ANGPTL3 Inhibitors

— Their Role in Cardiovascular Disease Through Regulation of Lipid Metabolism —

Eleni Geladari, MD; Panagiota Tsamadia, MD; Natalia G. Vallianou, MD, PhD

Elevated plasma lipid levels are linked to atherosclerosis, a hallmark for coronary artery disease (CAD), documented by animal studies as well as angiographic and clinical studies. The ability to treat hyperlipidemia through lifestyle changes and lipid-lowering agents has been related to the slow progression of atherosclerosis and decreased incidence of major coronary events. Angiopoietin-like proteins (ANGPTLs) are a family of secreted glycoproteins expressed in the liver that share common domain characteristics with angiopoietins, the main regulators of angiogenesis. Although ANGPTLs cannot bind the angiopoietin receptors expressed on endothelial cells, 2 ANGPTL family members (ANGPTL3 and ANGPTL4) have clinical importance because of their unambiguous effects on lipoprotein metabolism in mice and humans. The regulation of plasma lipid levels by ANGPTL3 is controlled via affecting lipoprotein lipase and endothelial lipase-mediated hydrolysis of triglycerides (TGs) and phospholipids. ANGPTL 3, along with the other 2 members, 4 and 8, is a key to balancing the distribution of circulating TGs between white adipose tissue (WAT) and oxidative tissues. Thus, ongoing trials with newly discovered medications in the form of monoclonal antibodies or antisense oligonucleotides with novel targets are under analysis and may represent a fresh frontier in the treatment of hyperlipidemia and CAD.

Key Words: Antibodies; Arteriosclerosis; Atherosclerosis

Cardiovascular disease (CVD) is among the major causes of death worldwide.¹ It is stated that deaths from coronary artery disease (CAD) in developed countries are expected to increase by almost 29% in women and by 48% in men in the years 1990–2020, and these numbers have been estimated to increase by 120% in women and 137% in men in developing countries.² In other words, in developing countries the estimated increases in deaths from CAD are much more pronounced than in developed countries. However, after applying appropriate interventions to modify and reduce the effect of risk factors that contribute to the development of CVD, a large percentage of heart disease, stroke and peripheral vascular disease could be favorably prevented.

Primary and secondary prevention of these diseases have been applied during the past 3 decades through efficient strategies. Pointedly, clinicians strongly recommend lifestyle changes (healthy diet, optimal weight, physical activity, moderate or no alcohol consumption, and smoking cessation), reduction of high blood pressure, control of diabetes mellitus and, chiefly, treatment of dyslipidemias.²

A therapeutic milestone was the discovery of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, known as statins, in 1976 (Figure 1).³ In 1994 a landmark study, the 4S Study, (Scandinavian Simvastatin Survival Study Group, 1994), proved that a 2nd-generation

statin, simvastatin, not only reduced acute coronary syndrome (ACS), but surprisingly prolonged survival in middle-aged people at a high risk of a coronary event.⁴ If patients cannot tolerate statins or fail to achieve low-density lipoprotein-cholesterol (LDL-C) goals, the addition of ezetimibe, which decreases cholesterol absorption in the small intestine, may be significant.^{5,6}

Another enzyme that was found to play a critical role in rising LDL-C levels in the plasma is called PCSK9 (pro-protein convertase subtilisin/kexin type 9). The mechanism by which this enzyme exerts its hyperlipidemic properties is its binding to hepatic LDL-receptors (LDL-R) and disrupting their subsequent return to the cell surface after internalization. The Fourier (Further cardiovascular Outcomes Research with PCSK9 Inhibition in subjects with Elevated Risk) trial studied evolocumab, a fully human monoclonal antibody, and showed that patients on a background of statin therapy receiving evolocumab had lower LDL-C levels and lower incidence of atherosclerotic CVD.⁷

Nonetheless, patients with mixed dyslipidemia still remain at high risk for cardiovascular events despite lowering LDL-C levels by 30–40%. For instance, in statin-treated patients with high-density lipoprotein (HDL) levels <39 mg/dL, there is an increased risk for ACS as opposed to those with HDL-C levels >55 mg/dL.⁸ However, that

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Department of Internal Medicine, Evangelismos General Hospital, Athens, Greece

Mailing address: Natalia G. Vallianou, MD, MSc, PhD, Department of Internal Medicine, Evangelismos General Hospital, 5 Pyramidon str, 19005, Municipality of Marathonas, Athens, Greece. E-mail: Natalia.vallianou@hotmail.com

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Figure 1. Mechanism of action for statins. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase catalyzes conversion of HMG-CoA to mevalonate, the precursor of cholesterol. Statins inhibit HMG-CoA reductase, resulting in decreased cholesterol concentrations in the cell and thereby upregulation of low-density lipoprotein receptors and low-density lipoprotein-cholesterol clearance from the circulation.

statement is now becoming argued. Specifically, specialized mechanisms that act by inhibiting the endothelial lipase (EL) gene, thus raising serum HDL levels, do not have an effect on the development of atherosclerosis.⁹ Similarly, hormone replacement therapy does not lower the risk of myocardial infarction despite reinforcing the expression of plasma HDL levels.¹⁰ It is of particular importance to mention the Atherothrombosis Intervention in Metabolic syndrome with low HDL cholesterol/High triglyceride and Impact on Global Health outcomes (AIM-HIGH) trial, in which case the addition of niacin to simvastatin indeed increased HDL-C levels and lowered TGs but did not lower the risk of adverse cardiovascular outcomes.¹¹

Furthermore, additional studies in statin-treated patients have shown that patients with TG levels <200 mg/dL had lower incidence of death, acute myocardial infarction (MI) or recurrent ACS than those with TGs >200 mg/dL. This suggests that it would be useful to address and treat other lipid imbalances, such as low HDL-C and or increased TGs, mainly with the addition of fibrates, niacin and omega-3 fatty acids.² Besides, the National Cholesterol Education Program has not recommended a specific target for managing either low HDL-C or high TGs. Interestingly, a late meta-regression analysis of intervention trials failed to prove that increasing HDL-C levels improved CV outcomes.⁸

In such cases, national treatment guidelines suggest a combination of lipid-modifying therapies. Hence, large ongoing clinical trials test statins' effects on reducing coronary accidents along with and on different time combinations with other lipid-lowering agents.^{2,8} However, results from these clinical trials are needed in order to ascertain which of these combinations may be clinically superior to statin therapy alone to reduce residual CV risk. At the same time, however, scientists are trying to find novel mechanisms to combat dyslipidemia in a more stable and consistent way.

As we pointed out earlier, lipid disorders are attributable not only to environmental influences but to genetic factors as well. Mutations in any of the genes that play a pivotal role in lipid metabolism may lead to the development of different Mendelian forms of hypercholesterolemia.^{1,12} Five different forms have been identified, with 3 of them showing autosomal dominant inheritance: familial hypercholesterolemia (FH), which is caused by mutations in LDL-R; familial defective apolipoprotein B (Apo B),

which is caused by a defect in Apo B that diminishes the ability of LDL-C to bind to the LDL-R; and FH type 3 caused by gain-of-function mutations in PCSK9, which increases the degradation of the LDL-R. Interestingly, loss-of-function (LoF) mutations of PCSK9 occur in 2.5% of African Americans and are associated with a decreased incidence of CAD. PCSK9 inhibitors have shown how critical is the translation of genetics into clinical therapeutics.^{1,12} Over the past decade, genome-wide association studies (GWASs) have tested thousands of samples from the population in order to identify the genes responsible for hypercholesterolemia.¹² Therefore, DNA sequencing studies are of critical importance because they continue to reveal targets for CAD in individual families, as well as in populations. Characteristically, the exome sequencing of a family with atypical hypobetalipoproteinemia (i.e., 4 of the offspring had very low levels of LDL-C, HDL-C and TGs) demonstrated that the affected offspring were homozygous for a mutation in angiopoietin-like protein 3 (*ANGPTL3*), which encodes angiopoietin-like 3 (an inhibitor of lipoprotein lipase (LPL) and EL).^{1,12-14} Reduced levels of triglyceride-rich lipoproteins (TRLs) in *ANGPTL3* deficiency reflect both activation of LPL-mediated lipolysis in peripheral tissues and decreased secretion of very-low-density lipoprotein (VLDL) TGs by the liver.¹⁵⁻¹⁷

ANGPTL3 as a New Target

ANGPTL3 is a secreted protein expressed in the liver. Its expression increases the plasma levels of TGs, LDL-C and HDL-C.¹³⁻¹⁸ Antibodies and antisense oligonucleotides targeting ANGPTL3 are being tested in current clinical trials and potentially could be developed into new therapeutic agents for the treatment of hypercholesterolemia and subsequently decreasing the incidence of CAD.^{15,19}

ANGPTL3: Insights Into Pathogenesis of Hypercholesterolemia

Familial combined hypobetalipoproteinemia (FHL2) is a newly discovered entity that is characterized by reduced levels of all major lipoprotein classes (VLDL, LDL, HDL) in plasma. This is a distinct form from other types of hypobetalipoproteinemia, such as FHL1 and abetalipoproteinemia (ABL), which is delineated as low, or absent levels of ApoB100, and LDL-C in plasma.²⁰ In the beginning, it

was thought that individuals with FHBL2 had a defect in the *APO B*, *PCSK9* and microsomal triglyceride transfer protein (*MTP*) genes; however, GWAS and exome sequencing studies have identified *ANGPTL3* variations that are associated with very low plasma LDL-C, HDL-C and TG.²⁰ *ANGPTL3* complete LoF mutations result in FHBL2, which manifests as a reduction in all lipoproteins, except lipoprotein a [Lp(a)]. In fact, clusters of people who are homozygous or compound heterozygous for null variants in *ANGPTL3* have up to 70% lower levels of plasma LDL-C and TGs than those without such variants.^{20–22}

LoF of *ANGPTL3* results in increased LPL and EL activity, as well as enhanced insulin sensitivity, without an increased prevalence of fatty liver disease, and decreased serum free fatty acids (FFAs). Subsequent data uncovered the beneficial cardiometabolic effects of blocking *ANGPTL3*, which constitutes a potential therapeutic goal for countering combined hyperlipidemia, a principal risk factor for atherosclerotic CAD.^{20–22}

ANGPTLs are a family of secreted glycoproteins that share common domain characteristics with angiopoietins, the main regulators of angiogenesis. Specifically, the common domains are a coiled-coil domain at the N-terminal region and a fibrinogen-like domain at the C-terminal, which are connected by a linker region. Although ANGPTLs cannot bind the angiopoietin receptors expressed on endothelial cells, 2 ANGPTL family members (*ANGPTL3* and *ANGPTL4*) have clinical importance because of their unambiguous effects on lipoprotein metabolism in mice and humans.^{23,24}

In humans, the *ANGPTL3* gene is located on chromosome 1 (p31.1–p22.3), where it encodes a 460-amino acid protein that originates exclusively in the liver. The regulation of plasma lipid levels by *ANGPTL3* is controlled via affecting

LPL- and EL-mediated hydrolysis of TGs and phospholipids. *ANGPTL3*, along with 2 other members, 4 and 8, is key to balancing the distribution of circulating TGs between white adipose tissue (WAT) and oxidative tissues. Any errors in the partitioning of TGs to specific tissues according to nutritional demand may cause such imbalance that leads to obesity, lipotoxicity and hypertriglyceridemia, representing excess TGs in WAT, non-adipose tissues and plasma, respectively.^{25,26}

In order for TGs to be released into the circulation, LPL needs to be activated. It is an extracellular enzyme mainly located in the vascular beds of many tissues and has a principal role in TG metabolism, because it catalyzes the hydrolysis of the TG component of chylomicrons and VLDL to generate FFAs and monoacylglycerol.²⁷ It is synthesized and secreted by adipocytes, macrophages, and muscle cells and then bound to the vascular endothelium by heparin sulfate proteoglycans and GPIHBP1 (glycosylphosphatidyl-inositol-anchored HDL-binding protein 1), a protein that has been recently discovered.^{27–29}

In metabolically active tissues, such as adipose, cardiac muscle and skeletal muscle, there is abundant expression of LPL, considering its vital presence for the release, storage and/or usage of fatty acids. Postprandial LPL activity rises in WAT, but diminishes in the heart and skeletal muscle, thereby guiding circulating TGs to WAT for storage; the reverse happens during fasting.^{27–30} LPL was first identified by Hahn, more than 7 decades ago.³⁰ He accidentally observed that heparinized plasma cleared diet-induced lipemia in dogs, but heparin by itself did not have this effect, indicating that heparin injection released a factor that cleared fat in the plasma. This factor was later recognized as LPL.^{30,31}

LPL is intrinsically unstable and its vivo activity is

regulated via proteins or other molecules that may stabilize or destabilize it. In particular, apolipoproteins C2 (ApoC2) and A5 (ApoA5), molecules that are specially associated with chylomicrons and VLDL, stimulate LPL activity by increasing its V_{max} , whereas ApoC1 and ApoC3 have inhibitory effects. A head-to-tail homodimer represents the active form of LPL. When detachment of this homodimer happens, the monomers that are formed are either capable of re-associating to form catalytically active LPL or they can undergo conformational changes, forming inactive, stable monomers.^{27–31}

We have already acknowledged that ANGPTL3 is a 460-amino acid polypeptide with an idiosyncratic signal peptide sequence, a N-terminal helical domain and a C-terminal globular fibrinogen homology domain. The sequence just after the N-terminal coiled-coil region (17–207 amino acids), specifically the amino acid domain 61–66, reversibly binds LPL and inhibits its catalytic activity thus affecting plasma TG levels (**Figure 2**). On the other hand, the fibrinogen-like domain (207–460 amino acids) binds to the integrin $\alpha v\beta 3$ receptor and affects angiogenesis. Furthermore, a short linker region (at 221–222 and 224–225) between the N- and C-terminal domains functions as a furin cleavage site. This shortened form of cleaved ANGPTL3 also displays enhanced inhibitory activity for LPL and EL.^{22–24}

ANGPTL4, and likewise ANGPTL3, has been shown to inhibit LPL activity and to regulate TG metabolism. Its expression is induced by fasting via the peroxisome proliferator-activated receptor in adipocytes. ANGPTL4's role is crucial in regulating LPL activity under conditions of fasting and exercise.^{22–24}

ANGPTL3 and ANGPTL4

Definitely, both ANGPTL3 and ANGPTL4 have been found to suppress LPL activity in vitro and in vivo, but enzyme kinetic analysis with purified recombinant proteins revealed key mechanistic differences between the 2 proteins. For instance, the catalytic activity of LPL was reduced by ANGPTL3 without significantly altering its self-inactivation rate, whereas ANGPTL4 completely suppressed LPL by accelerating the irreversible inactivation of LPL. Similarly, heparin was able to overcome the inhibitory effect of ANGPTL3 on LPL but not that of ANGPTL4. Although heparin was observed to protect LPL from ANGPTL3 at relatively low concentrations, possibly via competitive binding, it was not able to impede the inactivating effects of ANGPTL4.^{22–24}

ANGPTL3 in Animal Models

The first observation to demonstrate a link between ANGPTL molecules and lipoprotein metabolism was made in a laboratory in Tokyo, Japan. A colony of wild-type KK mice exhibited signs of obesity accompanied by hyperinsulinemia and hypertriglyceridemia.³² However, a mutant mouse strain, KK/San or KK/Snk, had low plasma lipid levels. Characteristically, the mutant strain, despite preserving the phenotype of obesity and diabetes, had a marked decrease (>90%) of plasma TGs as compared with the authentic KK mice. Interestingly, there was a reduction not only in pre- β lipoproteins (VLDL), but also in the β - and α -lipoproteins (LDL and HDL, respectively). The mouse mutation was discovered in the middle of chromosome 4,

in the gene encoding ANGPTL3. Homozygosity for a 4-bp insertion in exon 7 introduced a stop codon at position 347 and resulted in a truncated form of ANGPTL3, which led to a complete ANGPTL3 deficiency.³² Nevertheless, injection of adenoviruses encoding ANGPTL3 or recombinant ANGPTL3 protein into mutant KK/San mice raised plasma lipid levels. In order for the researchers to elucidate the effects of ANGPTL3 on lipid metabolism, they focused on the metabolic pathways of TGs. Hence, overexpression of *ANGPTL3* in KK/San mice resulted in a marked increase of plasma total cholesterol, non-esterified fatty acids, and especially VLDL TG-enriched lipoprotein. Secretion and clearance are the 2 main mechanisms that maintain plasma VLDL TG levels in balance. Subsequent studies affirmed that there was no significant difference between mutant and wild-type KK mice in the hepatic VLDL TG secretion rate, but in vitro analysis of recombinant protein revealed that ANGPTL3 directly inhibited LPL activity.^{32,33} These data fully supported the hypothesis that ANGPTL3 is a new class of lipid metabolism modulator, which regulates VLDL TG levels through inhibition of LPL activity.^{32,33}

Based on the data documenting that LoF variants in *ANGPTL3* are associated with decreased plasma levels of TGs, LDL-C and HDL-C researchers have examined whether such variants or therapeutic antagonism of ANGPTL3 is associated with a reduced risk of atherosclerotic CVD. Thus, the remarkable hypolipidemic phenotype in ANGPTL3 deficiency shows the potential of ANGPTL3 inactivation as a treatment for adjusting hyperlipidemia. In addition, the fact that in ApoE-deficient mice, deletion of ANGPTL3 is reported to reduce the development of atherosclerosis has made researchers consider the ANGPTLs as important regulators of lipoprotein metabolism and easy targets for modulation of lipid levels and CVD risk.^{27–33}

ANGPTL3 and Clinical Trials

In the DiscovEHR study, whole-exome sequencing was performed in 58,335 adult participants of European ancestry. More than 400 persons heterozygous for ANGPTL3 LoF variants were identified and eventually had approximately 50% lower ANGPTL3 levels than non-carriers and 39% lower odds of CAD.¹⁹ That study also evaluated the effects of pharmacologic antagonism of ANGPTL3 on lipid metabolism and atherosclerosis in a mouse model of atherosclerosis, and on lipid levels in human volunteers by using a human monoclonal antibody, named evinacumab.¹⁶ Indeed, there was a great reduction in plasma lipid levels and atherosclerosis similar to that reported previously in the same mouse model treated with atorvastatin. According to these results, combined hypolipidemia, low levels of TGs, LDL-C and even HDL-C, may be protective against CAD through exerting anti-atherogenic properties.¹⁹ It has been elucidated that TGs levels are reduced through disinhibition of LPL, while HDL-C reduction is arbitrated through disinhibition of EL. Still, it remains unclear how ANGPTL3 controls LDL levels. Interestingly, studies in LDL-R-deficient mice suggest that ANGPTL3 modulates LDL-C in a manner that is independent of LDL-R and other known mechanisms responsible for clearance of plasma LDL-C.^{19,21,33} Hence, individuals with a complete defect in LDL-R-mediated LDL-C uptake, such as homozygous FH patients, may certainly benefit in lowering LDL-C levels by applied therapeutic antagonism of

ANGPTL3. In human volunteers, the use of evinacumab was associated with dose-dependent reductions in LDL-C and TGs levels.¹⁹

Additionally, 9 adults with homozygous FH were involved in a single-group, open label study with evinacumab.¹² All study participants were already receiving an aggressive lipid-lowering regimen: statins, ezetimibe, lomitapide, PCSK9 inhibitor, or a portacaval shunt. After evinacumab treatment, LDL-C, ApoB100, non-HDL-C, TGs and HDL-C were notably decreased. These reductions were in addition to reductions in baseline levels already achieved with stable, aggressive lipid-lowering therapy. At least 1 adverse event was reported by all the participants, but no event led to treatment discontinuation.¹²

As well, preclinical studies and a randomized, double-blind, placebo-controlled phase 1 trial of antisense oligonucleotides (ASOs) targeting hepatic ANGPTL3 messenger RNA (mRNA) have been conducted in order to evaluate ANGPTL3 as a potential cardiometabolic therapeutic target for reducing the risk of CVD.²⁴ This strategy, selective antisense inhibition of ANGPTL3 expression in the liver, could potentially be better than administration of ANGPTL3 inactivating antibodies, because of avoiding immune responses that are associated with antibodies. Several experiments in different mouse models and in healthy volunteers have documented that the downregulation of ANGPTL mRNA and subsequent decreased expression and/or suppression of hepatic ANGPTL3 protein has significant cardiometabolic effects, halts atherosclerosis progression, decreases TG content and increases insulin sensitivity. These favorable effects are achieved through lowering LDL-C, TGs, non-HDL-C and VLDL-C. In various models, mice were administered ANGPTL3 ASO and there were observed significant reductions in the levels of TGs (reductions of 35–85%), LDL-C (7–64%) and HDL-C (3–23%), in addition to reduction in TGs within VLDL, intermediate-density lipoprotein, and LDL particles.^{24,25} These changes happen independently of the LDL-R pathway and occur in the absence or presence of an excess of ApoC3, the reduction of which further lessens TG levels. Importantly, the ANGPTL3 ASO did not cause substantial changes in liver mass, aminotransferase levels, or characteristic histopathologic lesions. Likewise, human trials showed that inhibition of hepatic ANGPTL3 may cause a significant reduction of TGs, LDL-C and VLDL-C levels, as well as reducing ApoC3 levels, a potent inhibitor of LPL, which strongly suggests that ANGPTL3 are important components of plasma TG homeostasis.^{24,25}

ANGPTL3 Inhibitors and HDL-C

A possible concern with the use of ANGPTL3 inhibitors is the decreased levels of HDL-C, probably attributable to an increase in EL activity, which genetic studies suggest should not increase the risk of CAD.^{34,35} The *EL* gene, a subclass of the lipase gene family, has been shown to be expressed by vascular cells in vivo, as well as by a wide range of nonendothelial cells in a variety of tissues. EL has predominantly phospholipase activity and to a lesser extent TG lipase activity.³⁶ Jaye et al demonstrated that high-level overexpression of EL in the liver by adenovirus-mediated gene transfer results in a significant decrease in HDL-C and ApoA-I, suggesting that EL could be a physiological regulator of HDL metabolism.³⁷ Although

data from in vitro and in vivo animal studies have indicated that EL may play a key role in modulating the metabolism of HDLs, data regarding the regulation of the metabolism of ApoB-containing lipoproteins are lacking.³⁷ Characteristically, elevated levels of LPL have been associated with an undesirable lipid profile, increased levels of TGs and ApoB concentrations, as well as increased levels of pro-inflammatory cytokine concentrations. Moreover, a rising prevalence of metabolic syndrome has been observed among individuals with high levels of EL.³⁷

Nonetheless, the belief that increased plasma HDL-C reduces the risk of an ACS is now being debated, because 2 Mendelian randomization analyses showed that raising plasma HDL-C by affecting genetic mechanisms did not seem to lower the risk of MI.³⁸ Although single-nucleotide polymorphisms altering upstream plasma LDL-C levels consistently reduced cardiovascular events, specific genetic variants in the *EL* gene (LIPG Asn396Ser) that raise plasma HDL-C did not lessen the risk of MI. The evidence that some genetic mechanisms raise HDL-C levels but do not lower the risk of MI leads to questioning if any intervention (lifestyle or pharmacologic) that reduces HDL-C leads to a substantial decreased risk of MI.³⁹ Moreover, in individuals with familial combined hypolipidemia, despite the striking reduction of plasma HDL-C and ApoA-I levels, no premature atherosclerosis has been reported so far.³⁹

The relationship between HDL-C and ANGPTL3 has been documented by a variety of studies in both humans and mice.^{34,35} ANGPTL3 binds to LPL and EL through its coiled-coil domain, thus inhibiting their ability to release FFAs and phospholipids from VLDL and HDL-C, respectively. As a result, TG plasma levels increase, provoking hypertriglyceridemia and the development of atherosclerotic plaque. Atherosclerotic plaque progression can be further enhanced after activation of the integrin V3 by the fibrinogen-like domain of ANGPTL3, leading to plaque neovascularization, intimal thickening, foam cell formation and inflammation.⁴⁰

Mice have a different lipoprotein metabolism because HDL-C accounts for approximately of total cholesterol levels, whereas in humans HDL-C accounts for only up to 20–30% of total cholesterol levels.²⁵ It is widely known that mice lack cholesteryl ester transfer protein (CETP) activity, and double transgenic expression of human CETP and ApoB100 in hCETP/ApoB100 mice results in shifting the lipoprotein-cholesterol distribution in a way that is similar to human normolipidemia.³⁹ Very recently, Xu et al demonstrated that in the above-mentioned “humanized” mice, no reduction in HDL-C was seen with ANGPTL3 silencing.⁴¹ They focused on determining the mechanism by which LoF ANGPTL3 mutations cause low LDL-C. They used a RNAi-mediated silencing approach to target hepatic ANGPTL3 expression in multiple mouse models and found that hepatic ANGPTL3 silencing was sufficient to reduce LDL-C levels. Their analysis using human hepatoma cells showed that ANGPTL3 silencing using an elegant CRISPR/Cas9 and deletion reduced ApoB100 secretion and increased LDL/VLDL uptake.⁴¹ Thus, they suggest that the reduction in LDL-C as a result of reduced ANGPTL3 is explained by both reduced hepatic ApoB secretion and increased hepatic LDL uptake. Surprisingly, using the hCETP/ApoB100 mice they found that downregulation of ANGPTL3 had no obvious effect on the HDL-C level, while still maintaining strong activity in reducing the LDL-C level.⁴¹ CETP plays a central role in

HDL catabolism by mediating the exchange of neutral lipids between HDL and VLDL/LDL.^{42–45} The CETP protein level inversely correlates with the HDL-C level and transgenic expression of hCETP markedly reduced HDL-C in mice.^{42–45} As described earlier, ANGPTL3 modulates HDL-C presumably through the inhibition of EL activity. The resistance of hCETP/ApoB100 mice to ANGPTL3 silencing perhaps largely comes from the much stronger activity of hCETP on the HDL-C level.^{42–45}

Taken together, the data indicate that ANGPTL3 and ANGPTL4 play unique roles in the modulation of lipid metabolism in vivo, through suppression of LPL activity.⁴⁶ These proteins lower the activity of LPL in a dose-dependent manner, and after many experimental procedures, it was concluded that ANGPTL4 specifically accelerates LPL inactivation, whereas ANGPTL3 suppresses its catalytic activity in the presence of substrates.^{46,47} LPL mediates the lipolysis of TG-rich lipoproteins and the delivery of fatty acids to adipose tissue and muscle. Hence, LPL's inactivation leads to a hypertriglyceridemic profile, which constitutes the target of the new pharmacologic platform, consisting of antibodies and small molecules, in the treatment of dyslipidemia. Preclinical studies and studies in humans have identified that increasing the LPL activity might ameliorate insulin resistance and reduce the risk of diabetes.^{48–50} Mutations in LPL that increase its activity reduce not only the risk of CAD, but also that of diabetes. The mechanism implies storage of TG in gluteofemoral rather than visceral fat. LoF variants in ANGPTL3 are associated with increased levels of LPL and EL activity, decreased levels of fatty acids in the serum and enhanced insulin sensitivity, without significant fatty liver disease.^{51,52} Homozygous or compound heterozygous individuals for ANGPTL3 have approximately 70% lower levels of plasma LDL-C and TGs than those without such variants.⁵²

ANGPTL3 ASOs are antisense oligonucleotides that target the hepatic messenger RNA (mRNA), and finally inhibit the ANGPTL3 protein. In the experimental stage, mice administered ANGPTL3 ASOs had obvious significantly higher insulin sensitivity than control mice and approximately 81% lower liver TG transfer protein (TTP), suppressed through TTP-specific ASO, and thereby *ANGPTL3* gene expression in LDL-R (LDLR^{-/-}) mice was inhibited, causing hepatic TG accumulation in hyperlipidemic mouse models.⁴⁹ Co-administration of the Mttp and ANGPTL3 ASOs is associated with lower lipogenic gene expression than either individual ASO.⁴⁹

Conclusions

Genetic and therapeutic antagonism of ANGPTL3 in humans undoubtedly leads to improved measures of insulin sensitivity, reduces the risk of diabetes and lowers plasma levels of total cholesterol, LDL-C and TGs.^{51,52} In future, new approaches that increase LPL activity will play a great role in the treatment of hypertriglyceridemia. In addition to ANGPTL3 targeting, we anticipate therapeutics targeting aApoC3, Lp(a) and interleukin 1b to help reduce the residual CVD risk.⁵³

Disclosures

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References

1. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors: Systematic analysis of population health data. *Lancet* 2006; **367**: 1747–1757.
2. Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases. Part I: General considerations, the epidemiologic transition, risk factors, and impact of urbanization. *Circulation* 2001; **104**: 2746–2753.
3. Endo A, Kuroda M, Tanzawa K. Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by ML-236A and ML236B fungal metabolites, having hypocholesterolemic activity. *FEBS Lett* 1976; **72**: 323–326.
4. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: The Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994; **344**: 1383–1389.
5. Phan B, Dayspring T, Toth P. Ezetimibe therapy: Mechanism of action and clinical update. *Vasc Health Risk Manag* 2012; **8**: 415–427.
6. Hammersley D, Signy M. Ezetimibe: An update on its clinical usefulness in specific patient groups. *Ther Adv Chronic Dis* 2017; **8**: 4–11.
7. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med* 2017; **376**: 1713–1722.
8. Khera AV, Ridker PM. Demystifying HDL cholesterol-A “Human Knockout” to the rescue? *Clin Chem* 2017; **63**: 33–36.
9. Ko KW, Paul A, Ma K, Li L, Chan L. Endothelial lipase modulates HDL but has no effect on atherosclerosis development in apoE^{-/-} and LDLR^{-/-} mice. *J Lipid Res* 2005; **46**: 2586–2594.
10. Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women: Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *JAMA* 1998; **280**: 605–613.
11. Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K, et al. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. *N Engl J Med* 2011; **365**: 2255–2267.
12. Goldstein JL, Brown MS. A century of cholesterol and coronaries: From plaques to genes to statins. *Cell* 2015; **161**: 161–172.
13. Gusarova V, Alexa CA, Wang Y, Rafique A, Kim JH, Buckler D, et al. ANGPTL3 blockade with a human monoclonal antibody reduces plasma lipids in dyslipidemic mice and monkeys. *J Lipid Res* 2015; **56**: 1308–1317.
14. Romeo S, Pennacchio LA, Fu Y, Boerwinkle E, Tybjaerg-Hansen A, Hobbs HH, et al. Population-based resequencing of ANGPTL4 uncovers variations that reduce triglycerides and increase HDL. *Nat Genet* 2007; **39**: 513–516.
15. Romeo S, Yin W, Kozlitina J, Pennacchio LA, Boerwinkle E, Hobbs HH, et al. Rare loss-of-function mutations in ANGPTL family members contribute to plasma triglyceride levels in humans. *J Clin Invest* 2009; **119**: 70–79.
16. 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.
17. Wang H, Eckel RH. Lipoprotein lipase: From gene to obesity. *Am J Physiol Endocrinol Metab* 2009; **297**: E271–E288.
18. Dewey FE, Gusarova V, O'Dushlaine C, Gottesman O, Trejos J, Hunt C, et al. Inactivating variants in ANGPTL4 and risk of coronary artery disease. *N Engl J Med* 2016; **374**: 1123–1133.
19. Dewey FE, Gusarova V, Dunbar RL, O'Dushlaine C, Schurmann C, Gottesman O, et al. Genetic and pharmacologic inactivation of ANGPTL3 and cardiovascular disease. *N Engl J Med* 2017; **377**: 211–221.
20. Di Costanzo A, Di Leo E, Noto D, Cefalù AB, Minicocci I, Polito L, et al. Clinical and biochemical characteristics of individuals with low cholesterol syndromes: A comparison between familial hypobetalipoproteinemia and familial combined hypolipidemia. *J Clin Lipidol* 2017; **11**: 1234–1242.
21. Stitzel NO, Khera AV, Wang X, Bierhals AJ, Vourakis AC, Sperry AE, et al. ANGPTL3 deficiency and protection against coronary artery disease. *J Am Coll Cardiol* 2017; **69**: 2054–2063.
22. Wang Y, Gusarova V, Banfi S, Gromada J, Cohen JC, Hobbs HH. Inactivation of ANGPTL3 reduces hepatic VLDL-triglyceride secretion. *J Lipid Res* 2015; **56**: 1296–1307.

23. Musunuru K, Pirruccello JP, Do R, Peloso GM, Guiducci C, Sougnez C, et al. Exome sequencing, ANGPTL3 mutations, and familial combined hypolipidemia. *N Engl J Med* 2010; **363**: 2220–2227.
24. Viney NJ, van Capelleveen JC, Geary RS, Xia S, Tami JA, Yu RZ, et al. Antisense oligonucleotides targeting apolipoprotein(a) in people with raised lipoprotein(a): Two randomised, double-blind, placebo-controlled, dose-ranging trials. *Lancet* 2016; **388**: 2239–2253.
25. Graham MJ, Viney N, Crooke RM, Tsimikas S. Antisense inhibition of apolipoprotein (a) to lower plasma lipoprotein (a) levels in humans. *J Lipid Res* 2016; **57**: 340–351.
26. Tikka A, Jauhiainen M. The role of ANGPTL3 in controlling lipoprotein metabolism. *Endocrine* 2016; **52**: 187–193.
27. Wang CS, Hartsuck J, McConathy WJ. Structure and functional properties of lipoprotein lipase. *Biochim Biophys Acta* 1992; **1123**: 1–17.
28. Wong H, Schotz MC. The lipase gene family. *J Lipid Res* 2002; **43**: 993–999.
29. Braun JE, Severson DL. Regulation of the synthesis, processing and translocation of lipoprotein lipase. *Biochem J* 1992; **287**: 337–347.
30. Hahn PF. Abolishment of alimentary lipemia following injection of heparin. *Science* 1943; **98**: 19–20.
31. Meneghetti MCZ, Hughes AJ, Rudd TR, Nader HB, Powell AK, Yates EA, et al. Heparan sulfate and heparin interactions with proteins. *J R Soc Interface* 2015; **12**: 20150589.
32. Shimamura M, Matsuda M, Kobayashi S, Ando Y, Ono M, Koishi R, et al. Angiotensin-like protein 3, a hepatic secretory factor, activates lipolysis in adipocytes. *Biochem Biophys Res Commun* 2003; **301**: 604–609.
33. Shimamura M, Matsuda M, Yasuno H, Okazaki M, Fujimoto K, Kono K, et al. Angiotensin-like protein 3 regulates plasma HDL cholesterol through suppression of endothelial lipase. *Arterioscler Thromb Vasc Biol* 2007; **27**: 366–372.
34. Graham MJ, Lee RG, Brandt TA, Tai LJ, Fu W, Peralta R, et al. Cardiovascular and metabolic effects of ANGPTL3 antisense oligonucleotides. *N Engl J Med* 2017; **377**: 222–232.
35. Musunuru K, Kathiresan S. Surprises from genetic analyses of lipid risk factors for atherosclerosis. *Circ Res* 2016; **118**: 579–585.
36. Hirata K, Dichek HL, Cioffi JA, Choi SY, Leeper NJ, Quintana L, et al. Cloning of a unique lipase from endothelial cells extends the lipase gene family. *J Biol Chem* 1999; **274**: 14170–14175.
37. Jaye M, Lynch KJ, Krawiec J, Marchadier D, Maugeais C, Doan K, et al. A novel endothelial-derived lipase that modulates HDL metabolism. *Nat Genet* 1999; **21**: 424–428.
38. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, et al. Plasma HDL cholesterol and risk of myocardial infarction: A mendelian randomisation study. *Lancet* 2012; **380**: 572–580.
39. Ando Y, Shimizugawa T, Takeshita S, Ono M, Shimamura M, Koishi R, et al. A decreased expression of angiotensin-like 3 is protective against atherosclerosis in apoE-deficient mice. *J Lipid Res* 2003; **44**: 1216–1223.
40. Lupo MG, Ferri N. Angiotensin-like 3 (ANGPTL3) and atherosclerosis: Lipid and non-lipid related effects. *J Cardiovasc Dev Dis* 2018; **5**: 39.
41. Xu YX, Redon V, Yu H, Querbes W, Pirruccello J, Liebow A, et al. Role of angiotensin-like 3 (ANGPTL3) in regulating plasma level of low-density lipoprotein cholesterol. *Atherosclerosis* 2018; **268**: 196–206.
42. Gaudet D, Gipe DA, Pordy R, Ahmad Z, Cuchel M, Shah PK, et al. ANGPTL3 inhibition in homozygous familial hypercholesterolemia. *N Engl J Med* 2017; **377**: 296–297.
43. ANGPTL3 targeting: The power of versatile lipid-lowering [Editorial]. *Atherosclerosis* 2018; **268**: 185–187.
44. Agellon LB, Walsh A, Hayek T, Moulin P, Jiang XC, Shelanski SA, et al. Reduced high density lipoprotein cholesterol in human cholesteryl ester transfer protein transgenic mice. *J Biol Chem* 1991; **266**: 10796.
45. Shan L, Yu XC, Liu Z, Hu Y, Sturgis LT, Miranda ML, et al. The angiotensin-like proteins ANGPTL3 and ANGPTL4 inhibit lipoprotein lipase activity through distinct mechanisms. *J Biol Chem* 2009; **284**: 1419–1424.
46. Sonnenburg WK, Daiguan Yu, Lee EC, Xiong W, Gololobov G, Key B, et al. GPIHBP1 stabilizes lipoprotein lipase and prevents its inhibition by angiotensin-like 3 and angiotensin-like 4. *J Lipid Res* 2009; **50**: 2421–2429.
47. Gaudet D, Gipe D, Hovingh K, Ahmad Z, Cuchel M, Shah P, et al. Safety and efficacy of evinacumab, a monoclonal antibody to ANGPTL3, in patients with homozygous familial hypercholesterolemia: A single-arm, open-label, proof-of-concept study. *Atherosclerosis* 2017; **263**: e9.
48. Miller M. Dyslipidemia and cardiovascular risk: The importance of early prevention. *Q J Med* 2009; **102**: 657–667.
49. Fodor JG, Frohlich JJ, Genest JJJ, Ruth P, McPherson P; for the Working Group on Hypercholesterolemia and Other Dyslipidemias. Recommendations for the management and treatment of dyslipidemia. *CMAJ* 2000; **162**: 1441–1447.
50. Zhang R. The ANGPTL3-4-8 model, a molecular mechanism for triglyceride trafficking. *Open Biol* 2016; **6**: 150272.
51. Alan R. Tall AR. Increasing lipolysis and reducing atherosclerosis. *N Engl J Med* 2017; **377**: 280–283.
52. Yuan Y, Zhang L, Cao H, Yang Y, Zheng Y, Yang XJ. Reduction in lipoprotein-associated apoC-III levels following volanesorsen therapy: Phase 2 randomized trial results. *J Lipid Res* 2016; **57**: 706–713.
53. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 2017; **377**: 1119–1131.