Cholesteryl ester transfer protein genotypes associated with venous thrombosis and dyslipoproteinemia in males

Short Title: CETP genotype associated with venous thrombosis

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Although numerous genetic and acquired factors are appreciated as risk factors for venous thromboembolism (VTE) (1,2), only recently have male gender (3,4), dyslipoproteinemia (5), and silent atherosclerotic vascular disease (6) been linked to VTE. We recently found that HDL deficiency is a key feature of a pattern of dyslipoproteinemia that is associated with VTE in males and found that the common *Taq*I B1 variation in the cholesteryl ester transfer protein (CETP) gene is significantly linked to VTE (5). However, the TaqI B1/B2 SNP itself is unlikely to affect directly CETP activity, but it is linked to non-synonymous CETP SNPs Ala373Pro and Arg451Gln (7-9). Here we demonstrate that these two CETP variations are associated with VTE and low plasma HDL levels in males.

We analyzed male patients less than 55 years old with objectively documented VTE (n=49) who were matched for age (± 2 years) and ethnic group (92% non-Hispanic white) in the previously described Scripps Venous Thrombosis Registry (5). VTE was classified as unprovoked for 82% (40/49) of cases. The protocol was approved by the Institutional Review Board of Scripps Clinic and subjects provided written informed consent.

Serum lipids and lipoprotein particle subclass concentrations were determined by proton NMR spectroscopy as described (5). Four SNPs in the CETP gene (TaqI B1/B2, Ala373Pro, Ile405Val, and Arg451Gln) were assayed by PCR and restriction digestion (5,7). For the comparison of the allele distribution between VTE patients and controls, chi-squared analysis was used. ANOVA was used to test the relationship of genotype and HDL associated parameters (Prizm™ 4.0 software, GraphPad Software, San Diego, CA).

Heterozygosity for Pro373 was identified in 20% of VTE cases (10 of 49) and 2% of controls (1 of 49) (p=0.004) while heterozygosity for Gln451 was identified in 16% of VTE cases (8 of 49) and 0% of controls (p=0.003). No homozygotes for Pro373 or Gln451 were found. All subjects

carrying Gln451 presented with Pro373. After excluding those with factor V Leiden and prothrombin 20210A in patient and control groups, statistical significance was retained for genotype difference between patients and controls for both Pro373 (7/31 vs 1/45) and Gln451 (8/31 vs 0/45) (p=0.01 and 0.001, respectively). The CETP TaqI B2 and the Val405 alleles were less common in VTE cases than controls (0.33 vs 0.47, 0.20 vs 0.31, respectively) (5). Allele frequency values for the four studied CETP variations for our Scripps controls resembled published population values (7-9). In contrast, all allele frequency values for our Scripps VTE patients exceeded values for our Scripps controls.

A previous study inferred the existence of at least fourteen different CETP haplotypes, each with an estimated prevalence of > 0.5% (8). Only one of those 14 haplotypes carries Gln451, while 2 of 14 haplotypes carry Pro373. The more prevalent of these two haplotypes carries TaqI B1, Pro373, Ile405, and Gln451, and these four CETP SNPs determined in our study were found to be in excess in VTE patients compared to controls. We speculate that the 16% of our VTE patients who present with both Pro373 and Gln451 carry this reported CETP haplotype carrying Gln451 (8). Due to the limited number of subjects, we couldn't define haplotypes or quantitate the linkage disequilibrium between Pro373 and Gln451; we can simply note that all subjects carrying Gln451 also had Pro373, TaqI B1 and Ile405.

HDL parameters for the 8 VTE patients carrying Pro373/Gln451-CETP were compared to those for both controls and other VTE patients who presented with the normal Arg451-CETP (Figure 1). Plasma levels of HDL cholesterol and HDL particles were significantly lower in patients carrying Pro373/Gln451 compared with either controls or other VTE patients (p=0.01 and 0.0009, respectively). In addition, plasma ApoAI levels were lower (p=0.047) in the 8 Pro373/Gln451 patients compared with normal Arg451 genotype groups (data not shown). The VTE subjects carrying both Pro373 and Gln451 did not differ significantly from other subjects in

the concentrations of VLDL particles or LDL particles (data not shown). Previous population studies have clearly shown that Pro373 and Gln451 in CETP are associated with higher plasma levels of CETP antigen and lower levels of HDL cholesterol (7,9). Thus, we suggest that the combined presence of Pro373 and Gln451 in CETP in these male VTE patients significantly helps to explain the finding of HDL deficiency associated with venous thrombosis (5).

Our study of HDL in VTE patients carrying Pro373 and Gln451 in CETP is limited by their small number. Nonetheless, all 8 VTE patients with both Pro373 and Gln451 had HDL cholesterol levels ≤ the 25th percentile of controls and 6 of these 8 VTE patients had HDL particle concentrations ≤ the 25th percentile of controls (Figure 1). For VTE patients with normal CETP Arg451 genotype, 54 % had HDL cholesterol levels that are below the 25th percentile of controls while 68% of VTE patients had HDL particle concentrations ≤ the 25th percentile of controls (Figure 1). Thus, a substantial number of VTE patients with the common CETP genotype encoding Ala373 and Arg451 also had low HDL parameters (Figure 1), and we conclude that although the finding of Pro373 and Gln451 in CETP contributes to HDL deficiency in some VTE patients, there are other factors, genetic and/or environmental, that contribute significantly to HDL deficiency in venous thrombosis.

This study has the limitation of employing a relatively small number of patients. However, the findings intrinsically possess strong statistical significance as well as biological plausibility because HDL is recognized to exert antithrombotic activities. The study is also limited because only males were studied (3-5) as we were driven by the observations that male gender itself is a risk factor for venous thrombosis. Further research on VTE linked to dyslipoproteinemia and HDL deficiency in males are needed as are studies of associations between VTE in females and HDL..

In summary, venous thromboembolism in males was significantly linked to two relatively rare CETP variations, Ala373 to Pro and Arg451 to Gln, which are known to cause decreased levels of HDL.

Acknowledgements

This work was supported in part by NIH grants (HL52246, HL21544, HL07195 and NIH-GCRC M0100833) and the Skaggs Clinical Scholar Program and its director, Professor Ernest Beutler, to whom we are particularly grateful.

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Figure 1. HDL cholesterol (HDL-C) and HDL particle concentrations for three groups of subjects: controls (n=49), VTE patients with normal CETP Arg451 genotype (n=41), and VTE patients carrying two uncommon CETP variants, Pro373 and Gln451 (n=8). Among the first two groups of subjects, those carrying the normal CETP Ala373/Arg451 genotype are indicated by open circles while three subjects (one control and two VTE patients) carrying the uncommon variant Pro373 and the common Arg451 are indicated by "X". The solid lines indicate mean levels and the dotted line indicates the 25th percentile of controls.