Identification of 677C>T Polymorphism of LRP1 Gene by PCR-RFLP Method

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Abstract **– The 677 C>T (rs1799986) polymorphism is located at position 677 in exon 3 of the LRP1 gene. The aim of this study was to determine the allele and genotype frequencies of the polymorphism in Bosnian population. The study included 100 unrelated healthy individuals. Genotyping of 677 C>T polymorphism of the LRP1 gene was performed with PCR-RFLP method. The most frequent allele was the C allele (91%), while the T allele was represented by 9%. These results suggested that presence of the 677 C>T polymorphism of the LRP1 gene in our population should be a base for further case-control association or population genetics studies.**

Keywords **– LRP1 gene, PCR-RFLP method.**

1. Introduction

The LRP1 gene is located on human chromosome 12q13-14 and contains 89 exons and spans 85kb [1]. It encodes low-density lipoprotein receptor-related protein 1. The encoded pre-protein LRP1 is proteolytically processed by furin to generate 515 kDa and 85 kDa subunits that form mature receptor.

DOI: 10.18421/TEM74-17
https://dx.doi.org/10.18421/TEM74-17

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Received: 21 August 2018.
Accepted: 19 October 2018.
Published: 26 November 2018.

The protein LRP1 is ubiquitously expressed in most tissues and abundantly exists in the liver and the brain [2]. It is a multifunctional receptor involved in several cellular processes, including intracellular signaling, lipid homeostasis and clearance of apoptotic cells and factor VIII in the blood coagulation process [3].

During the embryonic development, the LRP1 is essential for the maintenance of vascular integrity while in adult life it plays crucial role in protection against atherosclerosis reducing smooth vascular muscle cell proliferation and regulatation of levels of platelet-derived growth factor receptor in the vessel wall [4]. The LRP1 is also involved in regulation of proteases and protease inhibitor complexeses pathways [5].

To date sequence analysis of the LRP1 gene has revealed several single nucleotide polymorphisms: 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) [6]. The common polymorphism in the LRP1 is the 677 C>T single nucleotide polymorphism at position 677 in exon 3 of the LRP1 gene (database identifier dbSNP ID: rs1799986). This silent polymorphism does not change the amino acid sequence of the protein but can influence the splicing efficiency of exon 3, leading to a small but still significant decrease in full length of the LRP1 mRNA [6]. The encoded protein is necessary for the alpha 2-macroglobulin-mediated clearance of secreted amyloid precursor protein and beta-amyloid. According to the LRP1 role in signaling pathways of inositol, Ras and Src activation and the activation of mitogen-activated protein kinases and protein kinase A, several authors have suggested that LRP1 might act as a sensor for necrotic cell death leading to proinflammatory immune responses [7, 8].

Due to lack of published data of the prevalence of the LRP1 gene in Bosnian population and its importance in many cellular processes, the aim of this study was to determine the allele and genotypes

frequency of 677 C>T polymorphism of the LRP1 gene in a sample of healthy Bosnian population.

2. Material and methods

Subjects

The study included 100 unrelated healthy Bosnian individuals, 50 females aged 21 to 74 and 50 males aged 23 to 78. The mean age for entire sample was 47.17 years. The criteria for including the participants in the study was absence of known risk factors for Alzheimer's disease, metabolic syndrome, venous thromboembolism, Diabetes mellitus, breast cancer, atherosclerosis and obesity.

The blood samples of the participants were collected in ''Plava poliklinika'' Tuzla, Tuzla Canton, Bosnia and Herzegovina.

The participants were fully informed about the study protocol by the head of the project and have agreed to participate in the study by signing the written consent.

Methods

Total genomic DNA was isolated from EDTA anti-coagulated whole blood using the FlexiGene DNA isolation Kit (Qiagen, GmbH, Hilden, Germany).

The genotyping of the 677 C>T polymorphism of the LRP1 gene was done by polymerase chain-restriction fragment length polymorphism method (PCR-RFLP). The amplification of exon 3 of the LRP1 gene was performed by PCR according to modified method of Kang et al. [9]. PCR reactions in a final volume of 25 μL were carried out containing: 100 ng of the genomic DNA, 1x PCR buffer, 1,5 MgCl (Sigma Aldrich, Munich, Germany), 0.2 mM of each dNTP (Sigma Aldrich, Munich, Germany), 0.4 mM of each primer (F 5'-CCA TAG CCA GCT TGT TCA TG-3' and R 5'-ACG GGA GAG TAG AGA GTG G-3'), 1.5 U Taq DNA polymerase (Sigma Aldrich, Munich, Germany).

The exon 3 of the LRP1 gene was amplified in thermal cycler (Applied Biosystems by Roche Molecular systems Inc, New Jersey, the USA) according to the next protocol: initial denaturation at 94°C for 2 minutes followed by 30 cycles at 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds, and a final extension step at 72°C for 10 minutes.

The 212 bp PCR products of the LRP1 gene were digested by FokI enzyme (New England Biolab, the UK) at 37°C for one hour. The identification of genotypes of the 677 C>T polymorphism of the LRP1 gene were performed by electrophoresis in 4% agarose gel (Sigma Aldrich, Munich, Germany) stained with ethidium bromide.

Statistical analysis

Deviation of allele and genotype distribution from the Hardy-Weinberg equilibrium was assessed by the chi square test. Statistical significance was set at a *P* value of <0.05.

3. Results

The exon 3 of the LRP1 gene was successfully amplified by PCR for each participant (Figure 1.). Lines 1 to 10 show amplified PCR product of the LRP1 gene. The size of the PCR product was in the total length of 212 bp. Line 11 shows 100 bp molecular weight marker (New England BioLab, the UK).

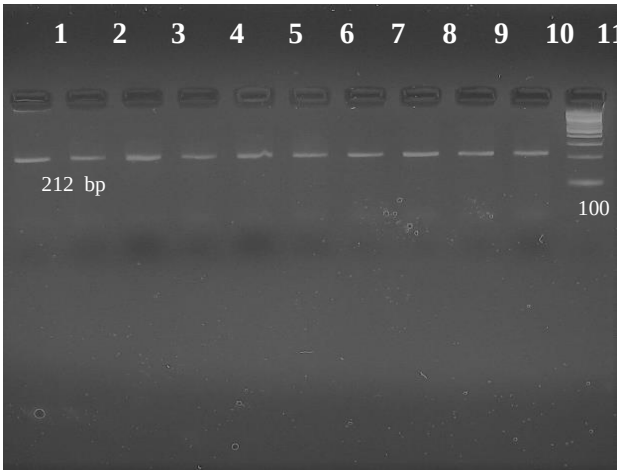

Figure 2. The amplified PCR product of exon 3 of the LRP1 gene

Amplified PCR products of the LRP1 were digested with FokI enzyme. After restriction digestion individuals homozygous for the C allele demonstrated one fragment of 212 bp in the 4% agarose gel, individuals heterozygous for the 677 C>T polymorphism of the LRP1 gene demonstrated three fragments of 212, 151 and 60 bp. The homozygous for the T allele (showing two fragments of 151 and 60 bp after digestion) were not identified in the study. The restriction digestion (RFLP) assay of the 212 bp PCR product of the LRP1 gene is shown in Figure 2.

The PCR fragments of the LRP1 gene (212 bp) digested with FokI enzyme were separated on 4% agarose gel stained by ethidium bromide.

Lines 2, 4, 5, 6, 7 and 8 show the CC genotype (individual homozygous for the C allele); lines 1, 3 and 9 show CT genotype (individual heterozygous for the rs1799986 polymorphism of the LRP1 gene; Line 10 shows 100 bp molecular weight marker (New England BioLab, the UK).

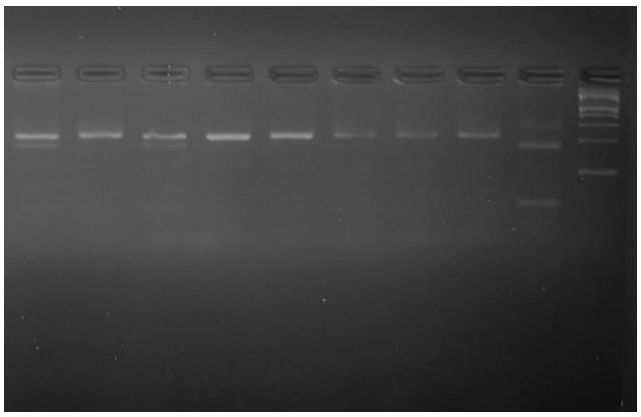

Figure 2. RFLP assay of 677 C>T (rs1799986) polymorphism of the LRP1 gene.

The most frequent genotype in a sample of 100 unrelated healthy Bosnian individuals was CC genotype (homozygous for the C allele) with 91%. The CT genotype (heterozygous for the polymorphism) was represented with 9%.

Table 1. shows the allele and genotypes frequencies of the 677 C>T polymorphisms of the LRP1 gene according to gender. The allele and genotypes frequencies of the 677 C>T polymorphisms did not differ significantly between genders.

Table 1. The allele and genotypes frequencies of 677 C>T polymorphism of the LRP1 gene in a sample of Bosnian population

Genotypes/alleles	**Males N (%)**	**Females N (%)**	X^2	**P**
CC genotype	45 (90%)	38 (76%)	3.473	0.062
CT genotype	5 (10%)	12 (24%)		
TT genotype	0	0		
The C allele	95 (95%)	88 (88%)	3.15	0.075
The T allele	5 (5%)	12 (12%)		

Comparison of the allele and genotype frequencies of the 677C>T polymorphism of the LRP1 gene between different populations is shown in Table 2. We did not find statistically important difference of the frequency of the polymorphism between the compared world populations.

Table 2. Comparison of the allele and genotype frequencies between populations

Population	N	Genotype frequencies				
		CC	CT	TT	X^2	P
The USA	822	0.83	0.16	0.01		
European	2939	0.74	0.24	0.02	6.81	0.33
Chinese	267	0.85	0.13	0.02		
Our study	100	0.83	0.17	0		

Population	N	Allele frequencies			
		C	T	X^2	P
The USA	822	0.91	0.09		
European	2939	0.86	0.14	3.83	0.27
Chinese	267	0.94	0.06		
Our study	100	0.91	0.09		

4. Discussion

In this study the 677 C>T polymorphism of the LRP1 gene has been genotyped in a sample of 100 unrelated Bosnian individuals.

Recent studies have shown that the prevalence of the C allele of the 677C>T polymorphism of the LPP1 gene is 85% and the T allele is 14% in Europe with significant decreasing of the frequency of the C allele from the north to the south of Europe [6, 10]. The meta-analysis of distribution of the polymorphism has found that frequency of the C allele is 91% and the T allele is 9% in the USA population, while in the Chinese population it is 94% (the C allele) and 6% (the T allele) [10]. The results of our study showed similar frequencies of the C allele (91%) and the T allele (9%) counted in the whole sample. We did not find statistically important difference in distribution of the 677 C>T polymorphism of the LRP1 gene between female and male individuals and between compared populations.

According to the multifunctional role of the LRP1 gene in many cellular processes, the association of the polymorphisms of the LRP1 gene with many diseases has been studied in the last 20 years. The most investigated role of the LRP1 gene is in the pathogenesis of the Alzheimer's disease. The LRP1 protein mediates the uptake of lipoproteins through the binding of the APOE and clear amyloid from the brain [11]. It is the main component of amyloid plaques found in Alzheimer patients. Recent studies reported its potential role in the pathogenesis of the Alzheimer's disease (AD) [12]. On the other hand, studies [13, 14] are showing conflicting results in relation to its contribution to the AD. The heterogeneity of associations of the LRP1 gene and the AD imply that the 677 C>T polymorphism does not play the main role in the pathogenesis of the AD and that might be considered as a modifier of the the AD phenotype.

Data findings linked the LRP1 gene functions to lipid metabolism and obesity [15]. However, the role of the LRP1 gene in glucose homeostasis has not been clarified yet. Vucinic et al. [6] have found the association of the polymorphism in exon 3 of the LRP1 gene with metabolic syndrome (MS) in a sample of Serbian population. The study provided on knockout mice suggested that the LRP1 is essential for modulating hepatic insulin action and highlight a

pivotal mechanism in the development of the MS [16].

Also, literature data shows that the LRP1 gene polymorphisms might be associated with coronary artery disease in Caucasian population [17, 18, 19, 20], and with coronary thrombosis [21, 22]. On the contrary, other studies have reported contradictory and less significant association of the LRP1 genes with coronary or venous thrombosis events in Caucasian population [23].

5. Conclusion

Due to the best of our knowledge this is the first study of genotyping this polymorphism in Bosnian population. The presence of the T allele of 677 C>T polymorphism of the LRP1 gene in a sample of the Bosnian population and its role in the pathogenesis in the above reported diseases implies the necessity for further case-control association or population genetics studies in the Bosnian population.

Although the studies suggested that the LRP1 gene might not be highlighted as a main factor of pathogenesis of investigated diseases, it can be considered together with other genetics and environmental risk factors contributing in the imbalance of lipid or glucose homeostasis.

Acknowledgements

This work was supported by the Federal Ministry of Education and Science of Bosnia and Herzegovina under Grant (number 01/2-3680-IV-9/16).

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