

APOLIPOPROTEIN C-III INHIBITORS: TRANSLATING LOCAL ALLELES TO WORLDWIDE THERAPIES

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BACKGROUND

In 2007, researchers at the Amish Research Clinic in Strasburg, PA, reported a curious metabolic phenomenon in the Lancaster County Old Order Amish community.¹ After participants in a study drank milkshakes that contained large amounts of fat and sugar, approximately 5% of all participants did not experience the expected steep rise in serum triglyceride concentration. Furthermore, this subgroup had baseline triglyceride levels that were approximately 40-50% below those of their peers, as well as elevated concentrations of high-density lipoprotein (HDL). The investigators noted that this metabolic profile appeared to be inherited, and they turned to genetic assays to explain the underlying biochemical mechanism.

The researchers found that these individuals carried a mutation in *APOC3*, the genetic locus that encodes apolipoprotein C-III. It had first been isolated and identified in the 1960s, and at the time of the Amish Research Clinic study apolipoprotein C-III was known to be an inhibitor of lipoprotein lipase (LPL).² The null mutation* identified in the research participants, known as p.R19G or R19X, led to an approximate 50% reduction in circulating apolipoprotein C-III, thus enhancing the activity of LPL and accelerating the clearance of triglyceride-rich lipoproteins (LPL) from the bloodstream. The acceleration of triglyceride clearance due to upregulation of LPL leads to a favorable increase in cardio-protective reverse cholesterol transport via HDL particles.³

After the discovery of the R19X allele in the Amish, and the apparent cardiovascular benefits it confers on carriers, population-based genetic sequencing programs determined its estimated frequency to

be less than 0.1% across various demographic groups in the general population. In addition to its low frequency, the mutation was found to have a diffuse geographical distribution with no discernible focal point.⁴ Interestingly, the only “founder” population for the R19X mutation discovered thus far outside Lancaster County is in the small town of Anogia on the Greek island of Crete, where the residents carrying R19X bear a high degree of phenotypic resemblance to the Amish carriers.⁵

Other loss-of-function mutations in *APOC3* with similar phenotypic effects have been identified in approximately 0.50-0.67% of the general population worldwide.^{6,7} These variants protect against the development of atherosclerotic cardiovascular disease, reduce the production of very low-density lipoprotein (VLDL), an LDL precursor, and decrease the progression of diabetes in animal models.^{2,4,7}

THERAPEUTIC IMPLICATIONS

The pharmaceutical industry has taken note of these findings, and is investing heavily in pharmacotherapies that mimic the effects of *APOC3* R19X and similar mutations. Their research is examining not only the reduction in triglycerides but the potential effect on insulin resistance and diabetes mellitus. As a result, inhibitors of apolipoprotein C-III may become the first class of drugs designed to treat the “the metabolic syndrome,” a complex that combines dyslipidemia, obesity, and insulin resistance, and affects nearly one quarter of the U.S. adult population.^{8,9}

The first apolipoprotein C-III inhibitor to reach Phase III trials is volanesorsen, an antisense oligonucleotide** inhibitor of *APOC3* manufactured by Ionis

*Null mutation: A mutant copy of a gene that lacks that gene's normal function.

**Oligonucleotides are short fragments of nucleic acids, typically 15-25 bases long. Antisense agents are fragments that are developed to be complementary to specific portions of “sense,” meaning messenger RNA (mRNA). When the antisense oligonucleotide hybridizes with its cognate mRNA target and forms a hybrid, the unnatural structure leads to the destruction of mRNA by RNase H, a cellular ribonuclease that degrades hybrid nucleic acids. The result, as described above in the text, is inhibition of the transfer of genetic information from DNA to the protein encoded by the target mRNA.

Pharmaceuticals.^{10,11} Unlike monoclonal antibody inhibitors of proprotein convertase subtilisin/kexin type 9 (PCSK9), which was approved in 2015 for treatment of hypercholesterolemia,¹² antisense inhibitors such as volanesorsen target not the protein product responsible for an adverse phenotype such as hypertriglyceridemia, but instead the messenger RNA (mRNA) responsible for translation of the protein in question.

Antisense inhibitors as a therapeutic class have developed from discoveries related to microRNA, an endogenous system of post-transcriptional regulation. Thus, volanesorsen demonstrates the value of “translational medicine,” not only in its targeting of the *APOC3* locus that was discovered through human population genetics, but in its mechanism of action as well.¹³ Volanesorsen has recently been reported to decrease plasma apolipoprotein C-III concentrations by up to 80% in Phase II trials, and Phase III trials

targeting multiple disorders of lipid metabolism are ongoing.^{10,14-16}

THE FUTURE

The R19X mutation discovered in Lancaster County nearly a decade ago launched a journey of discovery in many countries and continents that probed the very content of the genetic fabric of human life. Health care is progressing towards individualized medicine while it simultaneously advances the delivery of population-based care. Meanwhile, discoveries in the field of human genetics, including many made in Lancaster County, will contribute to the identification of both new targets for pharmacotherapy and new mechanisms for its delivery. By promoting genomic and translational medicine, Lancaster General Health is continuing its tradition of leadership.

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