

Commentary

An unsolved mystery of promoter variation in CETP gene and atherosclerosis

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Plasma cholesteryl ester transfer protein (CETP) mediates CE and triacylglycerol (TG) transfer or exchange among lipoproteins and is a major regulator of high density lipoprotein (HDL) cholesterol levels as well as non-HDL cholesterol in humans. By inhibiting CETP, diet-induced atherosclerosis was attenuated in cholesterol-fed rabbits, suggesting a novel therapeutic approach toward coronary artery disease with low HDL levels in humans [1–3]. However, there are considerable concerns regarding the efficacy of chemical CETP inhibitor in humans, as suggested in recent genetic population studies.

Extensive genotyping has been performed in the CETP gene, revealing its complex role in atherogenicity [4]. Several nonsynonymous coding polymorphisms, such as D442G and I405V, were reported to have direct or indirect effects on CETP and HDL levels. Population studies suggested that the genotypes associated with lower CETP levels are pro-atherogenic in men and women [5,6].

However, promoter variation or its linked polymorphism may have different consequences. TaqIB polymorphism, first analysed a decade ago, provides a mystery to be solved. This site is located at the +277 nucleotide of intron 1. Despite no apparent functional sequence in the TaqIB site, the TaqIB2 allele (no restriction sequence) was associated with lower CETP and/or higher HDL. This TaqIB2 genotype has been consistently anti-atherogenic in several different population studies [7–9]. Population admixture is unlikely to explain this association. Thus, the controversial association among low CETP, high HDL and antiatherogenicity of TaqIB2 is opposite to the results in I405V and D442G studies. Functionally, the TaqIB2 genotype may play an anti-atherogenic role in humans, probably mediated by a modified very low density lipoprotein (VLDL) secretion rate, increased low density

lipoprotein (LDL) size [9], and direct effects of higher HDL and lower LDL. Another explanation would be linkage disequilibrium between undefined causative promoter variation and TaqIB.

Indirect effects via linkage disequilibrium with near regulatory nucleotide variations or those in a nearby gene may exist with the TaqIB2 allele. Such examples, –629C/A and [gaaa] repeat, were disclosed recently [10,11]. This promoter variation of the –629A allele, located at the Sp1/Sp3 recognition site, had 25% decreased promoter activity, 23% lower CETP, and 12% higher HDL levels [10]. In this issue of the *European Journal of Clinical Investigation*, Kakko *et al.* examined the association between –629C/A and several polymorphisms in the CETP gene [12]. They found that the –629 A/A genotype increased HDL levels by 16% only in women, independent of plasma CETP activity. The –629A-TaqIB2 allele is strongly linked with 373 A in exon12, and 451R in exon15. Also, they suggested three category groups of –629C/A-TaqIB, A373P-R451Q and I405V as independent and interactive variables of CETP, HDL, and carotid atherosclerosis. Interestingly, 7–10% of the variation in carotid atherosclerosis could be explained by CETP gene polymorphisms. Similarly, Corbex *et al.* [13] examined CETP gene polymorphisms extensively, and showed associations of 373A with high HDL, and 451R with low HDL, revealed by four stratified genotypes of 373 and 451, although such an effect was not detected by analysis of each single genotyping. By studying two common polymorphisms of A373P and R451Q, Agerholm-Larsen *et al.* [14] demonstrated that the 373P-451Q allele is associated with lower HDL levels, but paradoxically, is also associated with lower coronary risk in women not receiving hormone replacement therapy. However, an opposite trend was found in men and women administered with hormone replacement therapy. Interestingly, the 373P-451Q allele is completely linked with –629C-TaqIB1 (known as pro-atherogenic in previous studies), in the study by Kakko *et al.* [12]. In addition, the association with carotid atherosclerosis in 373P was opposite between the sexes: pro-atherogenic in women, but antiatherogenic in men [12].

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Thus, results analysed by single polymorphism genotyping may be confounded by other polymorphism(s) in linkage disequilibrium. It is likely that marginal effects on phenotype could not be detected by univariate analysis of each of two sites because of linkage disequilibrium between them, when they have opposite effects on phenotype. Similarly, phenotypic effect would be exaggerated by the analysis of single polymorphism genotyping when another polymorphism in linkage disequilibrium has a similar effect.

In future, we should learn more regarding the promoter variation of CETP gene in longer lengths. Some population studies have shown that TaqIB2 and -629A alleles are associated with increased HDL levels, independent of plasma CETP activity [4,7,12], although the underlying mechanism has not been clarified. This phenomenon could be explained by environmental effects such as alcohol intake, or confounding effects by linkage disequilibrium between one polymorphism with low CETP and another with high CETP. Thus, whether nucleotide variation(s) of CETP promoter or its linked polymorphism(s) associated with lower CETP levels is really antiatherogenic or an incidental finding should be investigated.

This complexity noted in CETP gene association studies suggests that haplotyping or analysis of selected polymorphisms evaluated by linkage disequilibrium parameter is needed for genotype-phenotype association before mass screening. Also, careful interpretation is required in genetic epidemiological surveys when determining any disease associations.

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