ApoE phenotype is associated with inflammatory markers in middle-aged subjects

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Abstract. Objectives: Apolipoprotein (apo) E phenotype has been associated with inflammation markers. The determinants of these associations and the relationship between novel inflammation marker, resistin, and apoE phenotype are studied here.

Methods and Results: Middle-aged subjects of the population-based cohort (n = 526) of the OPERA– study were studied. Intima-media thickness (IMT) was measured with carotid ultrasound. The results suggest that, apoE phenotype was a significant independent predictive factor for resistin (p < 0.01) and hsCRP (p < 0.01) levels. The association of ApoE phenotype with hsCRP was seen among the subjects with the normal renal function (p = 0.005). ApoE4 was associated (p < 0.01) with the lowest hsCRP in the lowest IMT quartile while its relation with the highest resistin levels was evident in the highest IMT quartile.

Conclusions: ApoE phenotype is an independent determinant of plasma resistin and hsCRP levels. The extent of atherosclerosis and renal function seem to modify the effects of apoE phenotype on inflammatory parameters.

Key words: HsCRP – Resistin – ApoE phenotype – Atherosclerosis

Introduction

Apolipoprotein (apo) E has been shown to play an important role in lipoprotein metabolism [1]. Three common alleles, e2, e3 and e4, determine the six apoE phenotypes E2/2, E2/3, E2/4, E3/3, E4/3 and E4/4 [1]. The phenotypic pattern of apoE seems to modulate the risk for coronary heart disease [2, 3]. Subclinical chronic inflammation, where apoE plays a central role [4, 5], is an important pathogenetic factor in the development of atherogenesis. ApoE phenotype may influence the levels of inflammation markers, like high-sensitivity C-reactive protein (hsCRP), with apoE4 isoform having the lowest concentrations [6–9]. The latter observation is paradoxal considering the atherogenic effect of E4.

Resistin is a newly described hormone secreted from adipose tissue with a suggested role in insulin resistance in animals [10] while in humans inflammatory cells seem to be the major source of resistin [11] having perhaps more a role in inflammation. The recent results of our study imply that high plasma resistin level is associated with enhanced hsCRP and leukocytes [12]. Resistin could mediate the inflammatory effects on arterial wall and contribute to the development of atherosclerosis [13]. The latter notion was supported by the recent report showing that resistin is present in atherosclerotic lesions in apoE(-/-) mice [14]. No studies examining the relationship between apoE phenotype and plasma resistin concentration exist.

In the present study we were interested in whether resistin levels differ in apoE phenotype categories. Our hypothesis was that since apoE phenotype has been associated with the levels of inflammation markers, also resistin, correlating with hsCRP, might show an association with apoE phenotype. Specific stimuli may induce a release of inflammatory cytokines [15]. The severity of coronary atherosclerosis may affect plasma levels of inflammation parameters and higher hsCRP levels have been reported in subjects with more advanced state of atherosclerosis [16]. Therefore, we studied also the relationship of apoE phenotype with the levels of inflammation markers in various stages of early atherosclerosis.
Subjects and Methods

This study is a part of the OPERA (Oulu Project Elucidating Risk of Atherosclerosis) project, which is a population-based, epidemiological study designed to address the risk factors and disease endpoints of atherosclerotic cardiovascular diseases. The study population and selection criteria have been previously described in detail [17]. Briefly, the hypertensive cohort was randomly selected by age stratification (15 men and 15 women per year) from the Social Insurance Institute register for reimbursement of antihypertensive medication. For each hypertensive subject, an age- and sex-matched control was randomly selected from the national health register (including all inhabitants) excluding the subjects with the right to reimbursement for hypertension medication. Middle-aged subjects of the population-based control cohort (n = 526) of OPERA—study were examined in this study. The participants visited the research laboratory of the Department of Internal Medicine for laboratory tests, physical examination, and a detailed interview. The study was approved by the Ethical Committee of the Faculty of Medicine, University of Oulu. Blood pressure was measured according to the recommendations of the American Society of Hypertension in a sitting position from the right arm with an oscillometric device (Dinamap® model 18465X, Criticon Ltd., Ascot, UK) after an overnight fast and after a 10-15-minute rest. Three measurements were made at 1-minute intervals and the means of the last two were used in the analyses. Waist circumference was measured to the nearest 0.5 cm with a tape measure midway between the lower rib margin and the iliac crest in light expiration. An informed consent was obtained from each participant.

Laboratory Analyses

All laboratory samples were obtained after an overnight fast, and plasma was separated by centrifugation and stored at −20 °C. The plasma high-density lipoprotein (HDL), low-density lipoprotein (LDL), and total cholesterol and triglyceride concentrations were measured as previously described [17]. The creatinine concentrations were determined with standard methods in the routine laboratory of Oulu University Hospital. Estimated glomerular filtration rate (GFR) was calculated using the formula: eGFR (mL/min/1.73 m2) = 186 x (serum creatinine in mg/dl) -1.154 x (Age)0.203 x (0.742 if female) [18]. The apolipoprotein E phenotype was determined from delipidated plasma with isoelectric focusing and immunoblotting techniques [19], using commercial antibodies (Daichi Pure Chemical, Tokyo, Japan; Bio-Makor, Rehovot, Israel).

Fasting plasma total insulin-like growth factor 1 -binding protein 1 (IGFBP-1) concentration was determined using commercial kit (IEMA test, Oy Medix Biochemical, Kauniainen, Finland) and plasma highly sensitive C-reactive protein (hsCRP) concentration by commercially available ELISA kit (Diagnostic Systems Laboratories, Webster, Tex., USA). Plasma leptin was determined with the double-antibody RIA (Linco Research, Inc., St. Charles, MO, USA). Plasma resistin was measured in duplicate using a commercially available enzyme-linked immunosassay kit (Linco Research Inc., USA; intra- and interassay coefficients of variation 4.5 and 7.4 %, respectively) as described earlier [12].

Intima-media thickness (IMT) was measured with the carotid ultrasound procedure by one trained radiologist without knowledge of clinical data. A duplex ultrasound system with 7.5 MHz scanning frequency in B-mode, pulsed doppler mode and colour mode was used (Toshiba SSA-270A, Toshiba Corp., Tokyo, Japan). The reproducibility of the IMT measurements was assessed from the videotapes of 31 randomly selected study subjects by 2 radiologists blinded to the original results. The intra-reader variability and correlation coefficient (Pearson) were 3 % and 0.97 for the mean IMT and 9.9 % and 0.94 for the maximal IMT, respectively. The respective interreader variability and correlation values were 7.2 % and 0.93 (mean IMT) and 12.8 % and 0.92 (maximal IMT).

The far-wall IMT was measured at three different locations on both sides: one measurement from internal carotid artery (ICA), one measurement from bifurcation enlargement (BIF) and three measurements from common carotid artery (CCA) (proximal, middle and distal from the bifurcation) [20]. The mean IMT was defined as the mean of ICA, BIF, and the 3 highest CCA measurements.

Statistical methods

All statistical calculations were made with the SPSS (version 9.0; SPSS, Inc.) statistical package. P-value <0.05 was regarded as statistically significant. A χ2-test was performed to assess whether the number of current smokers in apoE phenotypes differed from each other. To compare the means of continuous variables between apoE phenotypes the analysis of variance (ANOVA) and analysis of covariance with adjustments (ANCOVA) were used. Study group was divided into four subgroups, quartiles, according to their carotid IMT and plasma resistin level. To compare the means of continuous variables measured between the resistin and IMT quartiles the ANOVA and ANCOVA were used. Linear regression analysis was used to test the determinants of resistin concentration by including apoE and all the factors correlating significantly with plasma resistin levels in our earlier study [12] in the model. To normalize the distribution a logarithm transformation was applied to resistin, leptin, IGFBP-1, hsCRP, leucocytes, hemoglobin A1c (HbA1c), total and HDL cholesterol, triglycerides and to all IMT measurements.

Results

The e2, e3 and e4 allele frequencies among the subjects 0.046, 0.762 and 0.192, respectively. In the analysis we have combined the groups so, that the E2 are subjects with the phenotypes E2/2, E2/3 and E2/4, E3 the subjects with the phenotype E3/3 and E4 subjects those with the phenotypes E4/4 and E4/3.

The clinical and inflammatory parameters and apoE phenotype

The clinical data of the subjects by the apoE phenotype are presented in Table 1. ApoE phenotype was associated significantly with age (P<0.01 for the difference between E2 and E3), creatinine and eGFR (P<0.01 between E2 and E3 or E4), systolic blood pressure (P<0.05 between E3 and E4), total and LDL-cholesterol (P<0.01 between E2 and E3 or E4), HDL-cholesterol (P<0.05 between E2 and E4) and mean IMT (P<0.01 between E2 and E3 or E4).

After adjustment for conventional risk factors (age, sex, smoking, systolic blood pressure and LDL-cholesterol) apoE phenotype was not associated with IMT.

ApoE phenotype was associated with plasma hsCRP levels (adjusted for age, sex, BMI, smoking) (p = 0.033). E4 subjects had the lowest hsCRP levels. The association was clearer among females (Table 2). In linear regression analysis apoE phenotype was a significant predictive factor for hsCRP levels (p<0.01) when age, sex, body mass index and smoking were added to the model.

Resistin levels (adjusted for age, sex, BMI, smoking and hsCRP) varied significantly according to apoE phenotype (p = 0.019). When genders were considered separately (Table 2), male control subjects with the E4 had the highest resistin levels (p = 0.005). The adjustment for the differences in blood pressure with apoE phenotype did not change the associations.

Is the apoE phenotype independently associated with plasma resistin concentration independently?

We wanted to study whether apoE phenotype is a significant predictive factor for plasma resistin concentration by in-