Association of Frequent Gene-Smoking Interactions with Elevated Plasma Apolipoprotein B Concentration in Absence of FH or any Criterion of the Metabolic Syndrome

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INTRODUCTION

Smoking, LDL-Cholesterol (LDL-C) and non-HDL-Cholesterol (non-HDL-C) are well known risk factor of cardiovascular disease (CVD) and important targets for intervention. Plasma apolipoprotein (apo) B has also proven to be an efficient marker of CVD risk in various studies. HyperapoB lipoproteinemia (hyperapoB) is a strong correlate of atherosclerosis, even in patients with normal LDL-C levels.1 Plasma apoB concentration is affected by several genetic and environmental factors including the habit. In particular, apoB is often elevated in presence of the metabolic syndrome (MetS), a worldwide increasingly prevalent phenotype influenced by the diet and sedentary. More than 1600 polymorphisms, in at least 65 genes, contribute to modulate plasma apoB levels. The number of genetic combinations and gene-environment interactions possibly affecting apoB concentration is huge but those having a large size effect on apoB variance are less frequent. Familial hyperapoB lipoproteinemia (FHL) is an example of a monogenic trait having a strong effect on LDL and CVD risk. However, the effect of most genetic variants, combination of variants and environmental factors affecting apoB concentration on CVD risk is much less established.

ApoB gene polymorphisms, the peroxisome proliferator-activated receptor (PPAR) and liver X receptor (LXR), as well as a loss-of-function (LoF) hepatic lipase (LPL) gene mutation, are examples of frequent variants modifying apoB-containing lipoproteins metabolism. PPARα, PPARγ1, and PPARβ/δ mutation and several LPL gene variants have been associated with higher apoB levels.2–4 The differential contribution of frequent apoB gene polymorphisms (APOB, 3 and 4) to apoB-containing lipoprotein metabolism and plasma LPL/ apoB concentrations is also well documented. APOA4 is associated with higher apoB-LDL-C and cholesterol apolipoprotein B concentration ratio than APOE3 (wild-type) whereas APOE2, in the homozygous state, is associated with lower LDL cholesterol (LDL-C) clearance, increased number of LDL particles (LDL-particles) and higher concentration of LDL-apoB, increasing the risk of dysbetalipoproteinemia (type III) and CVD.4

OBJECTIVE

The aim of this study was to evaluate the effect of smoking, components of the metabolic syndrome (MetS) and genetic factors on apoB catabolic pathways on plasma apoB concentrations.

METHODS

This study comprised a sample of 7,992 French Canadians selected on the basis of having a positive family history of dyslipidemia or type 2 diabetes. Subjects with familial chylomicronemia (complete LPL deficiency) were excluded.

Subjects were classified according to the presence of MetS components: (i) waist circumference ≥102 cm in men or ≥88 cm in women; (ii) triglycerides ≥1.7 mmol/L; (iii) HDL-Cholesterol <1.0 mmol/L in men and <1.35 mmol/L in women; (iv) blood pressure ≥130/85 mmHg or systolic or diastolic blood pressure treatment; (v) diabetes mellitus or impaired fasting glucose, or drug treatment for elevated glucose levels. The presence of 3 or more of these components confirmed MetS.5

Smoking habits were classified according to the number of cigarettes smoked daily (0 to 10 (non-smokers) vs. more than 10 (heavy smokers)). Blood samples were obtained after a 12-hour overnight fast. Cholesterol and triglyceride levels were enzymatically measured on a analyzer CKT (Beckman). ApoB levels were determined using nephelometry.

Define FH diagnosis was based on the presence of a FH-causing mutation in the LDLR gene or on Simon Broome Registry criteria. Genotyping of other frequent hyperapoB variants was performed using multiplex PCR-FLP-based methods, as previously described.2 LPL gene variants included those with a combined prevalence of at least 5% in the Eastern Quebec French Canadian population (P207L, G181R) or reported as prevalent worldwide (DRN2 and DIN91S).

Categorical variables were compared using the Pearson χ2 statistic, whereas group differences for continuous variables were compared with the Student’s unpaired two-tailed t-Test or ANOVA. Multivariate logistic regression models were built in order to calculate signed effects of interactions between smoking habits and the presence of hyperapoB genotypes on the relative odds (odds ratio (OR)) to exhibit plasma apoB concentrations >0.9 mmol/L. All statistical analyses were performed with the SPSS package (release 20.0, SPSS, Chicago IL).

This project is a sub-analysis of the Study of genetic determinants of T2D, CVD and dyslipidemia in the French Canadian population which has received the approval of the Chicoutimi Hospital Ethics Committee or IRB Services.

RESULTS

Table 1. Subjects’ characteristics according to MetS expression

Table 2. Interaction between smoking and the presence of 21 hyperapoB genotypes on the relative odds to exhibit apoB >0.9 g/L, among CAD-free subjects with LDL-C <3.5 mmol/L, and without any component of the MetS

Figure 1. Proportion of subjects (%) with plasma apoB >0.9 g/L, according to LDL-C and the number of MetS components

Figure 2. Plasma apoB concentration according to FH diagnosis and the number of MetS components

Figure 3. ApoB concentration (%) in non-FH subjects with LDL-C <3.5 mmol/L, after stratification for smoking habits and the presence of other hyperapoB genotypes

Figure 4. The differential contribution of frequent APOB genotypes (apoB-containing lipoproteins metabolism and plasma LPL/apoB concentrations is also well documented. APOA4 is associated with higher apoB-LDL-C and cholesterol apolipoprotein B concentration ratio than APOE3 (wild-type) whereas APOE2, in the homozygous state, is associated with lower LDL cholesterol (LDL-C) clearance, increased number of LDL particles (LDL-particles) and higher concentration of LDL-apoB, increasing the risk of dysbetalipoproteinemia (type III) and CVD.

The proportion of subjects with plasma apoB >0.9 g/L, in the absence of MetS components only when LDL-C <3.5 mmol/L (p=0.001). In the LDL-C < 3.5 mmol/L group, more than one fourth (24%) of individuals without any MetS component had plasma apoB >0.9 g/L. None of them were FH.

In absence of FH and in presence of low-LDL-C, smoking and the presence of other frequent hyperapoB variants in the LDL, apoE or PPARα genes tended to be associated with higher plasma apoB concentration, whether or not the individuals met criteria of the MetS. However, apoB concentrations were higher and the effect was greater in presence of the MetS.

Table 2. Interaction between smoking and the presence of 21 hyperapoB genotypes on the relative odds to exhibit apoB >0.9 g/L, among CAD-free subjects with LDL-C <3.5 mmol/L, and without any component of the MetS

There is a significant interaction between the presence of 21 hyperapoB variants in either LPL, APOE, PPARα genes and smoking habits on the risk to exhibit apoB >0.9 g/L, but only among subjects with low risk profile (MetS(-) and LDL-C <3.5 mmol/L). This interaction remains significant even among individuals who are in primary prevention (Table 3).

DISCUSSION/CONCLUSION

Our study suggests that frequent gene-smoking interactions could be associated with higher apoB concentration and eventually contribute to CVD risk, even when LDL-C levels are not elevated and in absence of FH or any criterion of the MetS. ApoB measurement, combined with life habits assessment may detect a significant proportion of patients at higher risk of CVD than predicted by guidelines (no component of MetS, no FH and LDL-C <3.5 mmol/L, in primary prevention). Life habits, including the diet and sedentarily importantly contribute to the expression of the MetS. Smoking and the MetS are well documented risk factors of atherosclerosis. Our results are in accordance with the fact that frequent genetic combinations involving key players in the VLDL catabolic pathway and lifestyle habits interactions, involving smoking, sedentarily and the diet, explain a significant proportion of CVD risk.

It is known that smoking-gene interactions influence atherosclerotic process, including the development of early vascular lesions and fat mass modification.6 Our results are in accordance with previous studies suggesting that smoking may alter the expression of genes, particularly those influencing LDL metabolism or TG and LDL particle size.7 The epigenetic signature of smoking in presence of hyper apoB is under study.

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