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ORIGINAL ARTICLE

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The C766T low-density lipoprotein receptor related protein polymorphism and coronary artery disease, plasma lipoproteins, and longevity in the Czech population

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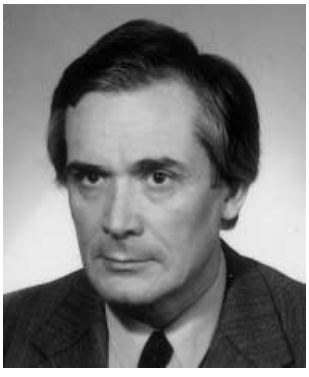

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Abstract Low-density lipoprotein receptor related protein (LRP) is a multifunctional endocytic receptor involved in various biological processes including the regulation of the coagulation-fibrinolysis balance, the lipoprotein metabolism, and cellular migration, all of which relate to the development of atherosclerosis. Polymorphisms affecting the function or expression of LRP may thus influence the individual risk of atherosclerosis development. This study investigated the association between the C766T LRP polymorphism, coronary artery disease (CAD), and plasma lipoprotein levels in a large sample of Caucasian subjects of Czech nationality. In addition, the 4G/5G promoter polymorphism of the gene coding for plasminogen activator inhibitor 1 (PAI-1), the known ligand of LRP with strong antifibrinolytic potential, was ascertained to investigate its possible association with CAD. Both polymorphisms were studied using polymerase chain reaction analysis in 654 patients with angiographically confirmed CAD and in 525 controls. No statistically significant differences in allele frequencies of the polymorphisms studied were detected between patients and controls, even when men, women, hypertonic, and type II diabetic subjects were compared separately. However, the frequency of the T allele of the LRP polymorphism was significantly higher in patients than controls when only subjects with the 5G/5G PAI-1 genotype were analyzed. In addition, the T LRP allele frequency was significantly lower in subjects aged 60 years or over than in those who were younger in both groups. No significant association was observed between the LRP or PAI-1 polymorphisms and plasma lipoprotein levels in the CAD patients. Our results demonstrate that the T allele of the C766T LRP polymorphism is negatively related to longevity, and that it increases the risk of CAD development in subjects with the 5G/5G PAI-1 genotype.

Keywords Low density lipoprotein receptor related protein · Plasminogen activator inhibitor 1 · Coronary artery disease · Genetic polymorphism · Longevity · Plasma lipoproteins

Abbreviations *CAD:* Coronary artery disease · *LRP:* Low-density lipoprotein receptor related protein · *MI:* Myocardial infarction · *PAI-1:* Plasminogen activator inhibitor 1 · *PCR:* Polymerase chain reaction

Introduction

Low-density lipoprotein receptor related protein (LRP) is a multifunctional endocytic receptor that is able to bind and endocytose several structurally and functionally distinct ligands, including α_2-macroglobulin–protease complexes, plasminogen activators, plasminogen activator/inhibitor complexes, lipoprotein lipase, chylomicrons, and β-migrating very low density lipoproteins [1, 2]. The protein structure of LRP is highly homologous to the low and very low density lipoprotein receptors [2]. LRP plays an important role in lipid metabolism by mediating the cellular uptake of chylomicrons [3]. The expression of LRP is induced in atherosclerotic lesions in smooth muscle cells and macrophages [4], and this receptor may thus contribute to cellular lipid accumulation and to foam cell formation [5]. Kang et al. [6] found a common C766T polymorphism in exon 3 of the LRP gene. Although this polymorphism does not affect LRP protein structure, the CC genotype was previously shown to be associated with Alzheimer's disease [6, 7], suggesting that this polymorphism is of functional importance or is a marker of other functional polymorphisms located in this gene. Due to the suggested function of LRP in lipoprotein metabolism and atherogenesis, this study was undertaken to investigate an association between the C766T LRP polymorphism, coronary artery disease (CAD) and plasma lipoprotein levels.

In addition, we investigated the 4G/5G promoter polymorphism of the gene for plasminogen activator inhibitor 1 (PAI-1) [8], a known ligand of LRP with strong antifibrinolytic potential [2, 9], for a possible association with CAD. The 4G allele of this polymorphism was previously shown to increase plasma PAI-1 concentrations [9] and to be associated with an increased risk of myocardial infarction (MI) [10, 11]. However, our previous work did not confirm this association in a sample of young Czech men [12].

Materials and methods

Subjects

The patients enrolled in this study were those with angina pectoris or a history of MI who were referred between August 1996 and February 2000 to the Center of Cardiovascular Surgery and Transplantation in Brno, to the Department of Cardiopulmonary Testing, University Hospital Brno-Bohunice, and to the Cardioangiology Department of the First Clinic of Internal Medicine. Patients with valvular diseases or cardiomyopathies were excluded. Each patient's disease status was defined according to the angiography of the coronary vessels. Significant CAD, defined as an at least 50% reduction in the diameter of the left main stem or an at least 70% reduction in the diameter of one of the major coronary arteries or their branches, was confirmed in 654 patients (527 men, 127 women; mean age 58.8±8.8 years, range 27–80 years). On the basis of the results of coronary angiography, the patients were divided into three groups: those with one-vessel (n=112), two-vessel (n=224), and three-vessel disease (n=314). A significant left main stem stenosis was classified as equivalent to two diseased vessels. The diagnosis of previous MI was ascertained from the patients' hospital records using the criteria of the World Health Organization. In addition, the patient's age when the first symptoms of myocardial ischemia (MI, angina pectoris) occurred in this group was recorded according to the patients anamnestic data; the overall mean was 54.7±9.3 years.

Table 1 Clinical characteristics of the CAD and the control groups

	CAD group (n=654)	Control group (n=525)
Age (years)	58.8±8.8	56.8±11.4
Men/women	527/127	402/123
Previous MI	444 (67.9%)	None
One-vessel disease	112	None
Two-vessel disease	228	None
Three-vessel disease	314	None
Diabetes mellitus	168 (25.7%)	123 (23.4%)
Hypertension	440 (67.3%)	280 (53.3%)

The control group consisted of 525 volunteers (402 men, 123 women; mean age 56.8±11.4 years, range 35–93 years) with no symptoms or clinical signs of CAD. They were recruited from various districts of the city of Brno by general practitioners from their clientele. The controls were selected to include a higher proportion of diseases which increase the risk of CAD (essential hypertension and type II diabetes mellitus) than reported previously in Czech population [13] to allow comparison of the polymorphisms studied between the CAD and the control group. Type II diabetes mellitus was diagnosed in the patients and the controls previously according to the WHO criteria. All diabetic subjects regularly attended a diabetic outpatient clinic, and an antidiabetic treatment was established. The diagnosis of essential hypertension in both groups was ascertained on the basis of a long-term antihypertensive treatment and/or according to repeated findings of blood pressure of 140/90 mmHg or higher. The presence of hypolipidemic drug treatment was ascertained in both groups from the subjects' personal records. Patients and controls were unrelated Caucasians of Czech nationality. Clinical characteristics of the CAD and the control groups are shown in Table 1.

Methods

Blood samples were collected after an overnight fast. Genomic DNA was extracted from peripheral blood leukocytes. The LRP C766T genotypes were determined by a polymerase chain reaction (PCR) and a subsequent restriction analysis with *Rsa*I restriction endonuclease as described elsewhere (Fig. 1) [7]. The PAI-1 4G/5G genotypes were determined by an allele-specific PCR as described previously (Fig. 1) [12]. In the CAD group, plasma concentration of total cholesterol, high-density lipoprotein cholesterol, and triglycerides were measured by enzymatic methods (Lachema Brno, diagnostic kits); apolipoprotein B and apolipoprotein AI by immunoturbidimetric methods (Boehringer-Mannheim); and low-density lipoprotein cholesterol was calculated using the Friedewald equation [13]. The lipoprotein and apolipoprotein data were available from 352 CAD patients not taking hypolipidemic drugs for total cholesterol; 288 for low-density lipoprotein cholesterol, 294 for high-density lipoprotein cholesterol, 328 for triglycerides, 232 for apolipoprotein B and 229 for apolipoprotein AI.

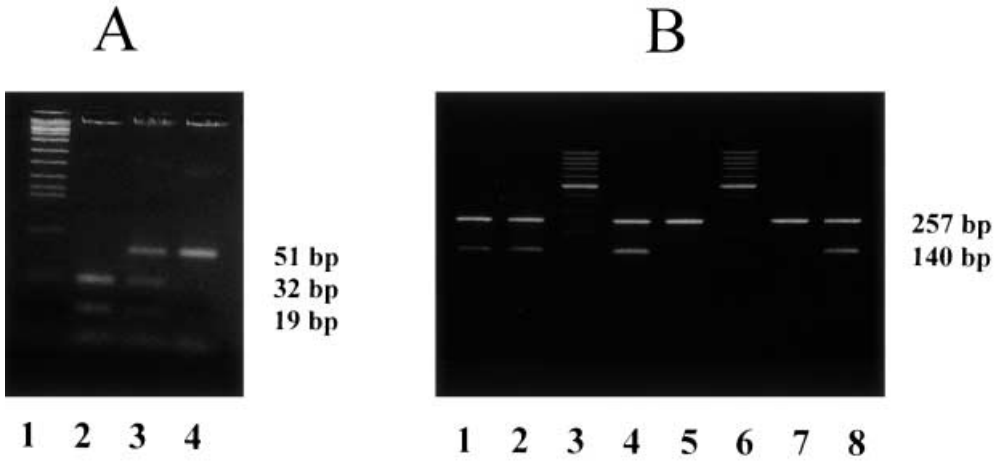

Fig. 1 **A** Typing of LRP polymorphism by restriction digestion of PCR products with *Rsa*I restriction enzyme. *Lane 1* DNA Molecular Weight Marker VIII (Roche); *lane 2* T/T homozygote; *lane 3* C/T heterozygote; *lane 4* C/C homozygote. **B** Typing of PAI-1 polymorphism by allele-specific PCR. *Lanes 3, 6* GeneRuler 50 bp Ladder (Fermentas); *lanes 1, 2* 5G/4G heterozygote; *lanes 4, 5* 5G/5G homozygote; *lanes 7, 8* 4G/4G homozygote. Each sample was amplified with 5G (*lanes 1, 4, 7*) or 4G allele specific primer (*lanes 2, 5, 8*). DNA fragment of 257 bp served as positive control

Table 2 Distribution of genotypes and allele frequencies of C766T LRP polymorphism in the CAD and the control groups and in both groups according to patients' age

	C/C	C/T	T/T	C	T	*P*
Overall set of patients						NS
CAD group (*n*=654)	462	180	12	0.844	0.156	
Control group (*n*=525)	377	136	12	0.848	0.152	
CAD group						0.018
≤60 years (*n*=375)	253	113	9	0.825	0.175	
>60 years (*n*=279)	209	67	3	0.869	0.131	
Control group						0.003
≤60 years (*n*=351)	240	100	11	0.826	0.174	
>60 years (*n*=174)	137	36	1	0.891	0.109	

Statistical analysis

Differences in genotype distributions from those expected for Hardy-Weinberg equilibrium were tested by the χ^2 test. The significance of differences in allele frequencies between groups was tested by Fisher's exact test. A multiple linear regression analysis was performed as an independent validation of the associations identified. The Kruskal-Wallis analysis of variance was used to test the differences between plasma lipoprotein parameters and LRP and PAI-1 genotypes. $P<0.05$ was considered statistically significant. For all statistical analyses CSS Statistics version 3.0 Program Package (Statsoft) was used.

Table 3 Distribution of genotypes and allele frequencies of 4G/5G PAI-1 polymorphism in the CAD and the control groups (differences nonsignificant)

	4G/4G	4G/5G	5G/5G	4G	4G
CAD group (*n*=654)	208	321	125	0.563	0.437
Control group (*n*=525)	171	261	93	0.574	0.426

Results

The distributions of the C766T LRP and the 4G/5G PAI-1 genotypes and allele frequencies in the samples studied are shown in Tables 2 and 3. In all the samples studied the allele frequencies of neither polymorphisms differed from Hardy-Weinberg equilibrium. No statistically significant differences were found in allele frequencies between the CAD and control groups either in the overall sample (Tables 2, 3) or when men and women were analyzed separately (data not shown). Similarly, no difference was observed between the CAD and the control groups when only hypertensive or diabetic subjects were compared (data not shown).

When the LRP allele frequencies between the CAD and the control group were compared according to PAI-1 genotype, the T LRP allele frequency was significantly higher in the CAD group than in the control group among PAI-1 5G/5G homozygotes ($P<0.05$) but not among 5G/4G heterozygotes or 4G/4G homozygotes (Table 4). To confirm this association, a multiple linear regression analysis was performed separately for each PAI-1 genotype subgroup with CAD as a dependent variable and age, gender, hypertension, type II diabetes, and LRP genotypes as independent ones. In the group of PAI-1 5G/5G homozygotes only the LRP genotype and age were significantly related to CAD ($P<0.05$; $P<0.01$) while no associations between LRP genotype and CAD were found in other PAI-1 genotype groups.

The LRP and the PAI-1 allele frequencies proved to be related neither to a history of MI nor to the severity of CAD in the CAD group (data not shown). No significant association was observed between the LRP or PAI-1 polymorphisms and plasma lipoprotein levels in subjects from the CAD group not taking hypolipidemic drugs.

Table 4 Distribution of genotypes and allele frequencies of C766T LRP polymorphism in the CAD and the control groups according to 4G/5G PAI-1 genotypes

	C/C	C/T	T/T	C	T	P
PAI 4G/4G						NS
CAD (*n*=208)	154	52	2	0.865	0.135	
Control (*n*=171)	120	48	3	0.842	0.158	
PAI 5G/4G						NS
CAD (*n*=321)	228	86	7	0.844	0.156	
Control (*n*=261)	184	70	7	0.839	0.161	
PAI 5G/5G						0.025
CAD (*n*=125)	80	42	3	0.808	0.192	
Control (*n*=93)	73	18	2	0.882	0.118	

In the CAD group the LRP allele frequencies differed significantly between subjects aged 60 years or older vs. those younger; a similar but more pronounced difference was observed in the control group (Table 2). In the overall set of subjects (patients plus controls) the C allele was significantly more frequent in older (>60 years) than in younger (≤60 years) subjects, but only among those with the PAI-1 5G/5G (0.902 vs. 0.798, 70/17/0 and 83/43/5, $P<0.01$) and 5G/4G (0.869 vs. 0.825, 166/54/2 and 246/102/12, $P<0.05$) genotypes, not in the 4G/4G homozygotes (0.875 vs. 0.843, 110/32/2 and 164/68/3).

Discussion

LRP is a multifunctional endocytic receptor playing a role in the regulation of plasma lipoprotein and plasma fibrinolytic parameters [1, 2] which both belong to the risk factors of CAD. We examined the hitherto unstudied relationship between the C766T LRP polymorphism, previously shown to be associated with Alzheimer's disease [6, 7], and CAD and plasma lipoprotein levels. This study also included the 4G/5G PAI-1 polymorphism, previously studied by us in young Czech men in relation to CAD or MI with negative results [12].

No significant associations between the LRP polymorphism and CAD, MI, or the severity of CAD were observed in the overall sample or when men, women, diabetic, or hypertonic subjects were analyzed separately. The only significant association between the LRP genotype and CAD was observed in the group of the PAI-1 5G/5G homozygotes, where the LRP T allele was more frequent in the CAD group. Interestingly, although the CAD patients were older than the controls in this subgroup (mean age 59.76±8.89 vs. 56.23±12.06 years), they had an increased frequency of the LRP T allele, which was found, however, to be less frequent in older subjects in the whole sample. An independent association between the LRP genotype and CAD in the PAI-1 5G/5G subgroup was confirmed by multiple linear regression analysis.

Complexes of plasminogen activators and PAI-1 belong to known ligands of LRP [1, 2]. Increased plasma concentration of PAI-1 is an established risk factor of CAD or MI [9]. The 4G/5G PAI-1 promoter polymorphism has been shown to influence the plasma PAI-1 concentration; the 5G allele is characterized by a lower transcription rate of the PAI-1 gene because of binding a transcriptional repressor protein which cannot be bound by the 4G allele [8]. Although some associations between the 4G/5G PAI-1 polymorphism and MI have been reported in certain studies [10, 11], we did not confirm those findings in our previous work [12]. A recent meta-analysis suggested that preexisting coronary atheroma and/or metabolic dysfunction are necessary to unravel the effect of the PAI-1 genotype [15]. In our previous study the apolipoprotein(a) TTTTA repeat polymorphism was found to be a CAD risk factor only in subjects with the PAI-1 4G/4G genotype [12]. The interaction between the PAI-1 and LRP genotypes described in the present study confirms a high interactive potential of the PAI-1 4G/5G polymorphism with other gene polymorphisms with respect to the CAD etiopathogenesis.

Concerning the relationship between the LRP polymorphism and plasma lipoprotein parameters, no association between this polymorphism and plasma lipoprotein and apolipoprotein levels was observed. Previously Hegele et al. [16] reported that another polymorphism in the LRP gene (the tetranucleotide repeat polymorphism in the 3′ untranslated region) was only weakly associated with total plasma cholesterol among Hutterites in Alberta, Canada. These results suggest that the effect of the LRP polymorphisms studied on plasma lipoprotein levels is only of minor importance.

An interesting finding was that the allele frequencies of the LRP polymorphism differed both in the CAD and control groups between subjects aged 60 years or over and those aged under 60 years, with a higher frequency of the C allele in the older subjects. This finding suggests that this polymorphism is a longevity factor especially in combination with the PAI-1 5G allele because the difference was observed only in subjects with this allele. In addition, the the LRP T allele is associated with CAD in the PAI-1 5G/5G homozygotes. The increased mortality for CAD in subjects with the LRP T allele and PAI-1 5G allele may thus be linked to the association observed. Age-dependent changes in the LRP gene expression have been observed in the CAD patients and the control subjects by Handschug et al. [17] however, these changes were in opposite direction in the CAD and the control group. The function of LRP as a longevity factor may be also related to its suggested role in tumor pro-

gression. LRP regulates proteinase activity, which is necessary for tumor invasiveness and neovascularization [18, 19]. Interestingly, the allele frequencies of the intron 5 Ins/Del polymorphism of the gene coding for receptor-associated protein [20] which serves as a molecular chaperone within the endoplasmic reticulum to assist the folding of LRP [21] differed also between the CAD subjects aged 60 years or over and those aged under 60 years, with a higher frequency of the Ins allele in the older subjects (unpublished observation).

In conclusion, we found that the T allele of the C766T LRP polymorphism represents a minor CAD risk factor, namely in the PAI-1 5G/5G homozygotes. This LRP allele in combination with the 5G PAI-1 allele seems to be less frequent in elderly subjects, suggesting that it may represent a genetic longevity factor in the Czech population.

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