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EDITORIAL COMMENT

ANGPTL3

A Gene, a Protein, a New Target? Aye, There's the Rub!*

Jacques Genest, MD

Angiopoietin-like protein 3 (ANGPTL3) is part of a family of secretory proteins that share homology with angiopoietins, key proteins that regulate angiogenesis. A 460-amino acid protein with an N-terminal helical domain that is predicted to form oligomeric structures, ANGPLT3 is secreted almost exclusively by the liver and binds reversibly to inhibit lipoprotein lipase (LPL) and decrease hydrolysis of triglycerides in chylomicrons and very low-density lipoproteins (VLDL) (1-3). Interestingly, a furin cleavage site at residues 222 to 224 appears necessary for this inhibitory activity (4). ANGTL3 also inactivates endothelial lipase, an enzyme found on the luminal aspect of vascular endothelial cells that contributes to the remodeling of high-density lipoproteins by hydrolyzing phospholipids into lysophospholipids (5,6).

The control of LPL activity is complex. Apolipoprotein (apo) CII and apoAV activate it; apoCIII and ANGPTL3 inhibit it. This system of checks and balances on energy (triglycerides) delivery to tissues might have evolved in mammals to preserve a precious fuel in times of fluctuating energy intake. Yet in modern humans, the complete absence of ANGPTL3 does not appear to cause ill health and even seems to reduce atherosclerosis risk. ANGPTL3 promotes the uptake of circulating VLDL triglycerides into white adipose tissue rather than skeletal muscle or brown adipose tissue in the heart (7). In the liver, inactivation of ANGPTL3 has little effect on the number of apoB-containing lipoproteins secreted; however, these apoB-containing lipoproteins are cleared more rapidly from the circulation (8).

ANGPLT3 remained an interesting protein until Musunuru et al. (9) showed the power of exome-wide DNA sequencing when loss-of-function (LOF) homozygosity (or compound heterozygosity) in *ANGPTL3* on chromosome 1p31 was identified as the cause of familial hypobetalipoproteinemia (FHBL2). Since then, several families with FHBL2 with mutations in ANGPTL3 have been identified and studied carefully (10-13). It is of interest that subjects heterozygous for an LOF in *ANGPTL3* do not appear to have a phenotype very different from noncarrier subjects (13), compared with homozygous or compound homozygous individuals. It is also of interest that *angptl3*-null mice exhibit a hypolipidemic phenotype and decreased atherosclerotic lesions (14).

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With this background, in this issue of the *Journal*, Stitziel et al. (15) examined the effect of ANGPTL3 deficiency on protection against coronary artery disease (CAD). In a series of elegant studies, they provide compelling evidence that ANGPTL3 is causally related to CAD. First, they examined 3 members of the family described by Musunuru et al. (9) with compound heterozygosity for LOF in the *ANGPTL3* gene and 3 unaffected siblings using computed tomography angiography of the coronary arteries. Second, they examined LOF mutations in a large population of patients with CAD (n = 21,980) and control subjects (n = 158,200). Third, they measured ANGPTL3 protein level in 1,493 patients with myocardial infarction and 3,232 control subjects.

They showed that subjects with LOF for the *ANGPTL3* gene have no discernible CAD compared with their siblings without such mutations based on computed tomography angiography. Using a clever assay, they reproduced each of the mutations in

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From the Research Institute of the McGill University Health Center, Montreal, Québec, Canada. Dr. Genest has reported that he has no relationships relevant to the contents of this paper to disclose.

ANGPTL3 identified in their cohort by examining the effect of adenovirus-mediated gene transfer in the *angplt3* knockout mouse obtained by CRISPR (clustered regularly interspaced short palindromic repeats) on a C57Bl6 background. They selected only those mutations that passed strict bioinformatics criteria for LOF and showed <25% activity compared with wild-type *ANGPTL3*. The allele frequency for *ANGPTL3* LOF mutations was 1 per 309 individuals, and heterozygous carriers had a significant reduction in plasma triglycerides (−17%) and low-density lipoprotein (LDL) cholesterol (−12%). Carriers had a 34% reduction in cardiovascular disease risk (95% confidence interval: 0.44 to 0.98). Finally, individuals with the lowest tertile of ANGPTL3 protein levels had a reduced risk of having a myocardial infarction.

The conclusions reached by the authors are well supported by the data provided: LOF mutations of *ANGPTL3* are associated with protection against CAD. Yet the association between LOF mutations and CAD (Figure 3 in Stitziel et al. [15]) reached significance at the 0.04 level (95% confidence interval: 0.44 to 0.98) obtained only by the meta-analysis of published data, of which none reached statistical significance individually. Importantly, these were not single-nucleotide polymorphisms but truly rare LOF mutations. Although the allele frequency in their population of *ANGPTL3* LOF mutation was 1 per 309, homozygosity (or compound heterozygosity) would be expected to be rare (approximately 0.0000026, or 1 case per 382,000 if the population was in Hardy-Weinberg equilibrium, which would make it rarer than homozygous familial hypercholesterolemia).

What then are the translational aspects of this study? It is unlikely that healthy individuals with a favorable lipid profile (albeit with low levels of high-density lipoprotein cholesterol) will require DNA analysis for diagnostic purposes. Being homozygous for LOF mutations in *ANGPTL3* is unlikely to raise more than a curious nod from the true lipidologist.

More important, is ANGPTL3 a therapeutic target? Stitziel et al. (15) make the point that a low plasma level of ANGPTL3 is associated with reduced cardiovascular risk. This association does not imply causality. Furthermore, the presence of 1 LOF allele was associated with modest changes in the average lipoprotein phenotype (Table 1 in Stitziel et al. [15]). This is important, because a small molecule that decreases ANGPTL3 activity might produce only a modest benefit compared with currently available lipid-lowering agents. To mimic the lipoprotein phenotype of homozygous (or compound heterozygous) LOF of *ANGPTL3* will require a drug (or monoclonal antibody or antisense RNA) that completely inhibits ANGPTL3 activity in plasma or its hepatic expression. A monoclonal antibody directed against ANGPTL3 improves the lipoprotein profile in mice and hypertriglyceridemic monkeys, which suggests that this approach might be clinically beneficial in specific human dyslipidemic states (16).

Then, there's the rub: we do not fully understand the physiological roles of ANGPTL3 and the mechanisms by which its inhibition lowers LDL cholesterol. The types of patients for whom such a drug would be considered are those with combined hyperlipidemia and the dyslipidemia of diabetes and metabolic syndrome. But these patients have an increased rate of production of apoB-containing particles (especially VLDL) from the liver. Increasing LPL activity by inhibiting ANGPTL3 could have the paradoxical effect of increasing the conversion of VLDL into LDL particles, an effect often seen with fibric acid derivatives. Therefore, it remains to be seen whether this finding will open therapeutic avenues for novel anti-ANGPTL3 therapies as an orphan drug and how might this extend to the more common dyslipidemias.

ADDRESS FOR CORRESPONDENCE: Dr. Jacques Genest, Faculté de Médecine, Université McGill, Institut de recherche du centre universitaire de santé McGill, 1001 Boulevard Decarie Bloc E, Office EM12212, Montréal, Québec H4A 3J1, Canada. E-mail: jacques.genest@mcgill.ca.

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