Short communication

**LPL polymorphism (D9N) predicts cardiovascular disease risk directly and through interaction with CETP polymorphism (TaqIB) in women with high HDL cholesterol and CRP**

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**Abstract**

Objective: We sought to determine whether concurrently high levels of HDL cholesterol and CRP predicted initial cardiovascular events in women, and to assess additional risk involving two genes encoding proteins involved in reverse cholesterol transport.

Methods: A graphical approach identified high-risk subgroups in a population-based female cohort. Polymorphism-associated risk was assessed for CETP (TaqIB [rs708272]) and LPL (D9N [rs1801177]) using multivariable analysis adjusted for clinical parameters and biomarkers.

Results: A high HDL-C/high CRP high-risk subgroup was identified. Multivariable modeling revealed D9N as predicting subgroup cardiovascular disease risk directly (minor allele-carriers versus major allele homozygotes: HR 5.16, 95% CI 1.43–18.54, p = 0.012) and through interaction with TaqIB (highest risk in minor allele carriers of both polymorphisms).

Conclusions: In women with high HDL-C and high CRP levels, an LPL polymorphism associated with risk and interacted with a CETP polymorphism such that the highest risk occurred in subjects with presumably decreased activities of both proteins.

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**1. Introduction**

Concurrently high levels of HDL cholesterol (HDL-C) and C-reactive protein (CRP) have recently been reported to be associated with increased cardiovascular disease (CVD) risk in men initially without CVD [1] and in postinfarction patients [2]. Furthermore, in postinfarction patients, a variant of the choleseryl-ester transfer protein (CETP) gene associated with decreased CETP mass and activity has been reported to predict risk in patients with high HDL-C and CRP levels [2]. This finding is consistent with impaired early phases of reverse cholesterol transport (RCT) as possibly playing a role in the observed risk-association by facilitating dysfunctional transformation of HDL.

To investigate this notion further, the current study aimed: to replicate the finding of a similar high HDL-C/high CRP high-risk subgroup in women without CVD and to explore in men and women, initially without CVD, the effect on risk of decreased activity of CETP and lipoprotein lipase (LPL), proteins both involved in HDL particle remodeling. To do this, we used outcome event mapping, a graphical exploratory data analysis tool for identifying high-risk subgroups [1–3] and Cox multivariable proportional hazards regression in conjunction with activity probes consisting of genetic polymorphisms of CETP (TaqIB [rs708272]) [4] and LPL (D9N [rs1801177]) [5] in male and female cohorts of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) population study [6].

**2. Methods**

2.1. Study population

The study group [1] comprised a cohort (N = 8592) of PREVEND [6], a prospective longitudinal study assessing albuminuria in predicting cardiovascular [7] and renal [8] disease. PREVEND was approved by the medical ethics committee of the University of Groningen, The Netherlands. PREVEND excluded insulin-using diabetics and pregnant women. Further exclusions for the present work included history of diabetes mellitus, renal disease, previous CVD, and incomplete laboratory results. Subjects with CRP levels ≥10 mg/L were excluded (to avoid confounding by inter-current ill-
ness); there was no exclusion based on HDL-C levels. Sub-cohorts of men (N = 3405) and women (N = 3619) resulted (see Supplement – Methods).

2.2. Blood markers and genotyping

Overnight fasted blood samples collected after 15 minutes of rest were stored at −20°C prior to analysis [9] (see Supplement – Methods). Genotyping of TaqIB (CETP) was performed as described previously [9]. Genotyping of D9N (LPL), was performed using TaqMan-MGB probes, primer, SDS 2.0 genotype calling software, and ABI 7900HT apparatus (Applied Biosystems, Applera Nederland, Nieuwerkerk aan de IJssel, The Netherlands).

2.3. Outcomes

Outcomes were combined incidence of CVD mortality and post-baseline hospitalisation [1]. Cardiovascular events included: acute MI, acute and subacute ischemic heart disease, coronary artery bypass grafting, and percutaneous transluminal coronary angioplasty. Median follow-up was 7.6 years (see Supplement – Methods).

2.4. Statistical analyses

Outcome event mapping [1–3] was used to identify high-risk subgroups (see Supplement – Methods). Statistica 9.0 (StatSoft, Inc., Tulsa, OK) was used for statistical and graphical analyses. Statistical tests (p < 0.05) included Pearson correlation, Mann–Whitney U, Kruskal–Wallis with Bonferroni correction, chi-square, Kaplan–Meier with log-rank statistic, and multivariable Cox proportional hazards regression (biomarkers treated as continuous variables: polymorphisms treated as minor allele carriers versus major allele homozygotes).

3. Experimental results

3.1. Study population

Qualitative identification in female subjects of two high-risk subgroups was reported previously [1]. Subsequent analysis (Supplement – Experimental results) revealed 879 subjects in a low HDL-C/high CRP subgroup (henceforth HR1); 508 subjects in a high HDL-C/high CRP subgroup (henceforth HR2); and 2232 subjects in a lower risk background subgroup (henceforth BG). Subjects in HR1 versus HR2 (Supplement – Table 1) were older, more hypertensive, have more MS and less alcohol use. Additionally, they demonstrated higher levels of cholesterol, triglycerides, non-HDL-C, glucose, apoB and apoB/apoA1 ratio, and lower HDL-C, apoA1, and HDL-C/apoA1 ratio, a rough measure of HDL particle size.

Correlation between HDL-C and triglyceride levels was used to indirectly assess HDL particle remodeling. Correlation coefficients (all significant) were: BG, −0.24; HR1, −0.29; and HR2, −0.15. Results of pairwise comparisons were: BG/HR1 (p = 0.16), BG/HR2 (p = 0.057), and HR1/HR2 (p = 0.0074). Thus in HR2, the magnitude of inverse correlation between HDL-C and triglycerides was significantly less than in HR1.

3.2. Genotype distributions

The TaqIB polymorphism (CETP) in females was in Hardy–Weinberg equilibrium; the D9N polymorphism (LPL) was not (for details and likely explanation, see Supplement – Experimental results). Significant differences (p < 0.01) in blood markers (HDL-C, apoA1, triglyceride, apoB, and total cholesterol) according to the polymorphisms (minor allele carriers versus major allele homozygotes) were for TaqIB: in HR1 and HR2, none; in BG, higher HDL-C and apoA1. For D9N, there were none.

3.3. Univariate biomarker and polymorphism risk within subgroups

Table 1 gives univariate significant Cox regression results for the high-risk subgroups. For TaqIB, the hazard ratio in both subgroups was non-significant; although for HR2, there was a tendency towards significance. For D9N, in HR2 the hazard ratio was significant; in HR1, Cox analysis was not applicable as minor allele carriers had no outcomes.

3.4. Polymorphism multivariable risk within subgroups

Multivariable modeling adjusted for significant clinical parameters and biomarkers (HR1 – age, BMI, hypertension, and cholesterol; HR2 – age, BMI, and hypertension; Supplement – Experimental results) was performed as a function of the polymorphisms. In HR1, TaqIB was non-significant; D9N analysis was excluded as noted above. In HR2, D9N remained significant with greater risk for minor allele-carriers (hazard ratio 5.16, 95% CI 1.43–18.54, p = 0.012); TaqIB tended towards significance (hazard ratio 2.15, 95% CI 0.48–9.73, p = 0.32).

To assess potential effects on HR2 of enrichment in PREVEND with albuminuric subjects, UAE and serum creatinine were forced into multivariable models. The D9N polymorphism remained significant. Additionally, a sensitivity analysis was performed on a PREVEND female sub-cohort not enriched with albuminuric subjects. High-risk subgroups similar to HR1 and HR2 resulted. In the corresponding HR2 subgroup, D9N tended towards significance (hazard ratio 5.02, 95% CI 0.59–42.70, p = 0.14).

3.5. Polymorphism interaction analysis within subgroups

To assess risk-associated interaction between the polymorphisms, Cox models were run including an interaction term.
Interaction was significant in HR2 but not in HR1. Fig. 1 illustrates the interaction in HR2 through Kaplan–Meier curves for minor allele carriers of both polymorphisms versus remaining subjects ($p = 0.0002$).

### 3.6. Polymorphism analyses in male PREVEND subjects

Characterization of male PREVEND subjects and identification of high-risk subgroups similar to females have been reported previously [1]. Similar analyses (Supplement – Experimental results) as above in males revealed non-significance for both polymorphisms in the corresponding high-risk subgroups.

### 4. Discussion

Results of the current study demonstrate in women initially without CVD a high-risk subgroup at high levels of HDL-C and CRP, similar to findings in men [1] and postinfarction patients [2]. Furthermore, investigations using multivariable risk-modeling within the subgroup with genetic markers to probe effects of decreased activities of CETP and LPL demonstrated D9N as a significant predictor of risk, and a trend towards significance for TaqIB. Moreover, interaction between D9N and TaqIB was significant with highest risk occurring in minor allele carriers of both polymorphisms, in each case, the allele reportedly associated with decreased protein activity [4,5]. An additional finding in women, as in men [1], was identification of a second high-risk subgroup at low HDL-C and high CRP levels enriched with metabolic syndrome subjects. This subgroup demonstrated a higher level of inverse correlation of HDL-C with triglycerides than the high HDL-C subgroup, a finding potentially indicative of less efficient HDL particle remodeling in the high HDL-C subgroup.

Corresponding findings were not seen in men. This may be related to the overall higher event rate in men (7.74%) versus women (2.95%) that when analyzed specifically in the context of event rates for D9N revealed similar values for minor allele carriers (men – 12.50%; women – 14.29%), but dissimilar values for major allele homozygotes (men – 7.62%; women – 2.43%). Thus, it appears that lack of D9N associated risk in men stems not from differences in the higher outcome rates of minor allele carriers but rather from the relatively higher risk in major allele homozygotes of men versus women.

LPL [10,11] is involved with triglyceride-rich lipoprotein metabolism and lipoprotein remodeling including HDL [12,13]. Consistent with our study, the reduced-activity minor allele of D9N is generally associated with increased CVD risk [5]. D9N-associated risk, especially in conjunction with TaqIB, suggests a role for altered HDL remodeling in pro-atherogenic transformation of HDL, a process likely potentiated by the inflammatory environment defining the subgroup. In this regard, high CRP may play a direct role in this transformative process. Recent reports [14] detail direct effects of CRP potentially relating to HDL transformation including myeloperoxidase release from human neutrophils, superoxide anion production via up-regulation of NAD(P)H oxidase in human foam cells; and superoxide release from rat macrophages. These effects could potentially link CRP to HDL dysfunction given recent reports of loss of HDL anti-atherogenic functionality through myeloperoxidase-mediated apoA1 oxidation [15]. Moreover like LDL [14], HDL outer layer phospholipids could be oxidized by CRP-mediated elaboration of oxidative species generating CRP binding sites on HDL particles that, again like oxidized LDL [14], could result in subsequent macrophage uptake of CRP-bound HDL.

Limitations in the current study included study population over-sampling with high-UAE subjects; however, findings regarding D9N-associated risk appeared little affected when UAE and creatinine were forced into multivariable models and when sensitivity analysis of a sub-cohort without albuminuric enrichment also gave similar results. Further study limitations related to the lack of evidence for the causal role of inflammation and oxidative stress in HDL dysfunctional transformation and lack of evidence for decreased activity of CETP and LPL in minor allele carriers. Future studies should be designed to address these issues.

The current study demonstrated in a population of women initially without CVD a high-risk subgroup defined by concurrently high levels of HDL–C and CRP. This subgroup was similar to high-risk subgroups previously identified in men initially without CVD [1] and in postinfarction patients [2]. Among high-risk women, carriage of the less active allele of D9N (LPL) was found to associate with risk and with simultaneous carriage of the less active allele of TaqIB (CETP) to intensify risk. We conclude that compromised early stages of RCT involving alterations in HDL particle remodeling likely play a role in establishing risk in women with high levels of HDL–C and CRP.

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### Disclosures

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2010.11.029.

### References


