Invited commentary

Apolipoprotein E mutations, type V hyperlipoproteinaemia and diet

The measurement of plasma lipid and lipoprotein levels is the basis of dyslipoproteinaemia classification. However, because the concentration of plasma lipoproteins is continuous and widely distributed in the population, this classification is somehow arbitrary. The identification of defined syndromes based on molecular defects is useful to understand the metabolic basis of lipid disorders and to develop appropriate therapeutic strategies.

Type V hyperlipoproteinaemia is probably the most mysterious dyslipoproteinaemia. It is defined by increased levels of both chylomicrons and VLDL in the post-absorptive state. Chylomicrons contribute little to plasma triacylglycerol levels during the post-absorptive state with the notable exception of an impairment of triacylglycerol hydrolytic capacity. For instance lipoprotein lipase or apolipoprotein C-II deficiency, which characterizes type I hyperlipoproteinaemia, result in the massive accumulation of plasma chylomicrons in affected subjects. In contrast to patients with type III hyperlipoproteinaemia, a metabolic disorder characterized by defective clearance of triacylglycerol-rich lipoprotein remnants, patients with type V have no evidence of chylomicron- and VLDL-remnant accumulation suggesting different metabolic alterations in the two metabolic syndromes. The molecular basis of this type V hyperlipoproteinaemia has not been clearly established.

Earlier studies have pointed out the possible implication of apolipoprotein E (apoE) in type V hyperlipidaemia (Ghiselli *et al*. 1982, 1992; Kuusi *et al*. 1988). ApoE is a normal constituent of VLDL and HDL. The primary function of apoE is to serve as a ligand for recognition of lipoproteins by cellular receptors. In addition, apoE interacts with various proteoglycans and is implicated in the anchoring process of lipoproteins to endothelial lipases. ApoE is a polymorphic protein coded by three common alleles on chromosome 19. The three common alleles of apoE code for three isoforms: E2, E3, E4. ApoE2 and apoE4 differ from apoE3 by a single cysteine and arginine interchange at amino acids 158 and 112. As the consequence of the cysteine–arginine interchange in position 158, located close to the binding site to the cellular receptors, apoE2 interacts poorly with the cells. The cysteine–arginine substitution at amino acid 112 has no impact on binding properties but confers to apoE4 an increased affinity for the triacylglycerol-rich lipoproteins.

The link between apoE and type V dyslipidaemia was established in case–control studies. These investigations reported higher frequencies of apoE4 isoform in patients with type V hyperlipidaemia than in the healthy normolipidaemic subjects. The fundamental problem behind this observation was to unravel the mechanism of the association between the a very common genetic polymorphism of apoE and a rare lipid disorder, type V hyperlipoproteinaemia. Although this intriguing problem was not solved, the findings were at least supported by data showing an association between the E4 isoform and triacylglycerol levels in large population studies, suggesting a broader role of apoE4 in triacylglycerol metabolism (Dallongeville *et al*. 1992). From a mechanistic point of view, the results of *in vitro* experiments showing that apoE4 isoform has a greater affinity for triacylglycerol-rich lipoprotein were of great interest. The hypothesis was that apoE4 at the surface of triacylglycerol-rich lipoprotein could interfere with the normal lipoprotein lipase mediated-process of triacylglycerol-rich lipoproteins resulting in a slow hydrolysis of chylomicron and VLDL in type V patients. The work reported in this issue of the *British Journal of Nutrition* by Vialettes and co-workers (Vialettes *et al*. 2000) extend these hypotheses to a rare apoE mutant.

ApoE Christchurch (136 Arg → Ser) was initially discovered in a patient with type III dyslipidaemia (Wardell *et al*. 1987). This subject was heterozygous for this mutation and for the more common apoE2 (158 Arg → Cys) variant. His VLDL contained approximately five times more apoE (136 Arg → Ser) than apoE2 (158 Arg → Cys), suggesting that apoE (136 Arg → Ser) had a greater affinity for triacylglycerol-rich lipoprotein than apoE2. This new apoE mutant appeared to contribute significantly to the patient's hyperlipidaemia. Later, five other subjects (one homozygous and four heterozygous) were found to bear this mutation in a sample of Spanish origin (Pocovi *et al*. 1996). Family studies were carried out and the rare apoE (136 Arg → Ser) variant was found to contribute significantly to cholesterol levels variability. This effect was attributed to the occurrence of cholesterol-enriched triacylglycerol-rich lipoproteins in the affected subjects suggestive of a defect in remnant clearance. Interestingly, the apoE (136 Arg → Ser) variant was associated with an incomplete dominance of type III hyperlipoproteinaemia. Altogether, these clinical observations were consistent with *in vitro* site directed mutagenesis experiments of apoE. These studies have demonstrated that key basic amino acids in the vicinity of residues 140–160 of apoE are important in mediating binding to the receptor and that mutation of the amino acid residues at position 136 resulted in defective binding to cellular receptor.

The patients described by Vialettes and co-workers (Vialettes *et al*. 2000) have in common with previously reported patients that the apoE (136 Arg → Ser) variant was associated with the common apoE2 (158 Arg → Cys) isoform. However, in contrast to the other patients in which the apoE

(136 Arg → Ser) mutant was usually associated with clinical or sub-clinical evidence of type III hyperlipoproteinaemia, a lipid disorder characterized by defective clearance of VLDL and chylomicron remnants, Vialettes *et al*. (2000) reported a clinical association between apoE (136 Arg → Ser) and type V hyperlipoproteinaemia, a lipid disorder that suggests defective lipolysis.

The results of the fat-load test showed a progressive increase in triacylglycerol levels after the fat meal in both affected subjects. Triacylglycerol rose progressively from the second hour to 6 or 7 h postprandially. This delayed triacylglycerol response pattern is more suggestive of a defect in triacylglycerol-rich lipoproteins clearance than of a defect in triacylglycerol-rich lipoprotein lipolysis. In the case of the latter, triacylglycerol levels tend to increase abruptly after fat intake. Then why do these patients express type V hyperlipoproteinaemia? Here is where all the complexity and interest of lipoprotein metabolism appears. Given the role of apoE in promoting triacylglycerol-rich lipoprotein anchoring to endothelial proteoglycans and in facilitating the interaction with lipoprotein lipase, it would have been important to know whether the 136 Arg → Ser mutation affects the ability of apoE to bind triacylglycerol-rich lipoproteins or whether it affects apoE interaction with proteoglycans. On a clinical point of view, it is well known that most apoE2 homozygotes are normolipidaemic despite the defective interaction between apoE2 and cellular receptors, pointing out a gap between *in vitro* experiments and clinical data. In fact, apoE2 alone is not sufficient to cause overt type III hyperlipoproteinaemia, suggesting that other genes or environmental factors are essential to convert the normolipidaemia to hyperlipidaemia. By extension, the clinical expression of the apoE (136 Arg → Ser) variant as type V hyperlipoproteinaemia suggests that some dietary factors such as alcohol or sugar consumption might favour type V expression rather than type III in French patients. In this respect, the improvement of hypertriacylglycerolaemia with dietary fat restriction support the contribution of diet to the phenotypic expression of type V hyperlipoproteinaemia.

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