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RESEARCH LETTER

Genetic determinants and early carotid atherosclerosis: is there a role for the ectonucleotide pyrophosphatase phosphodiesterase 1 (ENPP-1) K121Q polymorphism? Preliminary results in non diabetic individuals

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Introduction

Carotid intima-media thickness (CIMT) is an intermediate phenotype for early atherosclerosis and a strong predictor of myocardial infarction and ischemic stroke [1]. Several studies have described the effects of candidate genes on CIMT, being unclear, however, the relative contribution of genetic variation to the development of the early stages of atherosclerosis [2]. The ectoenzyme pyrophosphatase phosphodiesterase 1 (ENPP-1) inhibits insulin receptor signaling [3]. A non synonymous polymorphism (K121Q, rs1044498) of the ENPP-1 gene is responsible for a gain of function of the protein [3], resulting in an increased ability to inhibit insulin receptor signaling. This variant has been associated with insulin resistance and type 2 diabetes in most studies [4, 5]. In agreement with the link between insulin resistance and cardiovascular disease (CVD), the Q121 variant has been shown to modulate susceptibility to premature myocardial infarction [6] and ischemic stroke [7] and to increase pulse pressure, a marker of arterial stiffness [8]. Some of these associations have been shown to be stronger in obese subjects, suggesting a gene-by-obesity interaction in facilitating insulin resistance and atherogenic process [4, 9]. Up to date no results on ENPP-1 function on CIMT are available. Thus, we tested whether the ENPP-1 Q121 variant exerts an effect on CIMT measured at the common carotid artery (CCA) and at bulb and interacts with overweight–obesity in modulating CIMT.

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Patients and methods

Ninety-seven non diabetic Caucasian adults were consecutively recruited at the Cardiovascular Prevention Unit of Policlinico Umberto I (Rome, Italy). Inclusion criteria were age between 18 and 65 years and BMI <40 kg/m^2. Exclusion criteria were history of diabetes and cardiovascular disease, and any therapy affecting glucose and lipid metabolism. Each subject gave written informed consent, in accordance with the Helsinki declaration. Anthropometric parameters were measured in all patients. Fasting plasma glucose, serum triglycerides and HDL cholesterol levels were measured with commercially available enzymatic kits; LDL cholesterol was calculated using Friedewald formula. Carotid ultrasound was performed with a 7.5–10.0 MHz linear array transducer (Esaote MyLab25). Using antero-oblique insonation, far-wall carotid IMT was visualized bilaterally at common carotid artery (CCA-IMT) and carotid bulb (bulb-IMT). The images were digitally captured for offline measurements. The highest CIMT values were manually recorded by the same operator who was blinded to the patient's genotype. Mean CIMT values were calculated as the mean for the right and left measurements.

Genomic DNA was extracted from whole blood by standard methods. Genotyping of the K121Q polymorphism (rs1044498) was performed by TaqMan allele discrimination (assay C_16190162_10, Applied Biosystems, Forster City, CA) on the HT7900 platform (Applied Biosystems).

The failure rate was <1 %. Genotyping quality was assessed by including positive controls with known genotypes. The agreement rate was >99 %. In the study sample genotype distribution obeyed Hardy–Weinberg equilibrium (HWE).

Because of the low number of homozygous for the Q121 variant ($n = 6$), they were analyzed together with subjects carrying the K121/Q121 genotype and named as XQ individuals. Normally distributed data were expressed as mean ± SD, whereas variables with a skewed distribution were reported as median and interquartile range (IQ). Comparisons across genotype groups were performed using unpaired *t* test. Multivariate analyses were performed using linear models comprising age, gender, smoke, and hypertension. The product term gene * BMI change was added into the model when looking for gene-by-BMI interaction in modulating the outcome. A $p < 0.05$ was considered for statistical significance. All analyses were performed using SPSS version 13.0 for Windows (SPSS, Chicago, IL, USA).

Results

Of the 97 study participants, 35 % were males and 65 % females. Mean age was 51.8 (±9.3) years. Eighty-two percent of the subjects were overweight or obese (mean BMI 28.2 ± 3.6 kg/m^2), 34 % had hypertension, 23.7 % had glucose intolerance, and 22.7 % were smokers. Mean levels of total, LDL, HDL cholesterol, and triglycerides were 6.22 ± 1.00, 4.04 ± 0.79, 1.35 ± 0.38, and 1.85 ± 1.07 mmol/L.

No significant difference was observed between the two genotypes (KK = 72 subjects vs XQ = 25) in anthropometric parameters, lipid profile and prevalence of cardiovascular risk factors.

In the whole population median CCA-IMT and mean bulb-IMT values were 0.60 mm (IQ range 0.50–0.70) and 0.89 (±0.22) mm, respectively. CCA-IMT values did not differ between the two genotypes. On the contrary, a significant higher value of mean bulb-IMT was observed in XQ as compared to KK individuals (0.98 ± 0.24 vs 0.86 ± 0.21 mm, adjusted $p = 0.013$).

We also stratified the population according to classes of BMI: "normal weight" (BMI < 25 kg/m^2) versus "overweight–obese" (BMI ≥ 25 kg/m^2) subjects.

In the overweight–obese group, mean bulb-IMT value was significantly higher in XQ subjects ($n = 20$; mean bulb-IMT 1.05 ± 0.21 mm) as compared to KK ($n = 60$; mean bulb-IMT 0.85 ± 0.22 mm; adjusted $p = 0.001$; Fig. 1), thus suggesting a possible interaction of the polymorphism with the "overweight/obese" phenotype in modulating mean bulb-IMT values (Q121-by-overweight–obese interaction: adjusted $p = 0.002$).

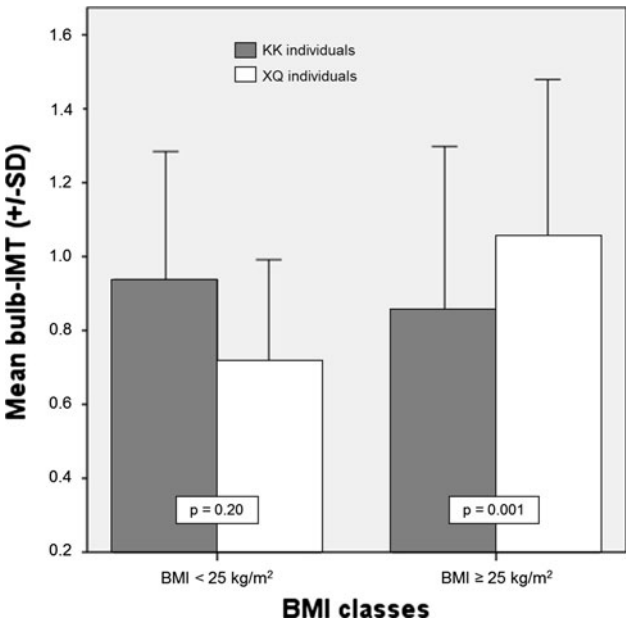

Fig. 1 Mean bulb-IMT values in the two genotypes (KK vs XQ individuals) according to "normal weight" and "overweight–obesity" phenotypes

Discussion

It is quite well established that CIMT is a strong predictor of CVD events. Numerous data have shown that the ENPP-1 Q121 variant plays a role in modulating the risk of premature atherosclerotic events, particularly in obese individuals. There is a growing body of evidence for association between genetics and CIMT, but the genes implicated remain undefined. In this study, we investigated if ENPP-1 K121Q polymorphism is associated with CIMT. In the population studied, despite comparable cardiovascular risk factors in the two groups, the K121Q polymorphism in ENPP-1 gene affects bulb-CIMT values. Indeed, this effect is marked in the overweight–obesity phenotype. This finding is in agreement with previous data demonstrating the presence of a Q121-by-BMI interaction in deteriorating insulin sensitivity and cardiovascular risk [4, 9]. Being adiposity an important modulator of insulin action [10], the coexistence of an acquired and hereditary increase in insulin resistance in overweight/obese individuals carrying the polymorphism, is a reasonable hypothesis to put forward to explain the risk of subclinical atherosclerotic damage. The small sample size is a noteworthy limit of our study and it must be considered in the interpretation of the results. Furthermore, we did not find difference in CCA–CIMT between the two groups. We are aware that the influence of polymorphisms in a single gene on a complex disease such atherosclerosis may be influenced by patient selection or sample size among others and that our findings represent only very preliminary results.

Nevertheless, if confirmed in larger studies, these results could provide useful insights for an early identification of individuals who are at increased risk of developing CVD events.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The authors declare that the study comply with the current laws of the country in which it was performed.

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