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NEWS AND COMMENTARY

APOC3 null mutation affects lipoprotein profile

APOC3 deficiency: from mice to man

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During the 1980s and early 1990s, the great majority of genes involved in lipoprotein metabolism were cloned, including the genes encoding the apolipoproteins. These proteins form the protein moiety of the lipoproteins and are essential for lipid metabolism. Subsequently, genetic variants that were associated with human dyslipidemias were identified. In parallel, the function of these genes has been thoroughly studied in knockout and transgenic mouse models. Generally, these rodent models recapitulated the human findings, and proved to be highly useful in studying the etiology of cardiovascular disease. In particular, mice with deficiencies of low-density lipoprotein receptor (*LDLR*) and apolipoprotein E replicating familial hypercholesterolemia and type III hyperlipidemias are commonly used as the background for studying the pathogenesis of atherosclerosis.

In several instances, mouse models were generated to understand gene function in the absence of the human-equivalent mutation. This is the case for apolipoprotein C3 (*APOC3*). *APOC3* has been established as an inhibitor for lipoprotein lipase, a gene that hydrolyzes triglycerides to generate free fatty acids before their uptake by muscle and adipose tissue (reviewed in Jong *et al*[1]). Mice with a high-level expression of human *APOC3* on a background of *LDLR* deficiency proved to be an excellent model for familial combined hyperlipidemia,[2] because they are disturbed in the breakdown of triglycerides. In contrast, mice lacking *Apoc3* show increased activity of LPL, which causes hypotriglyceridemia and protection from postprandial hypertriglyceridemia.[3] From these mice studies, it became clear that a deficiency of *APOC3* could cause a healthier lipoprotein profile, which is associated with protection from cardiovascular diseases. However, in the absence of *APOC3*-deficient subjects, this hypothesis was difficult to test directly.

To make the situation more complex, *APOC3* is located in a gene cluster – together with genes encoding the apolipoproteins A1, A4 and A5. Genetic evidence showed that sequence variation in the promoters and regulatory elements of *APOC3* and *APOA5* independently affects triglyceride levels.[4] However, there is strong functional interaction between the genes in this cluster. In the absence of structural mutations in single genes, it has proven to be complicated to genetically dissect the independent functions of these genes as has been done in rodent models.

A recent paper in *Science*[5] has now identified a null allele of *APOC3* and thereby substantially strengthened the evidence that heterozygous deficiency of the *APOC3* gene in humans leads to reduced levels of TG and is associated with reduced arterial plaque formation.

One of the reasons why the detection of *APOC3* deficiency in man took so long is that it is associated with a healthier lipoprotein profile, and such individuals are usually not screened for mutations in lipoprotein genes. The study by Pollin *et al* (2008)[5] included healthy participants who were studied for their postprandial response. This response was tested in 809 Old Order Amish individuals by giving the volunteers a high-fat drink and measuring the subsequent triglyceride levels after 1–6 h. Subsequently, all these individuals were genetically investigated using a genome-wide association screen with the 500 K array set of Affymetrix (Santa Clara, CA, USA). One SNP with a relatively low allele frequency (0.028) was strongly associated with low fasting triglyceride levels and low postprandial triglyceride levels. This SNP (rs10892151) was located at a distance of 800 kb from the *APOC3* gene cluster. A second SNP (rs681524), located at a distance of 40 kb from the cluster, with an allele frequency of 0.064, showed a much weaker association and was moderately correlated with rs10892151. On the basis of the relative proximity of the SNPs and the *APOC3* gene cluster, and given the phenotype that predicted a function for *APOC3*, this gene was sequenced, and a C → T substitution was found in exon 2, which predicted a premature stop codon at position 19 of the amino-acid sequence.

It is remarkable that a marker lying 800 kb away from the *APOC3* gene cluster was identified as the most strongly linked SNP and that a marker at a closer location was less strongly associated. It is likely that this fact can be explained because the study was carried out in an isolated population. The genetic structure of this population has been extremely well documented, and permitted the conclusion that the gene mutation was introduced in the early 1800s. Hence, the GWAS study should be regarded as an 'Identity by Decent (IBD)' approach, rendering a very large region around the markers as the candidate region for the mutation screen.

So what is the evidence that the *APOC3* gene mutation is the genuine defect in this study and not any other gene mediating the postprandial response in this subject? The method used implies that genes in a large region around the s10892151 marker are candidate genes; in addition, it cannot exclude mutations in non-coding sequences. In this case, all the evidence is strongly in favor of a function for *APOC3* to harbor the culprit mutation. Other genes with an established function in lipoprotein metabolism include the other genes in the *APOC3* gene cluster, and *ABCG4*. Furthermore, other genes of interest include *PCKS7* and interleukin 10 receptor α, but these genes currently have no documented function in postprandial lipoprotein metabolism.

Therefore, the *APOC3* null mutation found in the Old Order Amish population, residing close to a strongly associated SNP, in combination with a wealth of information from rodent studies and also human genetic studies provides a strong basis for the main conclusion of this paper that the *APOC3* gene is the causal gene. Nevertheless, to formally prove this finding, it will be necessary to

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obtain sequence information for the entire genetic interval. In the absence of such an excellent functional candidate gene, the study presented here would have required a more than substantial resequencing effort, as the genetic intervals to be sequenced in isolated populations tend to be large. Nonetheless, isolated populations are frequently enriched for interesting mutations, and could also facilitate follow-up studies to further understand the genotype–phenotype relationship ■

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