CARDIOVASCULAR ENDOCRINOLOGY

Is *ANGPTL3* the next *PCSK9*?

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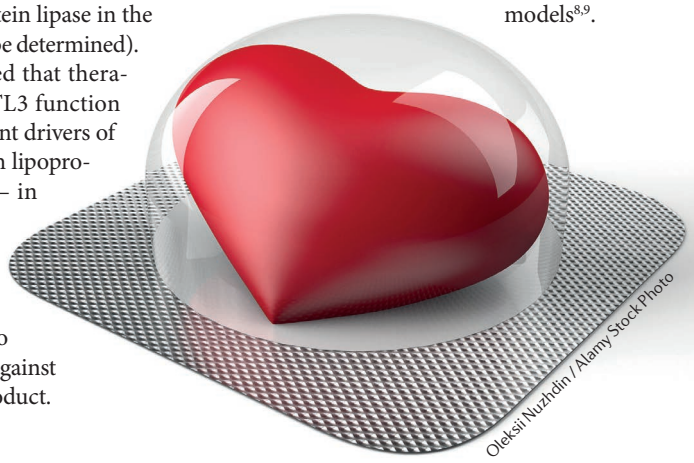
Recent reports suggest that molecular therapies targeting *ANGPTL3* and its encoded protein angiopoietin-like protein 3 have clinical potential comparable to therapies targeting *PCSK9* and its encoded protein proprotein convertase subtilisin/kexin type 9. By mainly affecting triglyceride-rich lipoproteins, ANGPTL3 inhibition might prove complementary to LDL cholesterol lowering with PCSK9 blockade.

Refers to Dewey, F. E. *et al.* Genetic and pharmacologic inactivation of ANGPTL3 and cardiovascular disease. *N. Engl. J. Med.* http://dx.doi.org/10.1056/NEJMoa1612790 (2017) | Graham, M. J. *et al.* Cardiovascular and metabolic effects of *ANGPTL3* antisense oligonucleotides. *N. Engl. J. Med.* http://dx.doi.org/10.1056/NEJMoa1701329 (2017).

The gene encoding proprotein convertase subtilisin/kexin type 9 (PCSK9) has become a celebrated example of the utility of human genetics to inform the development of novel therapeutics[1]. In just 14 years, *PCSK9* was discovered to cause familial hypercholesterolaemia when individuals harbour gain-of-function mutations; relatively common loss-of-function mutations in *PCSK9* in the general population were shown to be associated with substantially reduced blood levels of LDL cholesterol and risk of coronary heart disease (CHD); PCSK9 was determined to interact with the LDL receptor and reduce its levels at the plasma membrane, thus reducing LDL uptake from the blood; and both the gene and the protein were targeted by several novel molecular therapies, one of which has just been established to reduce the incidence of cardiovascular events in patients[2]. The success with *PCSK9* has engendered optimism that additional therapeutic gene targets will likewise emerge from human genetics.

One such gene target that now seems to be well poised for clinical translation is *ANGPTL3*, a hepatocyte-specific gene encoding a protein (angiopoietin-like protein 3) that is secreted into the bloodstream. First described in 1999, the mouse orthologue was reported in 2002 to be the cause of hypolipidaemia (reduced blood levels of triglycerides and cholesterol) upon positional cloning in a naturally occurring mutant mouse model[3]. *ANGPTL3* emerged as a candidate gene to influence triglyceride levels in humans; sequencing of the *ANGPTL3* coding region in population cohorts confirmed this hypothesis[4]. Subsequent investigation of a family with members affected by combined hypolipidaemia revealed compound heterozygous nonsense mutations in *ANGPTL3* to be the cause, thereby linking loss-of-function mutations in the gene to not only reduced blood levels of triglycerides but also LDL cholesterol and HDL cholesterol levels in humans[5]. In parallel, genome-wide association studies of blood levels of lipids identified common non-coding DNA variants near *ANGPTL3* that were linked to triglyceride and LDL cholesterol levels, later established to be due to altered expression of *ANGPTL3* in hepatocytes[6,7]. Intriguingly, *ANGPTL3* seems to regulate blood levels of triglycerides and LDL cholesterol via distinct mechanisms (the former via inhibition of lipoprotein lipase in the bloodstream; the latter yet to be determined). These observations suggested that therapeutic inhibition of ANGPTL3 function would reduce two independent drivers of CHD risk — triglyceride-rich lipoproteins and LDL cholesterol — in patients and, presumably, substantially reduce their risk of disease. This suggestion energized efforts by pharmaceutical companies to develop molecular therapies against *ANGPTL3* and its protein product.

Two reports recently published in the *New England Journal of Medicine* provide strong validation for the strategy of ANGPTL3 inhibition. In the first report, Dewey and colleagues evaluated a monoclonal antibody against ANGPTL3 (evinacumab) in healthy human volunteers who had elevated fasting levels of triglycerides (150–450 mg/dl) or LDL cholesterol (≥100 mg/dl) in a phase I randomized, placebo-controlled clinical trial[8]. The investigators observed a dose-dependent effect of evinacumab, with the participants who received the highest dose (20 mg/kg) intravenously experiencing a median reduction in triglyceride levels of 76% at day 4, a mean reduction in levels of LDL cholesterol of 23% at day 15, and a mean reduction in levels of HDL cholesterol of 18% at day 15 (compared with participants who received placebo). Substantial lipid-lowering effects were also observed when evinacumab was administered subcutaneously. In the second report, Graham and colleagues evaluated an antisense oligonucleotide against *ANGPTL3* messenger RNA in healthy human volunteers (fasting levels of triglycerides >90 mg/dl, LDL cholesterol levels >70 mg/dl) in a phase I randomized, placebo-controlled clinical trial[9]. Participants who received weekly subcutaneous injections of the highest dose of the therapy (60 mg/week) for 6 weeks experienced a mean reduction in triglyceride levels of 50% at day 43, a mean reduction in levels of LDL cholesterol of 33% at day 43, and a mean reduction in levels of HDL cholesterol of 27% at day 43 (compared with pretreatment baseline levels). In both studies, a reduction in lipid levels and decreased progression of atherosclerosis were observed upon repeated administration of the therapies in mouse models[8,9].



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With both therapies, study participants displayed the expected decreases in blood levels of lipids, triglycerides, LDL cholesterol and, to a lesser extent, HDL cholesterol. One crucial question is whether these therapies will also affect the risk of CHD in humans. In a recent report in the *Journal of the American College of Cardiology*, three siblings with familial combined hypolipidaemia resulting from two *ANGPTL3* nonsense mutations were observed to have no discernible coronary atherosclerosis, despite one of the individuals having substantial risk factors for disease, whereas two siblings without the mutations in the same family had a substantial burden of coronary atherosclerosis[10]. In the same report, tens of thousands of individuals were screened for loss-of-function mutations in *ANGPTL3*, and mutation carriers were determined to have a 34% reduction in CHD risk. Furthermore, in a population cohort in whom blood levels of ANGPTL3 were measured, individuals in the lowest tertile of ANGPTL3 levels had a 35% reduced risk of myocardial infarction compared with individuals in the highest tertile[10]. In their report describing the use of evinacumab, Dewey and colleagues also screened tens of thousands of individuals for loss-of-function mutations in *ANGPTL3* and found that carriers had a 39% reduction in CHD risk[8]. To date, no evidence exists of any adverse health consequences of carrying *ANGPTL3* loss-of-function mutations; indeed, the existing data suggest that such individuals might be mildly protected against diabetes mellitus. These observations strongly suggest that molecular therapies targeting ANGPTL3 — whether monoclonal antibody, antisense oligonucleotide, or other types of therapy — will ultimately prove to be safe and effective in reducing the incidence of cardiovascular events, just as is proving to be the case with *PCSK9* (REF. 2).

> “Reducing levels of triglyceride-rich lipoproteins represents a new way to reduce CHD risk…”

What is particularly enticing about ANGPTL3-targeting therapy is that it modulates blood levels of lipids by mechanisms that are distinct from, and possibly synergistic with, the mechanisms through which statins (which inhibit 3-hydroxy-3-methylglutaryl-coenzyme A reductase), ezetimibe (which inhibits Niemann-Pick C1-like protein 1) and PCSK9-targeting therapies act. Reducing levels of triglyceride-rich lipoproteins represents a new way to reduce CHD risk, one which ANGPTL3-targeting therapies are poised to exploit. Furthermore, combinations of therapies that target multiple pathogenetic mechanisms of CHD have the potential to sharply curtail the incidence of disease in patients at the highest risk and, perhaps, eventually the population at large.

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Competing interests statement

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