npg

NEWS AND COMMENTARY

APOC3 null mutation affects lipoprotein profile

• •

APOC3 deficiency: from mice to man

Marten H Hofker

• •

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

Professor Dr MH Hofker is at the Department of Pathology and Medical Biology, Medical Biology Section, Molecular Genetics, University Medical Center Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands.
Tel: + 31-50-3637932/5777; Fax: + 31-50-3638971;
E-mail: m.h.hofker@med.umcg.nl

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 ∎

1 Jong MC, Hofker MH, Havekes LM: Role of ApoCs in lipoprotein metabolism: functional differences between ApoC1, ApoC2 and ApoC3. *Arterioscler Thromb Vasc Biol* 1999; **19**: 472–484.

2 Masucci-Magoulas L, Goldberg IJ, Bisgaier CL *et al*: A mouse model with features of familial combined hyperlipidemia. *Science* 1997; **275**: 391–394.

3 Maeda N, Li H, Lee D, Oliver P, Quarfordt SH, Osada J: Targeted disruption of the apolipoprotein C-III gene in mice results in hypotriglyceridemia and protection from postprandial hypertriglyceridemia. *J Biol Chem* 1994; **269**: 23610–23616.

4 Talmud PJ, Hawe E, Martin S *et al*: Relative contribution of variation within the APOC3/A4/A5 gene cluster in determining plasma triglycerides. *Hum Mol Genet* 2002; **11**: 3039–3046.

5 Pollin TI, Damcott CM, Shen H *et al*: A null mutation in human APOC3 confers a favorable plasma lipid profile and apparent cardio protection. *Science* 2008; **322**: 1702–1705.