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Short communication

CETP deficiency due to a novel mutation in the CETP gene promoter and its effect on cholesterol efflux and selective uptake into hepatocytes

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ABSTRACT

Objectives: To identify the genetic variant in the CETP gene of the proband with high HDL-C and low CETP activity and to investigate whether HDL from the CETP-deficient subject was dysfunctional in the reverse cholesterol transport (RCT) pathway.
Methods: We sequenced the CETP gene and assessed its promoter activity. Cholesterol efflux and hepatic cholesteryl ester delivery studies were also performed using the proband's HDL.
Results: A proband was a compound heterozygote for a known D459G variant and a novel 18-bp deletion mutation in the CETP promoter. This promoter mutation markedly reduced the transcriptional activity in HepG2 cells. HDL2 from this subject increased SR-BI-mediated cholesterol efflux, whereas cholesteryl ester delivery into hepatocytes was maintained.
Conclusion: A novel deletion mutation in the CETP promoter is associated with high HDL-C and decreased promoter activity. HDL from this CETP-deficient subject was not dysfunctional in mediating two main steps of RCT assessed in vitro.

1. Introduction

Reverse cholesterol transport (RCT) is one of the major mechanisms by which HDL protects against atherosclerosis and involves a number of receptors, enzymes, and transfer proteins. Cholesteryl ester transfer protein (CETP) is one of the key players in the RCT pathway in humans. Genetic mutations in the gene encoding CETP are commonly found in subjects with very high levels of HDL-C or hyperalphalipoproteinemia (HALP) [1], especially in Japan, but information on the cause of HALP outside Japan is relatively scarce.

Whether CETP deficiency is associated with protection against atherosclerosis is still unclear. Earlier reports have shown that HDL-mediated cholesterol efflux was impaired in subjects with CETP deficiency [2,3]. Recent studies, however, have demonstrated that HDL from subjects with homozygous CETP deficiency enhances cholesterol efflux, but the underlying mechanism is conflicting [4,5]. Whether HDL from CETP deficiency is dysfunctional in later steps of RCT pathway is also unknown.

Our previous study in Thai subjects with HALP has discovered both known and novel mutations in the CETP gene [6], although not all subjects with lower plasma CETP activity were found to have mutations in the coding region. In this study, we examined the genetic variations in the entire CETP gene including its promoter in a proband who had HALP and low CETP activity and determined the function of the identified genetic variation in vitro. Furthermore, we performed additional experiments to test whether HDL from this subject with CETP deficiency was functional in mediating two main steps of RCT, cholesterol efflux from cells and selective uptake of cholesteryl ester (CE) into hepatocytes.

2. Materials and methods

Methods are described in online supplementary material. Data on clinical characteristics and the composition and concentrations of HDL of the proband and her family are shown in Supplemental Fig. 1A and B, Table 1.

3. Results and discussion

3.1. Genetic analysis of the CETP gene in the proband

The A21.1 proband had high HDL-C levels and low plasma CETP activity (6.8 pmol/μL/h vs. 20.1 ± 1.7 pmol/μL/h from 5 unrelated

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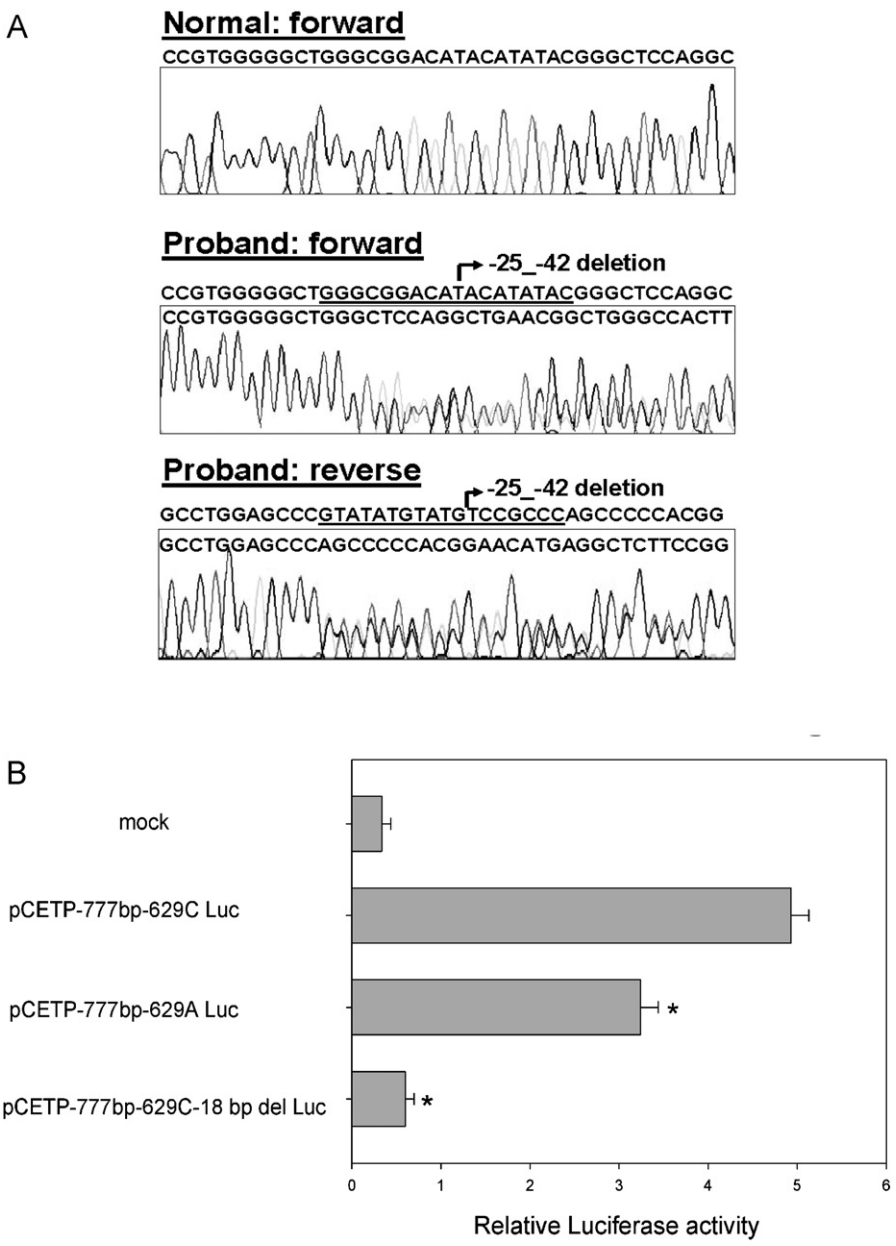

Fig. 1. (A) Genomic DNA sequence of the promoter of the CETP gene in the A21.1 proband showing a novel 18 bp deletion mutation, −25 to −42del GGGCGGACATACATATAC. (B) Transcriptional activity in HepG2 cells transfected with CETP promoter/reporter gene constructs. HepG2 cells were transiently transfected with wild-type or mutant constructs (−629C, −629A, and 18 bp deletion). Luciferase activity was measured in the cell lysates and was normalized to pRL-TK luciferase. Each value represents the mean ± SEM of three separate transfections, each performed in duplicate. *$P < 0.001$ vs. −629C.

healthy control subjects, Supplemental Fig. 1C). Sequencing the coding region of the CETP gene of the proband revealed that she was heterozygous for the c.1376A>G variant (rs2303790, p.Asp459Gly or D459G, also previously known as D442G) in exon 15 of the CETP gene. Because subjects with a heterozygous D459G variant have only mildly decreased CETP activity [7], a marked reduction in her CETP activity suggested that other sequence variation might be present. Further examination of the promoter region of the CETP gene revealed a novel heterozygous 18 bp deletion mutation from position −25 to −42 (relative to the transcription start site), −25 to −42del GGGCGGACATACATATAC (Fig. 1A, GenBank accession number HM191724). This novel mutation was not found in 115 unrelated normolipidemic subjects. The mutation was also not present in her son or her daughter (Supplemental Fig. 1A) but a heterozygous D459G variant of the CETP gene was found in both of her children. Therefore, the proband is a compound heterozygote with two different variants, an 18 bp deletion in the promoter and a D459G variant in the coding region, of the CETP gene on two different alleles.

3.2. Molecular pathology of the novel deletion mutation in the CETP gene promoter

Binding sites for both the Sp1 transcription factor and the TATA box have been reported in the −26 to −43 bp segment of the CETP gene promoter [8] and Sp1 has been shown to act as an activator of CETP gene expression at position −37 [9]. Therefore, the novel deletion mutation in this region found in our proband is predicted to disrupt these sites (Supplemental Fig. 2). We next performed transfection experiments to evaluate the transcriptional activity in HepG2 cells. The 777-bp DNA fragment of the promoter region of the CETP gene was cloned into the luciferase reporter vector and