-V, studies of its structure–function relationships may offer insight into domains that could be modulated to increase binding affinity for VLDLs, promote retention or increased stability of the protein in a lipid-bound state. Interestingly, among the *APOA5* variants identified by exome sequencing,

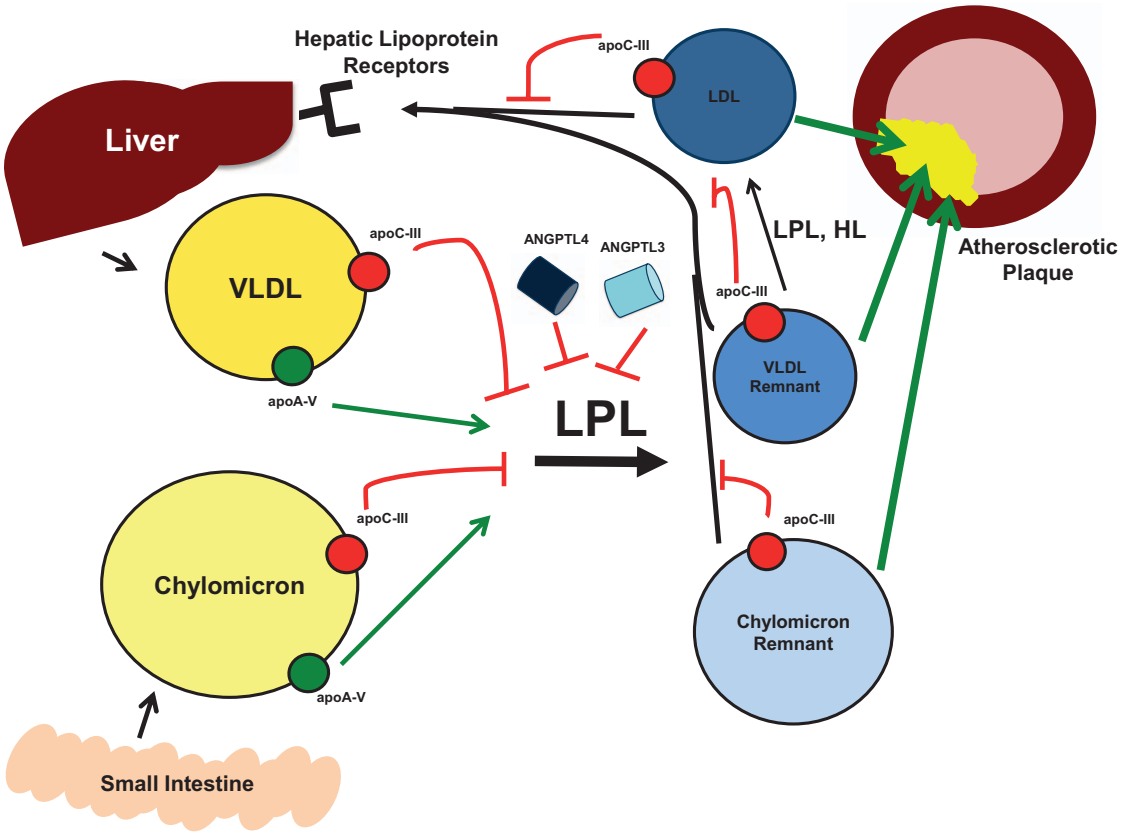

Figure. Roles of apoC-III and apoA-V in plasma triglyceride (TG) metabolism. ApoC-III and apoA-V are both found on TG-rich lipoprotein particles, such as very low-density lipoprotein cholesterol (VLDL) and chylomicrons, which are synthesized and secreted from the liver or intestinal enterocytes, respectively. ApoC-III inhibits lipoprotein lipase (LPL) activity, as do other secreted proteins ANGPTL3 and ANGPTL4. In contrast, apoA-V activates LPL activity. LPL-mediated TG lipolysis subsequently results in VLDL remnants from VLDL or chylomicron remnant particles derived from chylomicrons, both of which are enriched in cholesterol relative to TG. These particles may be taken up by the liver through interaction with specific lipoprotein receptors (VLDLR, LDLR, LRP1, and others), a process which is inhibited by apoC-III. VLDL remnants can be further modified by LPL and hepatic lipase (HL) to result in cholesterol-enriched LDL particles. If not removed from circulation, these various types of triglyceride-rich lipoproteins may be taken up by macrophages in the arterial wall, where they may contribute to vascular inflammation and atherosclerosis.

2 nonsynonymous missense variants were among those predicted to be deleterious by all 5 prediction algorithms used. These variants, Arg289Cys and Arg343Cys, both occur in the C-terminus of apoA-V, a region previously implicated as critical for lipid binding.[59] Further insight on apoA-V's structural composition and function will undoubtedly be gleaned from careful study of the most functionally deleterious coding variants identified from the exome and targeted sequencing. Such investigations are already underway; for example Sharma et al[60] demonstrated in the October 2014 issue of *ATVB* that 1 of the identified *APOA5* variants, Gly185Cys, disrupts apoA-V function by promoting aberrant disulfide bond formation of the mutant protein. This work combined the study of the variant through viral vector–mediated expression in *Apoa5* knockout mice with biochemical characterization of apoA-V from the plasma human carriers of the mutation. Such synergistic approaches will be necessarily to fully understand the implications of the many novel mutations identified and relate them to the physiology of plasma TG turnover.

These recent studies also raise interest in the prospect of targeting other regulators of LPL-mediated TG metabolism, including the angiopoietin-like (ANGPTL) proteins ANGPTL3 and ANGPTL4.[61] Like apoC-III, ANGPTL3 and ANGPTL4 are thought to inhibit LPL activity, leading to elevated plasma TG levels, although their respective mechanisms conferring LPL inhibition may be distinct.[61–66] Both common and rare variants in *ANGPTL3* are associated with plasma TG levels, and rare *ANGPTL3* LOF mutations underlie the Mendelian condition familial combined hypolipidemia, characterized by low plasma TG in addition to other lipid classes.[66–69] Although common variants at the *ANGPTL4* locus are associated primarily with HDL-C levels, rare coding mutations are robustly associated with reduced plasma TG.[66–68,70] These findings taken together suggest that pharmacological inhibition of these ANGPTLs could reduce plasma TGs by a mechanism similar to that of anti-*APOC3* focused therapies and result in reduced CAD risk. However, unlike the clear directional association of the *APOC3* variants to TG and CAD risk in the 2 *New England Journal of Medicine* studies, the evidence linking *ANGPTL3* and *ANGPTL4* LOF to CAD risk has been smaller or inconsistent.[71–74] The viability of targeting the ANGPTLs to reduce the risk of CAD will thus depend on both larger human genetics studies of clear LOF variants and better understanding of the physiological interplay of these proteins with different lipoprotein subclasses.

In summary, a remarkable confluence of robust human genetics findings for the past 6 months has convincingly and causally implicated triglycerides and TG-rich lipoproteins in the development of cardiovascular risk. Specifically, the LPL pathway and its reciprocal regulators apoC-III and apoA-V have been found to have a remarkably important influence on the risk of CAD (Figure). TRLs can be added to the list of apoB-containing lipoproteins, joining LDL and Lp(a), as causal risk factors of CAD. Whether novel interventions to reduce plasma levels of TRLs will be orthogonal and additive to LDL reduction in reducing cardiovascular risk remains to be established.

Sources of Funding

This work was supported by National Institutes of Health-National Heart, Lung, and Blood Institute grant R37-HL055323 to D.J. Rader. Additionally, S.A. Khetarpal is supported in part by National Institutes of Health grant F30-HL124967 and by the Perelman School of Medicine MSTP Program.

Disclosures

None.

References

1. Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, Joyal SV, Hill KA, Pfeffer MA, Skene AM; Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 Investigators. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N Engl J Med*. 2004;350:1495–1504. doi: 10.1056/NEJMoa040583.
2. LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart JC, Gotto AM, Greten H, Kastelein JJ, Shepherd J, Wenger NK; Treating to New Targets (TNT) Investigators. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. *N Engl J Med*. 2005;352:1425–1435. doi: 10.1056/NEJMoa050461.
3. Pedersen TR, Faergeman O, Kastelein JJ, Olsson AG, Tikkanen MJ, Holme I, Larsen ML, Bendiksen FS, Lindahl C, Szarek M, Tsai J; Incremental Decrease in End Points Through Aggressive Lipid Lowering (IDEAL) Study Group. High-dose atorvastatin vs usual-dose simvastatin for secondary prevention after myocardial infarction: the IDEAL study: a randomized controlled trial. *JAMA*. 2005;294:2437–2445. doi: 10.1001/jama.294.19.2437.
4. Sampson UK, Fazio S, Linton MF. Residual cardiovascular risk despite optimal LDL cholesterol reduction with statins: the evidence, etiology, and therapeutic challenges. *Curr Atheroscler Rep*. 2012;14:1–10. doi: 10.1007/s11883-011-0219-7.
5. Staniak HL, Salgado Filho W, Miname MH, Benseñor IM, Lotufo PA, Sharovsky R, Rochitte CE, Bittencourt MS, Santos RD. Association between postprandial triglycerides and coronary artery disease detected by coronary computed tomography angiography. *Atherosclerosis*. 2014;233:381–386. doi: 10.1016/j.atherosclerosis.2013.12.036.
6. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA*. 2007;298:299–308. doi: 10.1001/jama.298.3.299.
7. Langsted A, Freiberg JJ, Tybjaerg-Hansen A, Schnohr P, Jensen GB, Nordestgaard BG. Nonfasting cholesterol and triglycerides and association with risk of myocardial infarction and total mortality: the Copenhagen City Heart Study with 31 years of follow-up. *J Intern Med*. 2011;270:65–75. doi: 10.1111/j.1365-2796.2010.02333.x.
8. Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *JAMA*. 2007;298:309–316. doi: 10.1001/jama.298.3.309.
9. Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. *J Cardiovasc Risk*. 1996;3:213–219.
10. Miller M, Cannon CP, Murphy SA, Qin J, Ray KK, Braunwald E; PROVE IT-TIMI 22 Investigators. Impact of triglyceride levels beyond low-density lipoprotein cholesterol after acute coronary syndrome in the PROVE IT-TIMI 22 trial. *J Am Coll Cardiol*. 2008;51:724–730. doi: 10.1016/j.jacc.2007.10.038.
11. Kohli P, Cannon CP. Triglycerides: how much credit do they deserve? *Med Clin North Am*. 2012;96:39–55. doi: 10.1016/j.mcna.2011.11.006.
12. Janssens AC, van Duijn CM. Genome-based prediction of common diseases: advances and prospects. *Hum Mol Genet*. 2008;17(R2):R166–R173. doi: 10.1093/hmg/ddn250.
13. Kathiresan S, Srivastava D. Genetics of human cardiovascular disease. *Cell*. 2012;148:1242–1257. doi: 10.1016/j.cell.2012.03.001.
14. Shin SY, Petersen AK, Wahl S, et al. Interrogating causal pathways linking genetic variants, small molecule metabolites, and circulating lipids. *Genome Med*. 2014;6:25. doi: 10.1186/gm542.
15. Jansen H, Samani NJ, Schunkert H. Mendelian randomization studies in coronary artery disease. *Eur Heart J*. 2014;35:1917–1924. doi: 10.1093/eurheartj/ehu208.
16. Ference BA, Yoo W, Alesh I, Mahajan N, Mirowska KK, Mewada A, Kahn J, Afonso L, Williams KA Sr, Flack JM. Effect of long-term exposure to lower low-density lipoprotein cholesterol beginning early in life on the risk of coronary heart disease: a Mendelian randomization analysis. *J Am Coll Cardiol*. 2012;60:2631–2639. doi: 10.1016/j.jacc.2012.09.017.
17. Holmes MV, Dale CE, Zuccolo L, et al; InterAct Consortium. Association between alcohol and cardiovascular disease: Mendelian randomisation analysis based on individual participant data. *BMJ*. 2014;349:g4164.
18. Voight BF, Peloso GM, Orho-Melander M, et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *Lancet*. 2012;380:572–580. doi: 10.1016/S0140-6736(12)60312-2.
19. Rip J, Nierman MC, Ross CJ, Jukema JW, Hayden MR, Kastelein JJ, Stroes ES, Kuivenhoven JA. Lipoprotein lipase S447X: a naturally occurring gain-of-function mutation. *Arterioscler Thromb Vasc Biol*. 2006;26:1236–1245. doi: 10.1161/01.ATV.0000219283.10832.43.
20. Varbo A, Benn M, Tybjærg-Hansen A, Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG. Response: lipoprotein subclass profiling reveals pleiotropy in the genetic variants of lipid risk factors for coronary heart disease: a note on Mendelian randomization studies. *J Am Coll Cardiol*. 2013;62:1908–1909. doi: 10.1016/j.jacc.2013.08.1615.
21. Wittrup HH, Tybjaerg-Hansen A, Nordestgaard BG. Lipoprotein lipase mutations, plasma lipids and lipoproteins, and risk of ischemic heart disease. A meta-analysis. *Circulation*. 1999;99:2901–2907.
22. Humphries SE, Nicaud V, Margalef J, Tiret L, Talmud PJ. Lipoprotein lipase gene variation is associated with a paternal history of premature coronary artery disease and fasting and postprandial plasma triglycerides: the European Atherosclerosis Research Study (EARS). *Arterioscler Thromb Vasc Biol*. 1998;18:526–534.
23. Henderson HE, Kastelein JJ, Zwinderman AH, Gagné E, Jukema JW, Reymer PW, Groenemeyer BE, Lie KI, Bruschke AV, Hayden MR, Jansen H. Lipoprotein lipase activity is decreased in a large cohort of patients with coronary artery disease and is associated with changes in lipids and lipoproteins. *J Lipid Res*. 1999;40:735–743.
24. Gagné SE, Larson MG, Pimstone SN, Schaefer EJ, Kastelein JJ, Wilson PW, Ordovas JM, Hayden MR. A common truncation variant of lipoprotein lipase (Ser447X) confers protection against coronary heart disease: the Framingham Offspring Study. *Clin Genet*. 1999;55:450–454.
25. Groenemeijer BE, Hallman MD, Reymer PW, Gagné E, Kuivenhoven JA, Bruin T, Jansen H, Lie KI, Bruschke AV, Boerwinkle E, Hayden MR, Kastelein JJ. Genetic variant showing a positive interaction with beta-blocking agents with a beneficial influence on lipoprotein lipase activity, HDL cholesterol, and triglyceride levels in coronary artery disease patients. The Ser447-stop substitution in the lipoprotein lipase gene. REGRESS Study Group. *Circulation*. 1997;95:2628–2635.
26. Reymer PW, Gagné E, Groenemeyer BE, Zhang H, Forsyth I, Jansen H, Seidell JC, Kromhout D, Lie KE, Kastelein J. A lipoprotein lipase mutation (Asn291Ser) is associated with reduced HDL cholesterol levels in premature atherosclerosis. *Nat Genet*. 1995;10:28–34. doi: 10.1038/ng0595-28.
27. Lettre G, Palmer CD, Young T, et al. Genome-wide association study of coronary heart disease and its risk factors in 8,090 African Americans: the NHLBI CARe project. *PLoS Genet*. 2011;7:e1001300. doi: 10.1371/journal.pgen.1001300.
28. Teslovich TM, Musunuru K, Smith AV, et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature*. 2010;466:707–713.

29. Waterworth DM, Ricketts SL, Song K, et al; Wellcome Trust Case Control Consortium. Genetic variants influencing circulating lipid levels and risk of coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2010;30:2264–2276. doi: 10.1161/ATVBAHA.109.201020.
30. Do R, Willer CJ, Schmidt EM, et al. Common variants associated with plasma triglycerides and risk for coronary artery disease. *Nat Genet*. 2013;45:1345–1352. doi: 10.1038/ng.2795.
31. Ooi EM, Barrett PH, Chan DC, Watts GF. Apolipoprotein C-III: understanding an emerging cardiovascular risk factor. *Clin Sci (Lond)*. 2008;114:611–624. doi: 10.1042/CS20070308.
32. Sundaram M, Yao Z. Intrahepatic role of exchangeable apolipoproteins in lipoprotein assembly and secretion. *Arterioscler Thromb Vasc Biol*. 2012;32:1073–1078. doi: 10.1161/ATVBAHA.111.241455.
33. Eisenberg S, Patsch JR, Sparrow JT, Gotto AM, Olivecrona T. Very low density lipoprotein. Removal of apolipoproteins C-II and C-III-1 during lipolysis in vitro. *J Biol Chem*. 1979;254:12603–12608.
34. Aalto-Setälä K, Fisher EA, Chen X, Chajek-Shaul T, Hayek T, Zechner R, Walsh A, Ramakrishnan R, Ginsberg HN, Breslow JL. Mechanism of hypertriglyceridemia in human apolipoprotein (apo) CIII transgenic mice. Diminished very low density lipoprotein fractional catabolic rate associated with increased apo CIII and reduced apo E on the particles. *J Clin Invest*. 1992;90:1889–1900. doi: 10.1172/JCI116066.
35. Aalto-Setälä K, Weinstock PH, Bisgaier CL, Wu L, Smith JD, Breslow JL. Further characterization of the metabolic properties of triglyceride-rich lipoproteins from human and mouse apoC-III transgenic mice. *J Lipid Res*. 1996;37:1802–1811.
36. Gerritsen G, Rensen PC, Kypreos KE, Zannis VI, Havekes LM, Willems van Dijk K. ApoC-III deficiency prevents hyperlipidemia induced by apoE overexpression. *J Lipid Res*. 2005;46:1466–1473. doi: 10.1194/jlr.M400479-JLR200.
37. Jong MC, Rensen PC, Dahlmans VE, van der Boom H, van Berkel TJ, Havekes LM. Apolipoprotein C-III deficiency accelerates triglyceride hydrolysis by lipoprotein lipase in wild-type and apoE knockout mice. *J Lipid Res*. 2001;42:1578–1585.
38. Qin W, Sundaram M, Wang Y, et al. Missense mutation in apoc3 within the c-terminal lipid binding domain of human apoc-iii results in impaired assembly and secretion of triacylglycerol-rich very low density lipoproteins: evidence that apoc-iii plays a major role in the formation of lipid precursors within the microsomal lumen. *J Biol Chem*. 2011;286:27769–27780.
39. Sharma V, Forte TM, Ryan RO. Influence of apolipoprotein A-V on the metabolic fate of triacylglycerol. *Curr Opin Lipidol*. 2013;24:153–159. doi: 10.1097/MOL.0b013e32835c8c1a.
40. Pennacchio LA, Olivier M, Hubacek JA, Cohen JC, Cox DR, Fruchart JC, Krauss RM, Rubin EM. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. *Science*. 2001;294:169–173. doi: 10.1126/science.1064852.
41. The TG and HDL Working Group of the Exome Sequencing Project, NHLBI. Loss-of-function mutations in *APOC3*, triglycerides, and coronary disease. *N Engl J Med*. 2014;371:22–31.
42. Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG, Tybjærg-Hansen A. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. *N Engl J Med*. 2014;371:32–41. doi: 10.1056/NEJMoa1308027.
43. Pollin TI, Damcott CM, Shen H, Ott SH, Shelton J, Horenstein RB, Post W, McLenithan JC, Bielak LF, Peyser PA, Mitchell BD, Miller M, O'Connell JR, Shuldiner AR. A null mutation in human APOC3 confers a favorable plasma lipid profile and apparent cardioprotection. *Science*. 2008;322:1702–1705. doi: 10.1126/science.1161524.
44. Cohen JC, Stender S, Hobbs HH. Apoc3, coronary disease, and complexities of Mendelian randomization. *Cell Metab*. 2014;20:387–389.
45. Mendivil CO, Rimm EB, Furtado J, Chiuve SE, Sacks FM. Low-density lipoproteins containing apolipoprotein C-III and the risk of coronary heart disease. *Circulation*. 2011;124:2065–2072. doi: 10.1161/CIRCULATIONAHA.111.056986.
46. Shin MJ, Krauss RM. Apolipoprotein CIII bound to apoB-containing lipoproteins is associated with small, dense LDL independent of plasma triglyceride levels in healthy men. *Atherosclerosis*. 2010;211:337–341. doi: 10.1016/j.atherosclerosis.2010.02.025.
47. Johansen CT, Kathiresan S, Hegele RA. Genetic determinants of plasma triglycerides. *J Lipid Res*. 2011;52:189–206. doi: 10.1194/jlr.R009720.
48. Johansen CT, Wang J, Lanktree MB, et al. Excess of rare variants in genes identified by genome-wide association study of hypertriglyceridemia. *Nat Genet*. 2010;42:684–687. doi: 10.1038/ng.628.
49. Tang Y, Sun P, Guo D, Ferro A, Ji Y, Chen Q, Fan L. A genetic variant c.553G > T in the apolipoprotein A5 gene is associated with an increased risk of coronary artery disease and altered triglyceride levels in a Chinese population. *Atherosclerosis*. 2006;185:433–437. doi: 10.1016/j.atherosclerosis.2005.06.026.
50. Soufi M, Sattler AM, Kurt B, Schaefer JR. Mutation screening of the APOA5 gene in subjects with coronary artery disease. *J Investig Med*. 2012;60:1015–1019. doi: 10.231/JIM.0b013e3182686918.
51. Do R, Stitziel NO, Won HH, et al. Exome sequencing identifies rare ldlr and apoa5 alleles conferring risk for myocardial infarction [published online ahead of print December 10, 2014]. *Nature*. doi: 10.1038/nature13917. http://www.nature.com/nature/journal/vaop/ncurrent/full/nature13917.html.
52. Li B, Leal SM. Methods for detecting associations with rare variants for common diseases: application to analysis of sequence data. *Am J Hum Genet*. 2008;83:311–321. doi: 10.1016/j.ajhg.2008.06.024.
53. Purcell SM, Moran JL, Fromer M, et al. A polygenic burden of rare disruptive mutations in schizophrenia. *Nature*. 2014;506:185–190. doi: 10.1038/nature12975.
54. Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J Clin Invest*. 1973;52:1544–1568. doi: 10.1172/JCI107332.
55. Hazzard WR, Goldstein JL, Schrott MG, Motulsky AG, Bierman EL. Hyperlipidemia in coronary heart disease. 3. Evaluation of lipoprotein phenotypes of 156 genetically defined survivors of myocardial infarction. *J Clin Invest*. 1973;52:1569–1577. doi: 10.1172/JCI107333.
56. Graham MJ, Lee RG, Bell TA 3rd, Fu W, Mullick AE, Alexander VJ, Singleton W, Viney N, Geary R, Su J, Baker BF, Burkey J, Crooke ST, Crooke RM. Antisense oligonucleotide inhibition of apolipoprotein C-III reduces plasma triglycerides in rodents, nonhuman primates, and humans. *Circ Res*. 2013;112:1479–1490. doi: 10.1161/CIRCRESAHA.111.300367.
57. Gaudet D, Brisson D, Tremblay K, Alexander VJ, Singleton W, Hughes SG, Geary RS, Baker BF, Graham MJ, Crooke RM, Witztum JL. Targeting APOC3 in the familial chylomicronemia syndrome. *N Engl J Med*. 2014;371:2200–2206. doi: 10.1056/NEJMoa1400284.
58. Liu H, Labeur C, Xu CF, Ferrell R, Lins L, Brasseur R, Rosseneu M, Weiss KM, Humphries SE, Talmud PJ. Characterization of the lipid-binding properties and lipoprotein lipase inhibition of a novel apolipoprotein C-III variant Ala23Thr. *J Lipid Res*. 2000;41:1760–1771.
59. Beckstead JA, Wong K, Gupta V, Wan CP, Cook VR, Weinberg RB, Weers PM, Ryan RO. The C terminus of apolipoprotein A–V modulates lipid-binding activity. *J Biol Chem*. 2007;282:15484–15489. doi: 10.1074/jbc.M611797200.
60. Sharma V, Witkowski A, Witkowska HE, Dykstra A, Simonsen JB, Nelbach L, Beckstead JA, Pullinger CR, Kane JP, Malloy MJ, Watson G, Forte TM, Ryan RO. Aberrant hetero-disulfide bond formation by the hypertriglyceridemia-associated p.Gly185Cys APOA5 variant (rs2075291). *Arterioscler Thromb Vasc Biol*. 2014;34:2254–2260. doi: 10.1161/ATVBAHA.114.304027.
61. Kersten S. Physiological regulation of lipoprotein lipase. *Biochim Biophys Acta*. 2014;1841:919–933. doi: 10.1016/j.bbalip.2014.03.013.
62. Lee EC, Desai U, Gololobov G, et al. Identification of a new functional domain in angiopoietin-like 3 (ANGPTL3) and angiopoietin-like 4 (ANGPTL4) involved in binding and inhibition of lipoprotein lipase (LPL). *J Biol Chem*. 2009;284:13735–13745. doi: 10.1074/jbc.M807899200.
63. Yau MH, Wang Y, Lam KS, Zhang J, Wu D, Xu A. A highly conserved motif within the NH_2-terminal coiled-coil domain of angiopoietin-like protein 4 confers its inhibitory effects on lipoprotein lipase by disrupting the enzyme dimerization. *J Biol Chem*. 2009;284:11942–11952. doi: 10.1074/jbc.M809802200.
64. Larsson M, Vorrsjö E, Talmud P, Lookene A, Olivecrona G. Apolipoproteins C-I and C-III inhibit lipoprotein lipase activity by displacement of the enzyme from lipid droplets. *J Biol Chem*. 2013;288:33997–34008. doi: 10.1074/jbc.M113.495366.
65. Yin W, Romeo S, Chang S, Grishin NV, Hobbs HH, Cohen JC. Genetic variation in ANGPTL4 provides insights into protein processing and function. *J Biol Chem*. 2009;284:13213–13222. doi: 10.1074/jbc.M900553200.
66. Miida T, Hirayama S. Impacts of angiopoietin-like proteins on lipoprotein metabolism and cardiovascular events. *Curr Opin Lipidol*. 2010;21:70–75. doi: 10.1097/MOL.0b013e328333269e.
67. Romeo S, Yin W, Kozlitina J, Pennacchio LA, Boerwinkle E, Hobbs HH, Cohen JC. Rare loss-of-function mutations in ANGPTL family members contribute to plasma triglyceride levels in humans. *J Clin Invest*. 2009;119:70–79. doi: 10.1172/JCI37118.
68. Willer CJ, Schmidt EM, et al; Global Lipids Genetics Consortium. Discovery and refinement of loci associated with lipid levels. *Nature Genet*. 2013;45:1274–1283.

69. Musunuru K, Pirruccello JP, Do R, et al. Exome sequencing, ANGPTL3 mutations, and familial combined hypolipidemia. *N Engl J Med*. 2010;363:2220–2227. doi: 10.1056/NEJMoa1002926.
70. Romeo S, Pennacchio LA, Fu Y, Boerwinkle E, Tybjaerg-Hansen A, Hobbs HH, Cohen JC. Population-based resequencing of ANGPTL4 uncovers variations that reduce triglycerides and increase HDL. *Nat Genet*. 2007;39:513–516. doi: 10.1038/ng1984.
71. Talmud PJ, Smart M, Presswood E, Cooper JA, Nicaud V, Drenos F, Palmen J, Marmot MG, Boekholdt SM, Wareham NJ, Khaw KT, Kumari M, Humphries SE; EARSII Consortium; HIFMECH Consortium. ANGPTL4 E40K and T266M: effects on plasma triglyceride and HDL levels, postprandial responses, and CHD risk. *Arterioscler Thromb Vasc Biol*. 2008;28:2319–2325. doi: 10.1161/ATVBAHA.108.176917.
72. Hatsuda S, Shoji T, Shinohara K, Kimoto E, Mori K, Fukumoto S, Koyama H, Emoto M, Nishizawa Y. Association between plasma angiopoietin-like protein 3 and arterial wall thickness in healthy subjects. *J Vasc Res*. 2007;44:61–66. doi: 10.1159/000098153.
73. Korstanje R, Eriksson P, Samnegård A, Olsson PG, Forsman-Semb K, Sen S, Churchill GA, Rollins J, Harris S, Hamsten A, Paigen B. Locating Ath8, a locus for murine atherosclerosis susceptibility and testing several of its candidate genes in mice and humans. *Atherosclerosis*. 2004;177:443–450. doi: 10.1016/j.atherosclerosis.2004.08.006.
74. Folsom AR, Peacock JM, Demerath E, Boerwinkle E. Variation in ANGPTL4 and risk of coronary heart disease: the Atherosclerosis Risk in Communities Study. *Metabolism*. 2008;57:1591–1596. doi: 10.1016/j.metabol.2008.06.016.

KEY WORDS: apolipoprotein AV, human ■ apolipoprotein C-III ■ coronary artery disease ■ exome ■ myocardial infarction ■ triglycerides

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Triglyceride-Rich Lipoproteins and Coronary Artery Disease Risk: New Insights From Human Genetics

Sumeet A. Khetarpal and Daniel J. Rader

Arterioscler Thromb Vasc Biol. 2015;35:e3-e9; originally published online January 8, 2015;
doi: 10.1161/ATVBAHA.114.305172
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636