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Paraoxonase-1 and oxidized lipoprotein lipids. The Cardiovascular Risk in Young Finns Study

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ABSTRACT

Objective: Paraoxonase-1 (PON1) is suggested to have a role in the antioxidant activity of high-density lipoprotein (HDL). PON1 activity levels are strongly genetically determined by the rs662 polymorphism (PON1 *Q192R*). To clarify the role of PON1 in lipoprotein oxidation at the population level, we examined the relations between PON1 activity, the rs662 polymorphism and oxidized lipoprotein lipids in young adults.

Methods: A population-based cross-sectional study of 1895 Finnish adults ages 24–39 years (872 males and 1023 females). PON1 activity was determined with paraoxon as the substrate. Analysis of oxidized lipids in isolated HDL (oxHDLlipids) and low-density lipoprotein (oxLDLlipids) was based on the determination of conjugated dienes. Oxidized LDL was also measured with a method based on antibodies against oxidized Apo-B (oxLDLprot). Serum lipids and apolipoproteins were measured. Genotyping was performed with the Illumina Bead Chip (Human 670 K).

Results: In multivariable models, PON1 activity associated inversely with oxLDLlipids ($p = 0.0001$, semi-partial $R^2 = 0.09\%$), but it was not associated with oxHDLlipids ($p = 0.93$). There was a borderline significant association between PON1 activity and oxLDLprot ($p = 0.08$). PON1 rs662 polymorphism was strongly associated with PON1 activity (P-value < 0.0001), but not with oxidized lipoprotein lipids and oxLDLprot.

Conclusion: Higher PON1 activity is associated with decreased oxLDLlipids levels, but not with oxHDL-lipids in a population of young Finnish adults. These findings support the suggestion that PON1 activity may have a role in the oxidation of LDL lipids. There is a strong association between PON1 rs662 polymorphism and PON1 activity, but PON1 rs662 polymorphism is not associated with oxidized lipoprotein lipids and oxLDLprot.

1. Introduction

Serum paraoxonase (PON1) is a high-density lipoprotein (HDL) -associated hydrolytic enzyme, which is capable to hydrolyze a wide spectrum of substrates [1] and it is proposed that lactonase activity is likely to be the main physiologic function of PON1 [1]. The PON1 gene belongs to the paraoxonase gene cluster in the long arm of chromosome 7 [2] and PON1 rs662 polymorphism has a

Abbreviations: PON1, paraoxonase-1; oxHDLlipids, oxidized HDL lipids; oxLDL-lipids, oxidized LDL lipids; oxLDLprot, oxidized LDL proteins; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Apo-B, apolipoprotein-B; Apo-A1, apolipoprotein-A1; BMI, body mass index.

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strong effect on PON1 activity levels [3,4]. **PON1 rs662** (A) encodes a glutamine (Q) and PON1 **rs662** (G) encodes an arginine (R) [5]. PON1 activity is lower in a group with PON1 rs662 A/A genotype (QQ genotype) and higher in a group with PON1 rs662 G/G genotype (RR genotype) [4]. Population distribution of PON1 activity in Caucasians has been shown to be bimodal and PON1 allelic variants are associated with interindividual variability in enzyme activity [6].

The association between PON1 activity and cardiovascular risk seems presently controversial. Low PON1 activity is shown to be a risk factor for coronary events [7]. However, it has been shown that the inverse relation of PON1 activity with coronary artery disease was diluted after adjustment with HDL cholesterol and apolipoprotein-A1 (Apo-A1) in multivariable models [8], which might suggest that low PON1 activity is not independently associated with atherogenesis. On the contrary, high PON1 activity has been associated with an increased risk for acute myocardial infarction [9]. In line with this, genetically high PON1 activity has been associated with an increased risk for coronary heart disease [3] and acute myocardial infarction [10], respectively.

Because PON1 has ability to inhibit low-density lipoprotein (LDL) oxidation *in vitro* [11], it is suggested to be an anti-atherogenic enzyme [12]. It has additionally been shown in diabetic patients that PON1 has a role in the antioxidant activity of HDL as PON1 activity levels were demonstrated to be lower in type 1 diabetic patients compared to non-diabetic first degree relatives and this reduction was suggested to be able to influence the antioxidant capacity of HDL [13]. PON1 was also shown to regulate HDL-mediated cholesterol efflux from macrophages [14]. The ability to be an antioxidant for LDL has been suggested to be a possible atheroprotective mechanism of PON1 [15].

It is hypothesized that functional properties of HDL may reflect the atheroprotective effects of HDL better than HDL cholesterol levels alone [16], but it is still unclear, what are the main atheroprotective functional properties of HDL. One potential mechanism may be the lipid peroxide reverse transporting capacity of HDL [17]. In line with this, we recently demonstrated at the population level that an elevated cardiovascular risk profile is associated with lower levels of oxidized HDL lipids (oxHDLlipids) [18]. At present, PON-1 is proposed to be a factor in the anti-oxidant activity of HDL based on studies performed *in vitro* [13].

Therefore, to examine the associations of PON1 activity with oxidized lipoprotein lipids at the population level, we examined the associations of PON1 activity and genotype with oxHDLlipids and oxLDLlipids in The Cardiovascular risk in Young Finns Study.

2. Methods

The Cardiovascular Risk in Young Finns Study is a multicenter follow-up to evaluate risk factors and precursors of cardiovascular diseases (CVD) from childhood into adulthood. The study began in 1980 and the 21-year follow-up was conducted in 2001 with a total of 2283 subjects aged 24–39 years. Participants were randomly selected from the national register. The present study was restricted to subjects who had data on serum PON1 activity measured in the 2001 follow-up. Subjects who were pregnant ($N = 48$) were excluded from the study due to influence on metabolism. A total of 1895 subjects (872 males and 1023 females) were included in the final analyses. Participants gave written informed consent and the study was approved by local ethics committees. Details of the study design have been presented earlier [19].

Venous blood samples were collected after overnight fasting. PON1 activity was determined with paraoxon (O,O-diethyl-O-p-nitrophenylphosphate) as the substrate and the increase in the absorbance at 412 nm due to formation of 4-nitrophenol was measured spectrophotometrically [20]. The analysis of oxidized lipoprotein lipids was based on determination of the baseline level of conjugated dienes in lipoprotein lipids [21]. Phosphotungstic acid preparation was used to isolate serum HDL fraction (oxHDLlipids) [22] and serum LDL fraction (oxLDLlipids) was precipitated with buffered heparin [21]. Lipids were extracted from the isolated lipoproteins by chloroform-methanol (2:1), dried under nitrogen and redissolved in cyclohexane. The amount of peroxidized lipids was assessed spectrophotometrically at 234 nm [17]. The isolation procedures were validated for the purpose and did not affect the level of oxidized lipids [21]. Validation studies for the assay have ruled out interference by non-specific substances and shown that diene conjugation is a measure of oxidative HDL and LDL lipid modification found in all HDL and LDL lipid classes. Samples were stored at −80 °C until the analyses of lipid and protein oxidation in lipoprotein lipids were performed. The coefficient of variation (CV) was 4.4% and 5.2% for within-assay precision for determination of oxidized lipoprotein lipids, and 4.5% and 5.6% for the between-assay precision for LDL and HDL, respectively. We additionally measured LDL oxidation with another method, which was based on directing the mouse monoclonal antibody 4E6 against a conformational epitope in oxidized Apo-B-100 in serum [23]. LDL particles containing oxidized Apo-B (oxLDLprot) were evaluated with monoclonal antibody based enzyme-linked immunosorbent assay (Oxidized LDL ELISA kit, Mercodia, Sweden).

Height and weight were measured. Body mass index (BMI) was calculated with the formula weight (kg)/(height) $(m)^2$. Genotyping was performed with the Illumina Bead Chip (Human 670 K). Serum apolipoproteins A1 (Apo-A1) and B (Apo-B) were analyzed immunoturbidometrically (Orion Diagnostica, Espoo, Finland).

2.1. Statistical analyses

The normality assumptions were calculated by examining histograms and normal probability plots. Normalized rank transformation was made for PON1 due to skewed distribution. ANOVA was used to examine differences in characteristics between paraoxonase groups stratified by rs662 genotypes. P for trend was adjusted with age. Men and women were studied in combination, because there were no significant sex interactions between PON1 and other study variables. Pearson's correlation analyses were calculated to examine associations between two continuous variables and Spearman's correlation analysis was used in case of bivariate variables. In the multivariable models, a set of priori selected explanatory variables were forced in the models simultaneously with the aid of multicollinearity diagnostics. In a model for oxHDLlipids the explanatory variables were PON1, sex, Apo-A1, oxLDLlipids. age and BMI. The explanatory variables in a model for oxLDLlipids were PON1, sex, Apo-B, oxHDLlipids, oxLDLprot, age and BMI, and in a model for oxLDLprot the explanatory variables were PON1, sex, Apo-B, oxHDLlipids, oxLDLlipids, age and BMI. All statistical analyses were performed using Statistical Analysis System, SAS (version 9.3), and statistical significance was inferred at a two-tailed $P < 0.05$.

3. Results

The characteristics of the study subjects stratified by the rs662 genotype are shown in Table 1. The mean activity of serum PON1 was 51.8 U/L in a group with A/A genotype (QQ genotype), 126.6 U/L in a group with G/A genotype (QR genotype) and 152.5 U/L in a group with G/G genotype (RR genotype) (P-value < 0.0001). There were also statistically significant differences across rs662 genotypes in age and Apo-A1, but not in any other variables studied.

To study if PON1 is a significant explanatory variable for