Study on the Prevalence of Asn291Ser Mutation in the Lipoprotein Lipase Gene in a Population with Cardiometabolic Syndrome from North East Romania

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Metabolic syndrome associates atherosclerosis and cardiovascular disease risk, atherogenic dyslipidemia being one of its key features. The central role of lipoprotein lipase (LPL) in lipoprotein metabolism makes it a major candidate gene for coronary heart disease. Asn291Ser is one of the most common LPL gene variants that are known to be associated with reduced LPL activity and dyslipidemia. Our objective was to assess the frequency of Asn291Ser mutation in a group of 76 patients with cardiometabolic syndrome from North East Romania, and to characterize associated plasma lipid profiles. Two subjects were heterozygotes for this mutation, corresponding to a carrier frequency of 2.63%. The female carrier had a serum triglyceride level increased by 9.8% and an HDL cholesterol level decreased by 5.41 mg/dL compared with the mean value of the female subgroup. The male carrier had a serum level of HDL cholesterol decreased by 9.12 mg/dL, but the triglyceride level did not differ from that of the male subgroup. Both carriers had small dense LDL levels higher than the mean value of the corresponding subgroup (by 15 mg/dL for the female subject and by 10 mg/dL for the male subject). Our study suggests that the Asn291Ser mutation in the LPL gene may be a factor in the development of atherogenic dyslipidemia associated with cardiometabolic syndrome.

Keywords: cardiometabolic syndrome, lipoprotein lipase, Asn291Ser mutation, atherogenic dyslipidemia, cardiovascular disease

Subjects with cardiometabolic syndrome are much more prone to developing cardiovascular events compared with individuals that do not have this condition. The main features of cardiometabolic syndrome are: fasting hyperglycemia, obesity or overweight, arterial hypertension, and atherogenic dyslipidemia represented by increased serum triglycerides, decreased HDL cholesterol, increased small dense LDL particles [1,2].

An essential role in the metabolism of plasma lipoproteins is performed by lipoprotein lipase (LPL). This enzyme is involved in the catabolism of chylomicrons and VLDL by catalyzing the hydrolysis of triglycerides (TG) contained in these particles, thereby providing fatty acids to tissues as an energy source or for storage [3]. LPL requires a specific cofactor, apolipoprotein C-II, as an essential activator [4]. During LPL-mediated hydrolysis of TG-rich lipoproteins, surface lipids and apolipoproteins are transferred to HDL, so an additional role of LPL is to contribute to HDL particle formation [5,6]. LPL is distributed in a wide variety of extrahepatic tissues, mainly in the adipose tissue, skeletal muscle, and myocardium. The enzyme acts at the luminal surface of blood vessels, being anchored to vascular endothelium via heparan sulfate proteoglycans [7,8]. The enzymatic activity of LPL is regulated in a complex, tissue-specific manner in response to energy requirements and hormonal changes. Most notable is the action of insulin, which increases LPL synthesis at the transcriptional level and also stimulates LPL activity [4]. Giving its roles in lipoprotein metabolism, LPL produced by adipose tissue and muscle is considered to have a protective effect with respect to atherosclerosis.

LPL is also expressed by cell types found in the arterial wall, particularly by monocyte-derived macrophages. The enzyme having this location has pathophysiological actions that promote foam cell formation and atherosclerosis. The atherogenic role of LPL is linked to its ability to facilitate cellular uptake of lipoproteins independently of its catalytic activity [8]. The result of this LPL action is the increased retention and accumulation of lipoproteins in the arterial subendothelial matrix. Such trapped lipoproteins are more susceptible to atherogenic modification, and they are more rapidly taken up by macrophages, favoring foam cell formation [9-11].

The human LPL gene is located on chromosome 8p22, spans about 30 kb, and consists of 10 exons. It encodes a 475 amino acid polypeptide that yields a 448 amino acid mature protein after cleavage of a 27 amino acid signal peptide [12]. In view of its critical role in lipoprotein metabolism, LPL is a strong candidate gene for atherogenic lipid profiles and coronary heart disease (CHD).

The aim of this study was to assess the frequency of the Asn291Ser mutation, one of the common variations in the LPL gene, in a group of patients with cardiometabolic syndrome from North East Romania, and to make a correlation between the carrier status for this mutation and the plasma levels of triglycerides, HDL cholesterol and

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small dense LDL. This approach was motivated by the fact that, to our knowledge, the scientific literature lacks studies referring to the possible existence of LPL gene mutations among patients with cardiometabolic syndrome in this part of Romania. We intented that the results of our study would allow a better diagnostic approach, as well as an individualised therapy for patients with cardiometabolic syndrome.

Experimental part

Material and methods

Subjects

We evaluated 76 patients (55 males and 21 females) with cardiometabolic syndrome presenting abnormal values of the lipid parameters, the mean age being 49.10 ± 12.45 years. The patients originated from North East Romania.

The NCEP III (National Cholesterol Education Program) criteria for cardiometabolic syndrome are: abdominal obesity characterized by waist circumference > 102 cm for males and > 88 cm for females, fasting glycemia > 110 mg/dL or confirmed diabetes mellitus, serum triglycerides ≥ 150 mg/dL, HDL cholesterol < 40 mL/dL in males and < 50 mg/dL in females, arterial blood pressure ≥ 135/80 mmHg (Expert Panel on Detection). To ascertain the diagnosis of cardiometabolic syndrome, three of the five criteria must be met.

In our study we selected the patients that met the following three criteria: abdominal obesity, changes of at least one of the lipid parameters and changes of the arterial blood pressure. We chose to select patients in this way, focusing on the changes of the lipid profile, based on the fact that in the scientific literature dyslipidemia in the cardiometabolic syndrome is considered to be one of the most important risk factors in the pathogenesis of atherosclerosis. [13]. For the selected patients we searched for the presence of Asn291Ser mutation in the LPL gene and we determined the levels of lipid profile parameters.

Evaluation of the lipid profile

The values of the main lipid parameters were determined in serum samples, after an overnight fast. Triglyceride measurement was performed by a spectrophotometric assay using specific enzymes (Fossati and Principe method) [14], coupled with the Trinder's reaction.

Total cholesterol was measured using an enzymatic spectrophotometric assay. For the determination of cholesterol fractions specific reagents were used and the following steps were followed. (a) HDL cholesterol: selective precipitation of chylomicrons, VLDL and LDL particles with phosphotungstic acid and $MgCl_2$, followed by centrifugation and measurement of HDL cholesterol in the supernatant. (b) LDL cholesterol: a direct enzymatic cholorimetric method was used. This is based on the selective micellar solubilization of LDL cholesterol with a non-ionic detergent coupled to the use of a carbohydrate compound that interacts with VLDL and chylomicrons and prevents the reaction of cholesterol present in these particles with the reagents used in the assay. This allows the selective measurement of LDL cholesterol in serum. This method meets the NCEP (National Cholesterol Education Program) requirements of having a total analytical error ≤ 12% [15].

The serum concentration of small dense LDL particles (sLDL) was also determined, taking into account the atherogenic role of this lipoprotein fraction. A „two step" technique was performed using surfactant and specific enzymes. The assays described above were performed on a compact analyzer of wet chemistry of RX-Imola type.

DNA analysis for the detection of the Asn291Ser mutation

The Asn291Ser mutation in the LPL gene was detected by mismatch PCR followed by *Rsa* I restriction digestion, according to the method of Zhang et al. [16], adapted (regarding the PCR amplification program) in our laboratory. The following steps were performed:

a. DNA extraction. Genomic DNA was extracted from whole blood collected on EDTA, using a GeneJET purification kit and an automated Magnesia 16 magnetic bead extraction system. The DNA amount was estimated spectrophotometrically, by measuring the absorbance at 260 nm (for nucleic acids) and at 280 nm (for proteins), using the refference filter of 320 nm for wavelength correction. The OD_{280}/OD_{260} ratio was an indicator of nucleic acid purity.

b. DNA amplification. Exon 6 of the LPL gene was amplified using a 5'-PCR primer located in intron 5 near the 5' boundary of exon 6 (5'-GCCGAGATACAATCTTGGTG-3') and a 3'-mismatch PCR primer located in exon 6 near the Asn291Ser mutation (5'-CTGCTTCTTTTGGCTCTG ACTGTA-3'). PCR amplification reactions were performed with 5 μL of genomic DNA in PCR buffer containing 2 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, 1 μmol/L of each primer, and 0.6 U *Taq* DNA polymerase. The amplification was achieved on a SureCycler 8800 thermal cycler. The amplification program involved a first denaturation step at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 60 s, annealing at 51°C for 60 s, and elongation at 72°C for 45 s, ending with a final elongation step at 72°C for 5 min.

c. Restriction digestion of the PCR product. The PCR product (10 μL) was digested with 5 U Rsa I enzyme (from Rhodopseudomonas sphaeroides), 2 μL 10x buffer Tango, and 18μL nuclease-free water at 37°C for 1 h. The reaction was then stopped by keeping the samples at 80°C for 20 min. The digested fragments were separated on 2% agarose gels.

Results and discussion

In this study we searched for the presence of the LPL Asn291Ser mutation in the 76 patients with cardiometabolic syndrome. To detect this mutation we used a mismatch PCR primer as the 3'-PCR primer together with the normal 5'-PCR primer. DNA amplification of exon 6 with the two primers generates a 238-bp fragment. The Asn291Ser substitution in the LPL protein is caused by an A to G mutation located at nucleotide 1127 in exon 6 of the LPL gene. The use of a mismatch primer generates a C instead of the normal A (the mismatch) at nucleotide 1130 in the PCR fragments amplified from both the mutant and normal alleles. Thus, in the PCR fragment from the mutant allele, a recognition site for the *Rsa* I restriction endonuclease will be created: 5'-GTAC-3' (G_{1127} from the Asn291Ser mutation and C_{1130} from the mismatch). As a consequence, this 238-bp fragment will be cleaved into a 215-bp fragment and a 23-bp fragment. The PCR product from the normal allele will have a 52 -ATAC-32 sequence in this region and cannot be cleaved by the *Rsa* I enzyme, therefore remaining as a single 238-bp fragment (fig. 1).

In our population sample we found two subjects that were heterozygous for the LPL Asn291Ser mutation (one female and one male - fig. 2 and fig. 3, respectively), corresponding to a carrier frequency of 2.63%.

More than 100 naturally occurring mutations in the LPL gene have been identified in humans, the majority being single nucleotide polymorphisms. More than half of them are missense mutations, most of which are located on exons 5 and 6 [5]. The frequency of individual LPL mutations differs widely between populations, but the

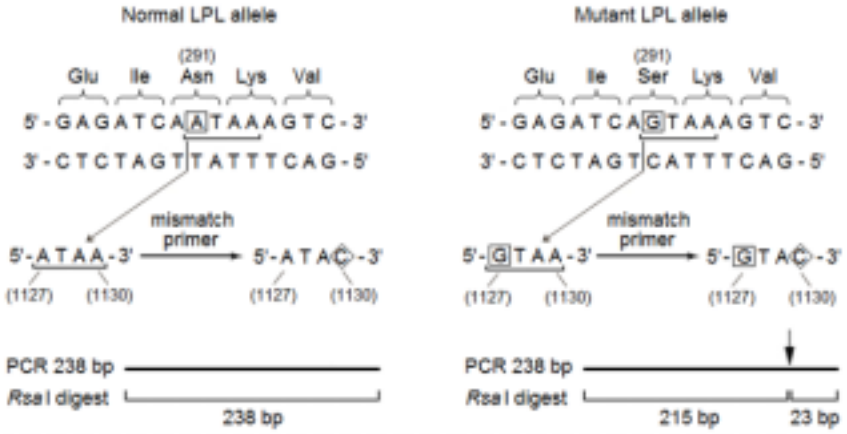

Fig. 1. The sequence of exon 6 of LPL gene containing the A to G substitution at nucleotide 1127, and the products of *Rsa* I digest of the PCR product of exon 6, for the normal and the mutant allele

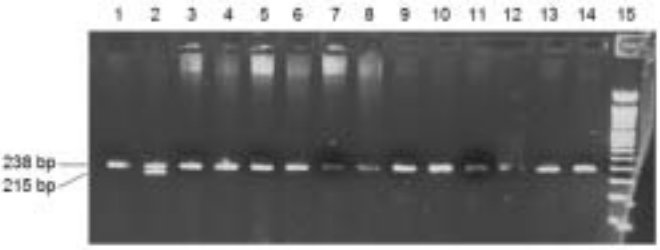

Fig. 2. Detection of the Asn291Ser mutation by PCR and *Rsa* I restriction digestion for the female patient. Lane 2: heterozygote; lanes 1 and 3-14: normal; lane 15: MW marker.

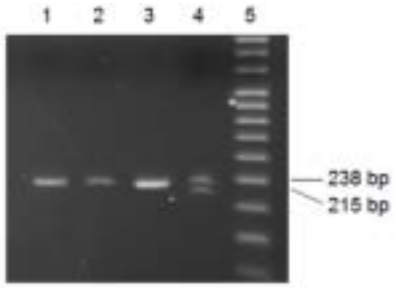

Fig. 3. Detection of the Asn291Ser mutation by PCR and *Rsa* I restriction digestion for the male patient. Lane 4: heterozygote; lanes 1-3: normal; lane 5: MW marker.

cumulative frequencies may approach 20% in Caucasian populations [11]. Many of the mutations in LPL gene are associated with a decrease in LPL catalytic activity to various degrees, and they have been shown to play a major role in the development of dyslipidemia, thus leading to premature atherosclerosis and increased risk of CHD [17]. Among the mutations reducing LPL activity, Asn291Ser is one of the most common, the frequency of heterozygous carriers being estimated as ranging between 2% and 5% in Caucasian populations [5]. Several studies have shown that the Asn291Ser variant in the LPL gene is a risk factor for dyslipidemia, characterized by hypertriglyceridemia and low HDL cholesterol levels [3,18,19].

In our study, the Asn291Ser mutation in the LPL gene was found with a carrier frequency of 2.63% among the 76 patients with cardiometabolic syndrome. This frequency is lower compared to that reported by other studies. The Asn291Ser mutation has been found with a carrier frequency of 5.2% in a group of 807 Dutch patients with CHD [20], 4.5% in a group of 899 men from the United States with CHD and low HDL cholesterol levels [21], and 3.3% in a group of 721 australian subjects with CHD [12]. We consider that this difference in the carrier frequency between our study and other reports might be explained by the fact that our group was significantly smaller compared with the others.

In this study we also aimed to analyse the presence of the Asn291Ser mutation in correlation with the serum levels of triglycerides, HDL cholesterol and small dense LDL. The results of fasting levels of the lipid parameters are presented in table 1.

We found that the female carrier subject of our study had a serum TG level increased by 9.8% and an HDL cholesterol level decreased by 5.41 mg/dL compared with the corresponding mean values of the female subgroup. These results are in accordance with those reported by several studies. A meta-analysis performed by Hu et al. [19] on 21 studies published up to 2004 and including ~19 000 subjects revealed that the Asn291Ser variant in the LPL gene was a risk factor for dyslipidemia, characterized by hypertriglyceridemia and low HDL cholesterol levels. Based on the evaluation of 19 studies published up to 2007 and including ~24 000 participants, Sagoo et al. [3] found that carriers of the Asn291Ser LPL mutation had lower HDL cholesterol levels (by 0.12 mmol/L) and higher TG levels (by 0.19 mmol/L, representing a 10% elevation) than noncarriers.

The male carrier subject of our study had a serum level of HDL cholesterol decreased by 9.12 mg/dL compared with the mean value of the male subgroup, but the TG level did not differ from that of the corresponding subgroup. These results are in accordance with those reported by Ferencak et al. [22], who studied two groups of Croatian subjects, with and without angiographically confirmed CHD, and found that the LPL Asn291Ser variant was significantly associated with CHD, but the TG levels did not show any significant difference between carriers and non-carriers in either group.

In our study we also analyzed the relationship between the presence of the Asn291Ser mutation and the level of small dense LDL particles. This LDL subfraction has been shown to coexist with elevated TG and low HDL cholesterol levels and to be associated with increased cardiovascular risk. The sLDL particles are more easily oxidized, have lower affinity for the LDL receptor, and are taken up more easily by arterial tissue, having a higher degree of retention in the arterial wall [23]. Both patients from our group that were carriers of the Asn291Ser mutation had sLDL levels higher than the mean value of the coresponding subgroup (by 15 mg/dL for the female carrier and by 10 mg/dL for the male

Table 1
SERUM LIPID VALUES IN THE STUDIED GROUP*

Subjects	Triglycerides	Total cholesterol	HDL cholesterol	LDL cholesterol	Small dense LDL
Males [n = 55]	428.85 ± 258	257.93 ± 57.95	46.20 ± 12.75	152.20 ± 53.21	48.47 ± 19.08
Females [n = 21]	298 ± 187.1	218 ± 45.28	48.50 ± 7.15	143.84 ± 43.87	68.11 ± 28.54

*Values are presented as the mean value ± SD and are expressed in mg/dL.

carrier). By being associated with a decrease in LPL activity, the Asn291Ser mutation leads to deffective VLDL catabolism and an elevation of VLDL concentration. The formation of a greater number of small dense LDL particles represents the final expression of the alteration of VLDL catabolism. Our results are in accordance with those reported by Lopez-Ruiz et al., who found that patients with familial combined hyperlipidemia also carrying the Asn291Ser mutation showed a smaller average diameter of LDL particles and were at higher cardiovascular risk [24]. We consider that the level of sLDL in carriers of the Asn291Ser mutation might be considered a more accurate predictive marker for coronary heart disease compared with the classic lipid variables.

The studies that were examined by us refer to patient groups much larger compared to ours. However, as far as we know, our study is the first one that reports the frequency of the Asn291Ser mutation, a common variation in the LPL gene, in a group of subjects with cardiometabolic syndrome from North East Romania. We consider the initiation of such a research, in which we optimized the method for detecting this mutation, as being appropriate.

Conclusions

Our study shows that one of the most common LPL mutations, Asn291Ser, may be a factor in the development of atherogenic dyslipidemia associated with cardiometabolic syndrome, characterized by hypertriglyceridemia, low HDL cholesterol levels and increased sLDL levels. We consider that the identification of such a genetic factor, that increases the cardiovascular risk, will help the patient, as well as his physician, to focus on decreasing or eliminating the modifiable risk factors (such as smoking or obesity), thus favoring therapeutic efficiency. We are aware of the limitations of this study in relation to the number of patients that were investigated. Nevertheless, the finding of two carriers among 76 subjects with cardiometabolic syndrome imposes the continuation of the research on a larger group of patients, as well as its extension in the general population. At the same time, we consider that the investigation of the first degree relatives of the two carriers would be appropriate, in order to prevent the early onset of cardiovascular disease, thus contributing to the improvement of life quality.

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