

Effect of Gene Polymorphisms on Lipoprotein Levels in Patients with Dyslipidemia of Metabolic Syndrome

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Summary

Dyslipidemia in the metabolic syndrome (MS) is considered to be one of the most important risk factors for atherosclerosis. It is characterized by hypertriglyceridemia, low concentration of plasma HDL-cholesterol, predominance of small dense LDL particles and an increased concentration of plasma apolipoprotein B (apoB). The pathogenesis of this type of dyslipidemia is partially explained, but its genetic background is still unknown. To evaluate the influence of cholesterol ester transfer protein (CETP) *TaqIB* polymorphism, lipoprotein lipase (LPL) *PvuII* and *HindIII* polymorphisms, hepatic lipase (LIPC) *G-250A* polymorphism and apolipoprotein C-III (APOC3) *SstI* gene polymorphism on lipid levels in dyslipidemia of the metabolic syndrome, 150 patients with dyslipidemia of metabolic syndrome were included. 96 % of patients had type 2 diabetes. The patients did not take any lipid lowering treatment. The exclusion criterion was the presence of any disease that could affect lipid levels, such as thyroid disorder, liver disease, proteinuria or renal failure. Gene polymorphisms were determined using the polymerase chain reaction and restriction fragment length polymorphisms. The genotype subgroups of patients divided according to examined polymorphisms did not differ in plasma lipid levels with the exception of apoB. The apoB level was significantly higher in patients with S1S1 genotype of *APOC3 SstI* polymorphism when compared with S1S2 group (1.10±0.26 vs. 0.98±0.21 g/l, p=0.02). Similarly, patients with H-H- genotype of *LPL HindIII* polymorphism had significantly higher mean apoB, compared with H+H- and H+H+ group (1.35±0.30 vs. 1.10±0.26 g/l, p=0.02). In the multiple stepwise linear regression analysis, apoB level seemed to be influenced by *APOC3 SstI* genotype, which explained 6 % of its variance. The present study has shown that the S1 allele of *APOC3 SstI* polymorphism and the H- allele of *LPL HindIII* polymorphism might have a small effect on apoB levels in the Central European Caucasian population with dyslipidemia of metabolic syndrome.

Key words

Apolipoprotein B • Dyslipidemia • Metabolic syndrome • Genetic polymorphisms

Introduction

The metabolic syndrome (MS) is characterized

by clustering of several cardiovascular risk factors, such as insulin resistance, glucose intolerance, abdominal obesity, dyslipidemia, hypertension, microalbuminuria, as

Table 1. Baseline characteristics of the study group.

<i>Sex (% males)</i>	27
<i>Age (years)</i>	62.3 ± 10.0
<i>Diabetes mellitus (%)</i>	96
<i>BMI (kg/m²)</i>	30.5 ± 5.3
<i>Glucose (mmol/l)</i>	8.13 ± 3.18
<i>HbA_{1c} (%)</i>	8.05 ± 2.47
<i>Hypertension (%)</i>	90
<i>Creatinine (μmol/l)</i>	90 (78; 108)
<i>Current smokers (%)</i>	14
<i>CAD (%)</i>	68
<i>Previous MI (%)</i>	30
<i>Previous stroke (%)</i>	24

BMI body mass index; HbA_{1c} glycosylated hemoglobin; CAD coronary artery disease; MI myocardial infarction.

well as procoagulation and proinflammatory states (Groop *et al.* 1993, Reaven 1994, Festa *et al.* 2000). MS is closely related to type 2 diabetes mellitus and may precede the diagnosis of type 2 diabetes by many years. Dyslipidemia of metabolic syndrome and type 2 diabetes mellitus is considered to be one of the most important risk factors in the pathogenesis of atherosclerosis. It is characterized by hypertriglyceridemia, low concentration of plasma HDL-cholesterol, predominance of small dense LDL particles (Ginsberg *et al.* 2000) and an increased concentration of plasma apoB, major protein component of LDL, IDL and VLDL particles (Wagner *et al.* 1999). Interventional studies have shown beneficial effects of statins and fibrates in treatment of this type of dyslipidemia resulting in lower incidence of cardiovascular and cerebrovascular events (Elkeles *et al.* 1998, Gavish *et al.* 2000).

Recently, research has been focused on the genetic background of atherosclerosis. The genotype may influence the development of atherosclerosis, e.g. by affecting lipoprotein levels, as well as by possible gene-environment interactions. Some authors have reported a similarity between dyslipidemia of metabolic syndrome and familial combined hyperlipidemia – the most common inherited disorder of lipid metabolism that substantially increases the risk of premature development of atherosclerosis (Shoulders and Naumova 2004).

In the present study we have concentrated on the gene polymorphisms of some enzymes and proteins which play an important role in the lipid metabolism. The

examined polymorphisms included cholesterol ester transfer protein (CETP) *TaqIB* polymorphism because of a key role of CETP in the transport of cholesterol esters and triglycerides between lipoprotein particles (Yamashita *et al.* 2000). Lipoprotein lipase (LPL) *HindIII* and *PvuII* polymorphisms were examined because of the key role of LPL in the metabolism of triglyceride-rich particles (TRL) (Goldberg 1996). We also examined apolipoprotein C-III (APOC3) *SstI* gene polymorphism because apoC-III also plays a key role in the metabolism of TRL as a noncompetitive inhibitor of lipoprotein lipase (Wang *et al.* 1985). Finally, hepatic lipase (LIPC) *G-250A* polymorphism was examined because of its important role in the metabolism of several lipoprotein particles, especially HDL particles (Hegele *et al.* 1993).

The aim of the present study was to look for the possible associations between the mentioned gene polymorphisms (*APOC3 SstI*, *CETP TaqIB*, *LPL HindIII*, *LPL PvuII*, and *LIPC G-250A*) and the lipid levels in the patients with dyslipidemia of the metabolic syndrome.

Methods

Subjects

One hundred and fifty patients with dyslipidemia of metabolic syndrome (39 males, 111 females) who were consecutively hospitalized in the Clinic of Internal Medicine of the University Hospital, were selected for the present study. The study was approved by the Hospital Ethics Committee. The metabolic syndrome and dyslipidemia of MS was diagnosed according to the criteria of NCEP ATP III. 96 % of patients had diabetes mellitus type 2 (according to the definition of the American Diabetes Association). The mean age of the study group was 62±10 years. The included patients did not receive any lipid-lowering treatment. Patients were excluded from the study if we detected any disease that could affect lipid levels, such as thyroid disorder, liver disease, nephrotic range proteinuria or renal failure. The demographic and clinical data of the patients are shown in Table 1.

Biochemical measurements and genotype detections

Blood was withdrawn after an overnight fasting for the determination of blood glucose, total cholesterol, HDL cholesterol and triglyceride levels, which were measured by routine biochemical assays. LDL cholesterol was calculated according to the Friedewald's formula: LDL cholesterol = total cholesterol – HDL cholesterol –

Table 2. Comparison of lipid levels among the genotypes.

Gene polymorphism	Genotype	Triglycerides (mmol/l)	Total cholesterol (mmol/l)	HDL cholesterol (mmol/l)	LDL cholesterol (mmol/l)
<i>CETP TaqBI</i> (n=109)	B1B1 (N=18)	2.24 (1.38; 2.99)	5.01 ± 1.10	0.90 ± 0.26	3.07 ± 1.00
	B1B2 (N=63)	2.47 (1.85; 3.79)	5.52 ± 1.45	1.00 ± 0.27	3.36 ± 1.27
	B2B2 (N=28)	2.03 (1.74; 3.03)	5.64 ± 1.00	1.09 ± 0.35	3.49 ± 0.89
	p	NS	NS	NS	NS
<i>LPL HindIII</i> (n=125)	H+H+ (N=43)	2.30 (1.95; 3.81)	5.52 ± 1.21	1.00 ± 0.28	3.36 ± 1.00
	H+H- (N=65)	2.09 (1.60; 3.26)	5.32 ± 1.31	1.01 ± 0.33	3.24 ± 1.20
	H- H- (N=10)	3.28 (2.24; 3.75)	6.20 ± 1.30	1.06 ± 0.16	3.78 ± 1.27
	p	NS	NS	NS	NS
<i>LPL PvuII</i> (n=111)	P+P+ (N=22)	1.99 (1.61; 3.83)	5.09 ± 1.44	1.02 ± 0.35	2.94 ± 1.11
	P+P- (N=57)	2.24 (1.72; 3.23)	5.57 ± 1.25	1.02 ± 0.31	3.39 ± 1.06
	P- P- (N=32)	2.72 (1.85; 3.59)	5.55 ± 1.25	0.99 ± 0.22	3.54 ± 1.19
	p	NS	NS	NS	NS
<i>LIPC G-250A</i> (n=108)	AA (N=41)	2.65 (2.04; 3.61)	5.34 ± 1.16	1.01 ± 0.27	3.22 ± 1.21
	AG (N=38)	2.01 (1.69; 3.11)	5.47 ± 1.38	1.05 ± 0.33	3.43 ± 1.18
	GG (N=29)	2.17 (1.46; 3.30)	5.49 ± 1.39	1.00 ± 0.31	3.24 ± 0.90
	p	NS	NS	NS	NS
<i>APOC3 SstI</i> (n=115)	S1S1 (N=92)	2.24 (1.71; 3.59)	5.56 ± 1.26	1.02 ± 0.29	3.40 ± 1.07
	S1S2 (N=23)	2.72 (2.03; 3.70)	5.07 ± 1.23	0.99 ± 0.31	2.99 ± 1.08
	S2S2 (N=0)	-	-	-	-
	p	NS	NS	NS	NS

Data are mean ± S.E., M. except of triglycerides given as median (25th, 75th percentile). NS – not significant

0.45 x triglycerides. Glycated hemoglobin and apoB were measured by immunoturbidimetric assays. The lipid profile was not completely examined in all the patients, apoB levels were missing in 37 patients.

DNA was extracted from blood cells by the salting out method and then was amplified by the polymerase chain reaction (PCR) using appropriate primers. The PCR products were further analyzed by standard restriction fragment length polymorphisms for each gene: *TaqIB* for *CETP* polymorphism (Brousseau *et al.* 2002), *HindIII* and *PvuII* for *LPL* polymorphisms (Ahn *et al.* 1993), *SstI* for *APOC3* polymorphism (Chhabra *et al.* 2002) and *G-250A* for *LIPC* polymorphism (Zambon *et al.* 1998).

Genotyping was performed in 150 patients. Genotype data for *CETP TaqIB*, *LPL HindIII*, *LPL PvuII*, *LIPC G-250A* and *APOC3 SstI* polymorphisms were available in 109, 125, 111, 108 and 115 patients, respectively.

Statistical analysis

Continuous variables are shown as mean ± S.D. with the exception of triglycerides and creatinine where median (25th, 75th percentile) is shown. Analysis of variance (ANOVA) and Student's t-test was used for the comparison of means among genotypes. Distribution of the categorical variables among genotypes was compared using the chi-square test. Multiple stepwise linear regression models were used in the multivariate analysis.

Results

One hundred and fifty patients were divided into three groups according to genotypes within each of the examined polymorphisms: *LPL HindIII*, *LPL PvuII*, *CETP TaqIB*, *APOC3 SstI* and *LIPC G-250A*. The groups were matched for age and gender representation. No significant difference was observed among individual genotype groups within each polymorphism in body mass

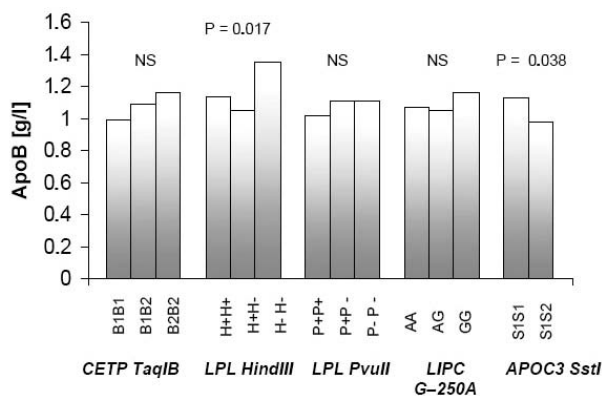


Fig. 1. Comparison of apolipoprotein B levels among particular genotypes.

index, blood pressure, creatinine, fasting plasma glucose or HbA1c values. Baseline characteristics of the whole study group are shown in Table 1.

When comparing the lipid levels, no significant effect of examined polymorphisms was observed on any lipid parameter with the exception of apoB (Table 2). A tendency towards higher mean values of triglycerides, total cholesterol and LDL-cholesterol was observed in H-/H- genotype of *LPL HindIII* polymorphism and in P-/P- genotype of *LPL PvuII* polymorphism. Similarly, we have observed a tendency towards higher levels of triglycerides in S1S2 genotype of *APOC3 SstI* polymorphism. In B1 allele carriers of *CETP TaqIB* polymorphism a tendency towards lower levels of HDL-cholesterol was observed.

When comparing the apoB levels, significant differences were observed only in *LPL HindIII* and *APOC3 SstI* polymorphisms (Fig. 1). Patients with S1S1 genotype of *APOC3 SstI* polymorphism (n=72) had significantly higher mean apoB, when compared with the S1S2 group (n=19) (1.10 ± 0.26 vs. 0.98 ± 0.21 g/l, $p=0.04$). After adjustment of apoB levels on sex, age, BMI, glycated hemoglobin and TSH, this association remained significant ($p=0.03$). We have also observed a significant association between *LPL HindIII* polymorphism and apoB levels. When the subjects with at least one H+ allele (n=84) were pooled and compared with H-H-homozygotes (n=7), significantly increased apoB levels were observed in H-H-homozygotes (1.35 ± 0.30 vs. 1.10 ± 0.26 g/l, $p=0.02$). After adjustment of apoB levels to sex, age, BMI, glycated hemoglobin and TSH, this association was not significant ($p=0.06$).

Factors predicting apoB levels were examined in the multiple stepwise linear regression analysis. In a multivariate model, with apoB as the dependent variable

and all the examined gene polymorphisms, age, gender, BMI, glycated hemoglobin and TSH as the independent variables, apoB level seemed to be influenced significantly by glycated hemoglobin ($p=0.02$) and *APOC3 SstI* genotype ($p=0.03$), each of them explained 6 % of its variance.

Discussion

The present study focused on the patients with dyslipidemia of MS has shown a significant association between both *APOC3 SstI* and *LPL HindIII* polymorphisms and apoB levels. We have observed significantly higher apoB levels in the patients with S1S1 genotype of *APOC3 SstI* polymorphism and with H-H-genotype of *LPL HindIII* polymorphism.

ApoB is the main protein component of LDL, IDL and VLDL particles. It reflects the number of TRL particles (VLDL and IDL) as well as the number of cholesterol-rich lipoprotein particles (LDL) (Wagner *et al.* 1999). In several prospective studies apoB has been shown to be a potent predictor of atherosclerosis development, both in the general population and in patients with type 2 diabetes mellitus (Lamarche *et al.* 1998, Wagner *et al.* 1999). Increased levels of apoB are considered to be a typical feature of dyslipidemia of metabolic syndrome and type 2 diabetes mellitus (Wagner *et al.* 1999).

The *APOC3 SstI* polymorphism results from a transversion from C to G in the position 3238 in the 3' untranslated region of exon 4 in APOC3 gene, which is located on the long arm of chromosome 11 (Bruns *et al.* 1984, Karathanasis 1985). Several studies have suggested an association between rare S2 allele (G3238) of SstI polymorphism and elevated apolipoprotein C-III, triglycerides (Talmud and Humphries 1997), total cholesterol levels and higher cardiovascular risk (Ordovas *et al.* 1991, De Lorenzo *et al.* 1994). In the Framingham Offspring Study, S2 allele was associated with elevated total cholesterol, LDL-cholesterol and apoB levels in women, but not in men (Russo *et al.* 2001). Similarly, in the study of a population of northern France, triglycerides and apoB levels were higher in women bearing the S2 allele. However, there was no evidence for any significant association between *APOC3 SstI* polymorphism and lipid disorders in this study (Dallongeville *et al.* 2000). In contrast, our results have shown an connection between S1 (but not S2) allele and higher apoB levels in the whole study group, and preferentially

in women. The mechanism by which APOC3 genotype influences apoB levels has not been clearly explained yet. The research of the *APOC3 SstI* polymorphism has focused more on the association between the rare S2 allele and susceptibility to hypertriglyceridemia (Stocks *et al.* 1987, Tybjaerg-Hansen *et al.* 1993, Hoffer *et al.* 1998). In the present study, when comparing triglyceride (TG) levels between S1S1 and S1S2 genotypes, a tendency towards higher levels of TG in S2 allele carriers was also observed. However, several studies in nondiabetic populations with diverse geographical and ethnical origin show contradictory results (Price *et al.* 1986, Marcil *et al.* 1996). Since the *SstI* polymorphism is located in the 3' untranslated region of APOC3 gene, some authors suggest that S2 allele may not be etiologic but in a linkage disequilibrium with other causative mutations hitherto unknown in APOC3 or a nearby gene involved in determining the TG levels (Chhabra *et al.* 2002). It has been speculated that the linkage disequilibrium between this polymorphic site and the causative mutation is weakened or absent in some populations (Shoulders *et al.* 1996). It has to be mentioned that there are no S2S2 homozygotes in our study group because of the very low frequency of S2 allele in the Central European population.

CETP TaqIB polymorphism represents a silent base change affecting the nucleotide 277 in the fMstI intron 1 of the gene (Kuivenhoven *et al.* 1997), resulting in the disruption of a restriction site for the enzyme *TaqI*. The relationship between B2 allele (absence of the *TaqI* restriction site) and higher levels of HDL cholesterol has been demonstrated in several studies in both diabetic and non-diabetic populations (Brousseau *et al.* 2002, Yilmaz *et al.* 2004). Individuals carrying the B2 allele showed lower CETP activity (Freeman *et al.* 1990) and lower CETP mass concentration (Fumeron *et al.* 1995) resulting in higher HDL and apoA-I levels (Gudnason *et al.* 1999). In this study we have observed only a tendency towards higher HDL levels in B2 allele carriers, which did not reach statistical significance. This could be explained by a lower statistical power of the study due to a relatively small number of included patients. The B1 allele has been associated with higher prevalence of coronary heart disease in several studies (Durlach *et al.* 1999, Ordovas *et al.* 2000) and this relationship was mediated by lower

HDL cholesterol levels as the recent meta-analysis has shown (Boekholdt *et al.* 2005).

LPL HindIII polymorphism is caused by the presence or absence of T→G transition in the position +495 in intron 8 of LPL gene (Gotoda *et al.* 1992). In some studies, the common H+ allele of the *LPL HindIII* polymorphism has been shown to be significantly associated with hypertriglyceridemia (Jemaa *et al.* 1995ab, Mattu *et al.* 1994), hypercholesterolemia (Heizmann *et al.* 1991), lower levels of HDL (Heizmann *et al.* 1991, Gerdes *et al.* 1995, Jemaa *et al.* 1995ab), increased apoC-III (Jemaa *et al.* 1995ab) and apoB (Mattu *et al.* 1994), and premature coronary heart disease (Mattu *et al.* 1994, Gerdes *et al.* 1995, Jemaa *et al.* 1995ab, Thorn *et al.* 1990). However, another reports have failed to note such an association (Vohl *et al.* 1995, Georges *et al.* 1996). In the present study, the H- allele was associated with significantly increased apoB levels. Furthermore, H- allele carriers tended towards higher levels of TG, total cholesterol, and LDL cholesterol.

In conclusion, the present study has shown that the S1 allele of *APOC3 SstI* polymorphism and H- allele of *LPL HindIII* polymorphism might have a small effect on apoB levels in Central European Caucasian population with dyslipidemia of the metabolic syndrome. Discrepancy between our results and other reports may be explained by the fact that the effect of the studied polymorphisms might be different in a specific population of predominantly diabetic patients included in the present study. Ethnic differences cannot also be excluded. Finally, the small sample size could be responsible for the inability to reproduce some of the previously reported associations. The above presented data suggest that the effect of examined polymorphisms on lipid levels is small and probably not of high clinical importance. These preliminary observations have to be verified in a larger study group. It also has to be determined in further pharmacogenetic studies, whether the above mentioned polymorphisms affect the response to lipid-lowering treatment in this high risk population.

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