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ORIGINAL ARTICLE

THE *LRP1* GENE POLYMORPHISM IS ASSOCIATED WITH INCREASED RISK OF METABOLIC SYNDROME PREVALENCE IN THE SERBIAN POPULATION

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ABSTRACT

The determination of genetic background in metabolic syndrome (MetS) represents one of the necessary steps to prevent the disorder, thus reducing the cost of medical treatments and helping to design targeted therapy. The study explores the association between individual alleles of the *LRP1* gene and the diagnosis of MetS to find correlation between the low-density lipoprotein receptor-related (*LRP1*) gene polymorphism and each individual anthropometric and biochemical parameter. The study included 93 males and females, aged from 19 to 65, divided into two groups. The genotype of each person was determined from the restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) profile. Results indicated the association of the T allele form of exon 3 *LRP1* gene with development and progression of MetS that further pointed out its negative impact on tested anthropometric and biochemical parameters. The presence of the T allele in patients multiplies the chance of occurrence of deviations from the reference values of body mass index (BMI), (4.24-fold) and low-density lipoprotein (LDL) (20.26-fold) compared to C allele carriers. The results showed that T allele presence multiplies the chance (4.76 fold) for the occurrence of MetS in comparison to C allele carriers. Correlation found that the T allele of the *LRP1* gene with MetS determinants is not negligible, therefore, the T allele may be considered as a risk factor for MetS development.

Keywords: Genetic predisposition; Lipid metabolism; Low-density lipoprotein receptor-related (*LRP1*) gene polymorphism; Metabolic syndrome (MetS); T allele.

INTRODUCTION

During the past 50 years, numerous dramatic changes in human environment as well as behavioral and lifestyle changes, have led to a global increase in obesity and type 2 diabetes mellitus (T2DM). Both diseases are reaching epidemic proportions in developed and developing countries and their co-occurrence represents one of the biggest health threats in the 21st century [1,2].

Metabolic syndrome (MetS) has been described as a cluster of risk factors for cardiovascular diseases (CVDs) and T2DM, primarily due to the existence of abdominal obesity and insulin resistance [3]. Patients with MetS have a 3-fold higher risk of experiencing a heart attack or stroke and a 2-fold higher risk of fatal outcome compared to the general population. Recent findings revealed that MetS increases the risk for the prevalence of microalbuminuria, which is crucial in this syndrome because it accelerates the progression of chronic kidney disease and increases the prevalence of cardiovascular events [4]. Fujita [5] recently demonstrated the possible involvement of aldosterone/ mineralocorticoid receptor activation in hypertension development and renal injury in obesity-induced hypertension with MetS. Pathophysiological abnormalities that contribute to the development of MetS include impaired mitochondrial oxidative phosphorylation and

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mitochondrial biogenesis, dampened insulin metabolic signaling, endothelial dysfunction, and associated myocardial functional abnormalities [6]. Introduction of drug therapy for MetS should not be delayed, as adherence to lifestyle modifications such as dietary changes, weight reduction, and exercise is not achieved in most cases [7].

All components of MetS are considered to be multifactorial traits. The identification of a genetic component of MetS is difficult due to the complexity of MetS and variability of lifestyle factors. However, linkage analysis, candidate gene approach and genome-wide association studies (GWAS) suggested that MetS is a polygenic and multifactorial disease, developing as a result of complex interactions of many genes and environmental factors [8]. Investigations of the genetic basis of this syndrome represent a major challenge [2-4]. Metabolic syndrome is a complex polygenetic disorder of metabolism including central obesity, dyslipidemia, hypertension, and hyperglycemia. Genetic predisposition is one of the risk factors that cannot be controlled, but the knowledge of genetic basis allows us to correct other, modifying, environmental factors, thereby disease can be delayed or prevented. Understanding the importance of genetic and environmental factors, as well as their interactions is critical for finding specific treatments and identifying individuals at high risk of becoming ill. Determining the genetic basis of MetS is one of the necessary steps in disease prevention, and in designing targeted therapies [9].

The *LRP1* gene is located on human chromosome 12q13-14 and encodes low-density lipoprotein receptor-related protein [5]. To date, several known mutations of this gene are: intron 2 (C>T), exon 3 (C>T), exon 6 (C>T), intron 6 (G>C), intron 19 (C>T), exon 22 (C>T), intron 38 (C>T), exon 61 (G>A) and intron 83 (G>A). Protein LRP1 is a multifunctional receptor with two main biological functions: endocytosis of many ligands and control and integration of intracellular signaling pathways that are essential for the survival of the cells themselves, but are also essential for the maintenance of basal cell function and development and survival of the organism. Because of its importance, LRP1 is expressed in almost all cells [10]. Mutation within exon 3 of the *LRP1* gene, C>T at position 667 that does not change the amino acid sequence of the protein, is associated with Alzheimer's disease [6,11], cerebral amyloid angiopathy [12], increased factor VIII (FVIII) level in plasma and the risk of venous thromboembolism [13]. It also affects the expression of markers for adipocyte differentiation and the maintenance of lipid levels in mature adipocytes, and has a key role in lipid metabolism [14,15]. Liu *et al.* [16] found that LRP1, which is critical in lipid metabolism, also regulates food intake and energy homeostasis in adult central nervous system. The investigation of Niemeier *et al.* [17] showed that among the low-density lipoprotein receptor (LDLR) family in human osteoblasts, LRP1 plays a predominant role in vitamin K1 uptake through chylomicron remnants endocytosis. Association between LRP1 and lung cancer was investigated in study of Meng *et al.* [18] pointing out that LRP1 expression is associated with improved lung cancer outcomes. The *LRP1* gene is highly variable in different populations. Looking at the entire human population, frequency of the T allele of exon 3 *LRP1* gene was 22.0%, and the C allele was 78.0%. To date, frequency of *LRP1* exon 3 alleles have not been analyzed in the Serbian population.

The LRP1 receptor is one of the major ApoE binding receptors in the liver, muscles, heart and adipose tissue. As participating in such a large number of physiological processes, functional clarification of these mechanisms and further identification of LRP1 partners can open up new aspects in the treatment of metabolic diseases, such as lipid metabolism disorders, atherosclerosis, obesity, Alzheimer's disease and inflammatory processes [9,19,20]. Diet-induced obesity and its serious consequences, such as diabetes, cardiovascular disease and cancer, rapidly became one of the biggest global health problems. Thus, the clarification and understanding of the cellular and molecular mechanisms by which fat food intake causes obesity and diabetes is essential to identify preventive and therapeutic strategies [21]. Data findings identify LRP1 as a critical regulator of adipocyte energy homeostasis, where functional impairment of LRP1 leads to reduced lipid transport, increased insulin sensitivity and muscle power consumption [22]. Thanks to its role in these processes, associations of the *LRP1* gene and various pathological conditions was investigated. Although a 667 (C>T) polymorphism does not change an amino acid sequence, this polymorphism influences the splicing efficiency of exon 3 in an exon-trapping assay, leading to a small, but still significant decrease in full-length LRP mRNA produced form the *LRP* gene with the C allele [23]. As far as we know, none of the diseases are associated with mutations in the *LRP1* gene coding region [15].

The main goals of the present study were to determine the association between individual alleles of the exon 3 *LRP1* gene and MetS development and correlation analyses between the *LRP1* gene polymorphism and individual anthropometric and biochemical parameters. To the best of our knowledge, this is the first study demonstrating the association of the *LRP1* gene polymorphisms with MetS incidence as well as with the individual components of MetS.

MATERIALS AND METHODS

Study Subjects. The study included 93 individuals (males and females), aged from 19 to 65 [X ± standard deviation (SD)], attending the Clinical Center of Vojvodina, Novi Sad, Serbia. All subjects were divided in two groups. The MetS group consisted of 63 unrelated men and women who were diagnosed as MetS patients. The control group consisted of 30 unrelated healthy men and women, sex- and age-matched with the test group. The International Diabetes Federation (IDF) definition was applied to define the MetS group of patients [24]. To be diagnosed as MetS, participants needed to fulfill the following criteria: to have central obesity defined by waist circumference (WC) at least 94 cm in men and 80 cm in women, plus two of the following, hyperglycemia defined as fasting plasma glucose of at least 5.6 mmol/L, high blood pressure (BP) defined as resting BP of at least 130/85 mmHg or known treatment of hypertension hypertriglyceridemia defined as triglycerides (TG) at least 1.7 mmol/L low high-density lipoprotein (HDL) defined as fasting HDL cholesterol less than 1.0 mmol/L in men and less than 1.3 mmol/L in women [24,25]. Signed informed consent was obtained from all participants and the study was approved by the Institutional Ethics Review Committee and was performed according to the Declaration of Helsinki.

Anthropometric Measurements and Biochemical Parameters Determination. Anthropometric measurements [body mass (BM), body height (BH) and WC), and cardiovascular risk factors assessment [systolic and diastolic BP, fasting serum lipids levels, glucose levels, C-reactive protein (CRP)] were determined. With the subjects wearing light indoor clothes and no shoes, BM and BH were measured using a calibrated beam-type balance to the nearest 0.1 kg and a Harpenden anthropometer to the nearest 0.1 cm, respectively, and body mass index (BMI) was calculated [BMI = (body weight) BW/BH^2 (kg/m^2)]. Waist circumference was measured using flexible tape to the nearest 0.1 cm at the level midway between the lowest point on the rib margin and the highest point on the iliac crest. Systolic and diastolic BP were measured in a fasting state, early in the morning, using sphygmomanometer by Scipione Riva-Rocci (Italian inventor of cuff-based version of the mercury sphygmomanometer for the measurement of blood pressure; 1863-1937) in sitting position after a 10-15 min. rest period. The mean of three measurements was taken as the most valid value. Total cholesterol (TC) and TG were determined using a commercial kit (Boehringer Manheim GmbH, Mannheim, Germany). High-density lipoprotein cholesterol was estimated using the method of precipitation with sodium phospho-wolframate, while LDL-cholesterol was calculated using the formula by Friedewald *et al.* [26]. Fasting plasma glucose was measured using the Dialab glucose GOD-PAP (Dialab GmbH, Wiener Neudorf, Austria), method. The CRP levels were done by Latex (Dialab GmbH) immunoturbidimetric method. All blood samples were drawn after an overnight 12-hour fast.

Genotyping of the *LRP1* Gene. Total genomic DNA was isolated from EDTA-anticoagulated blood using phenol chloroform isoamylalcohol extraction [27]. Exon 3 of the *LRP1* gene was amplified with standard primer set F (5'-CCA TAG CCA GCT TGT TCA TG-3') and R 5'-ACG GGA GAG TAG AGA GTG G-3') [19]. Polymerase chain reaction (PCR) amplification was done according to the modified method of Kang *et al.* [20]. The PCR reaction was performed in a 25 μL final volume containing 100 ng of genomic DNA as a template with 0.4 μM of each primer, 200 μM dNTPs (dATP, dTTP, dCTP, dGTP), 1 × Buffer, 1.5U Taq polymerase, 0.5 × Q solution and 4 mM. The *LRP1* gene was amplified for 30 cycles, consisting of 30 seconds at 94 °C, 30 seconds at 55 °C and 30 seconds at 72 °C. Final extension at 72 °C for 10 min. was applied. The PCR products were digested with *Fok*I over a minimum period of 5 hours at 55 °C.

The LRP1 fragments obtained were separated by electrophoresis on a 4.0% MetaPhor agarose gel in 1 × TAE buffer and visualized by ethidium bromide fluorescence. The genotype of each person was determined from the restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) profile.

Data Analyses. Statistical analysis of data was performed by software package STATISTICA, version 10.0 (http://www.statsoft.com/Products/STATISTICA-Features/Version-10) [28]. Odds ratio (OR) as estimate of relative risk for disease were calculated and 95% confidence intervals (CI) obtained by logistic regression, were used to test the relationship between MetS and LRP1 alleles, as well as their associations with selected anthropometric and biochemical parameters.

RESULTS

In the study 93 subjects were examined and analyzed, a group of 63 MetS patients and the control group of 30 healthy subjects. Exon 3 of the *LRP1* gene was successfully amplified for each subject, and the product was 212 bp in length. In both groups, the genotype of the *LRP1* gene for each person was successfully determined. The results of the *t*-test revealed significant differences ($p < 0.05$) in

Table 1. The Student's *t*-test results between the metabolic syndrome and control groups.

Parameters	Arithmetic Mean			
	Control Group	MetS Group	*t*-Test	*p* Value
Age (years)	38.70	42.10	–1.29	0.20
BM (kg)[a]	87.33	123.30	–4.98	0.00
BH (cm)	170.13	170.86	–0.16	0.87
BMI (kg/m^2)[a]	25.99	41.50	–8.97	0.00
WC (cm)[a]	88.90	125.67	–9.77	0.00
Systolic BP (mmHg)[a]	120.50	139.37	–4.91	0.00
Diastolic BP (mmHg)[a]	77.33	89.60	–4.20	0.00
TC (mmol/L)	5.18	5.48	1.01	0.31
Triglycerides (mmol/L)[b]	3.57	2.12	2.33	0.02
HDL cholesterol (mmol/L)[a]	1.45	0.97	5.47	0.00
LDL cholesterol (mmol/L)[a]	2.29	3.68	–5.19	0.00
Non-HDL cholesterol (mmol/L)	4.03	4.51	–1.87	0.06
LDL/HDL cholesterol (mmol/L)[b]	3.27	3.86	–2.79	0.01
Glycemia (mmol/L)	4.67	5.18	–1.93	0.06
IRI (μU/mL)	8.44	15.32	–3.97	0.00
HOMA IR (μU/mL)[a]	1.80	3.65	–3.76	0.00
C-reactive protein (mg/L)	1.88	12.62	–4.58	0.00

BM: body mass; BH: body height; BMI: body mass index; WC: waist circumference; Systolic BP: systolic blood pressure; Diastolic BP: diastolic BP; TC: total cholesterol; TG: triglycerides; HDL cholesterol: high-density lipoprotein cholesterol; LDL cholesterol: low-density cholesterol; IRI: insulin level HOMA IR: homeostasis model assessment of insulin resistance; CRP: C-reactive protein.

[a] Student's *t*-test: $p < 0.01$.
[b] *p* Value: <0.05.

means of anthropometric and biochemical measurements between the two analyzed groups for all values, except for glycemia, age and sex (Table 1).

There was a difference in the distribution of genotypes between the two groups. There was a higher frequency of genotypes CT and TT in the MetS group compared to the healthy group. In the MetS and healthy group, the CC genotype was found most often (MetS: 57.4%; control: 86.67%), while the TT genotype was determined only in the MetS group (7.94%). The most common genotype in the total sample was CC (66.67%). The presence of each allele of the *LRP1* gene was determined in the total studied sample as well as separately in each study group (Table 2).

Table 2. Frequencies of the *LRP1* exon 3 alleles.

Group	Allele		Total
	C (%)	T (%)	
MetS group	74.60	25.40	100.00
Control group	93.33	6.67	100.00
Total	80.65	19.35	100.00

In both groups, the most frequent allele was allele C (MetS: 74.60%, control: 93.33%), while the T allele (25.4%) was more common in the MetS group. The most frequent allele in the total sample was the C allele (80.65%). Based on the results of the χ^2 test of independence, it was found that there is an association between the presence of different exon 3 *LRP1* genotypes and values of BMI over 30 kg/m^2 ($p < 0.05$; $\chi^2 = 7.01234$) and LDL >3.0 mmol/L ($p < 0.01$; $\chi^2 = 15.9979$).

In order to test the relevance of the *LRP1* gene in determination of different anthropometric and biochemical parameters, we tested the total sample divided into three different groups of genotypes (CC, CT and TT). Single factor analysis of variance showed statistically significant differences for the parameters shown in Table 3, therefore, it can be concluded that there was a strong influence of different genotypes of exon 3 *LRP1* genes on the tested parameters (Table 3).

Odds ratio analyses (Table 4) showed that the presence of the T allele in patients multiplies the chance of deviation from the reference values of each anthropometric and biochemical parameter, especially for BMI (4.24-fold)

Table 3. One-way analysis of variance between the exon 3 *LRP1* gene and tested relevant anthropometric and biochemical parameters.

ANOVA	**Exon 3 *LRP1* Gene**	
Parameter	F = VA/VR	*p* Value
BMI (kg/m^2)	4.47	0.010
WC (cm)	6.59	0.000
Systolic BP (mmHg)	4.67	0.012
LDL cholesterol (mmol/L)	40.54	0.000
Index of atherosclerosis LDL/HDL	11.52	0.000

ANOVA: analysis of variance; F = VA/VR: statistics of F test is the ratio of factorial and residual variance; BMI: body mass index; WC: waist circumference; Systolic BP: systolic blood pressure; LDL: low-density lipoprotein/high-density lipoprotein cholesterol.

Table 4. Odds ratio for the exon 3 *LRP1* gene allele and measured anthropometric and biochemical parameters.

Parameters	**Allele**	**C**	**T**
BMI (<30 kg/m^2)	OR 95% CI *p* value	0.23 0.07-0.70 >0.05	4.24 1.42-12.65 <0.05
LDL cholesteror (>3 mmol/L)	OR 95% CI *p* value	0.04 0.00-0.37 >0.05	20.26 2.70-152.04 <0.05

BMI: body mass index; OR: odds ratio; 95% CI: 95% confidence interval; LDL cholesterol: low-density lipoprotein cholesterol.

and LDL cholesterol (20.26-fold) compared to the C allele carriers. Calculating the value of OR in relation to the occurrence of MetS, it was found that the presence of the T allele in patients multiplies the chance (4.76-fold) for the occurrence of MetS in comparison to the C allele carriers.

DISCUSSION

Based on the results presented in our study, the *LRP1* gene polymorphism was associated with the features of MetS. To the best of our knowledge, the polymorphism of exon 3 of the *LRP1* gene has not yet been analyzed in the Serbian population therefore, our results represent the first step in this domain in the Serbian population. In Europe, the 14.0% frequency of the T allele, and the C allele frequency of 86.0% was found [29]. The results of our study showed similar frequencies, where the C allele represented 80.65% and the T allele 19.35% in the total sample. Panza *et al.* [30] found that the frequency of allele C shows a significant decreasing trend from north to south Europe, with the associated increase in the frequency of the T allele, but only in patients with Alzheimer's disease.

In the MetS group, significantly higher values of BW, BMI, WC, systolic and diastolic BP, TG, HDL cholesterol, LDL cholesterol, index of atherosclerosis, insulin level (IRI), homeostasis model assessment of insulin resistance (HOMA IR) and CRP were noticed, which was expected, especially for the WC, systolic and diastolic BP, TG and HDL cholesterol, which are the determinants of MetS by the IDF definition. Although there were no significant differences for some expected parameters such as the level of non-HDL cholesterol (p = 0.06) and fasting glucose (p = 0.06), which is one of the parameters of the IDF MetS definition, p values are very close to the threshold of significance, indicating that a (possibly) greater number of subjects could lead to significant differences in these parameters. In the MetS group, the presence of the T allele (25.4%) was found to be more frequent compared to the control group (p <0.01). Benes *et al.* [31] also showed a significantly higher presence of the T allele (21.0%) in the group of women suffering from breast cancer compared to the control group (p <0.05).

The results revealed statistically significant connection between the presence of the CT genotype and the MetS group affiliation (p = 0.01). The TT genotype was detected only in the MetS group. The χ^2 test of independence revealed a statistically significant connection between the T allele itself and MetS (p = 0.0001), indicating that the T allele may be considered as a risk factor for the development of MetS. In the MetS group, eight subjects, T allele carriers, were younger than 30 years, thus pointing out the association between the T allele and MetS emergence, also

encouraging early diagnosis in order to improve quality of life and reduce the number of possible patients. Masson *et al.* [15] found that LRP1 plays a very important role in lipid metabolism, and its synthesis is very high during the adipocyte differentiation in mice and humans. Up to now, studies regarding the LRP1 expression and human fat tissue have been extremely scarce. There has not been any information in the literature available to us, regarding the polymorphism of exon 3 of the *LRP1* gene and individual anthropometric and biochemical parameters examined in our study, therefore, our results represent the first step in revealing these relationships. The LRP1 exon 3 T allele showed a significant association with the emergence of MetS. We analyzed the connection of the five individual components of MetS with LRP1 genotypes and found that carriers of the T allele are significantly more present among subjects with elevated BMI, WC, systolic BP, TC, LDL cholesterol and index of atherosclerosis (LDL/HDL), revealed by one-way analysis of variance (ANOVA). The difference in the parameter values between the CC/CT and CC/TT genotypes and between CT/TT genotypes indicates that the presence of the T allele in the genotype is responsible for statistically significant differences. Carriers of the T allele have significantly disrupted reference values of the abovementioned parameters, and that indicates its important role in the metabolism of lipids. Further processing of the data based on the OR results indicates that presence of the T allele in patients multiplies the chance of occurrence of reference value deviations of BMI, TC and LDL cholesterol compared to C allele carriers. After adjusting for all MetS components, T allele carriers showed a significant association with the emergence of MetS by OR analysis. Our present study showed that T allele carriers of the *LRP1* gene are 4.76-fold more likely to develop MetS compared to C allele carriers.

In our previous study, we investigated the connection between individual alleles of the apolipoprotein E (*apoE*) gene on one side and the appearance of MetS on the other, as well as the correlation between the *apoE* gene polymorphism and each of individual anthropometric and biochemical parameters in both control and MetS test groups [32]. The frequency of the apoe4 allele was significantly higher in the MetS group. In addition, positive correlation was revealed between the presence of the apoe4 allele and all measured parameters. It was observed that the apoe4 allele was associated with a significantly increased OR of MetS disorders defined by the IDF definition. These results suggested that apoe4 allele may act as one of the determinants for development of MetS. Determining the contribution of the *apoE* gene polymorphism and its receptors, including *LRP1*, is very important in the study of lipid and lipoprotein metabolism [32].

Conclusions. A molecular genetic approach is the most reliable in the diagnosis of most diseases. Studies show that the implementation of routine screening, particularly in patients with hereditary load, contributes to the early diagnosis and prevention, which delays the process leading to the formation and development of CVD, diabetes, obesity, IR, thus leading to MetS, improves the quality of life and reduces the number of patients. Very early confirmation of a genetic predisposition for CVD, diabetes, obesity, IR, leading to MetS, allowed us to change habits that contribute to the emergence and development of the disease, also saving the cost of treatment [4]. The results of correlation found for the T allele of the *LRP1* the gene with the determinants of MetS is not negligible, and the T allele may be considered as a risk factor for the development of MetS.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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