Polymorphism AvaII of the LDL receptor (rs5925) is associated with carotid-intima media thickness in patients with diabetes mellitus type 2

Polimorfizem AvaII receptorja za holesterol LDL (rs5925) je povezan z debelino intime medije pri bolnikih s sladkorno boleznijo tipa 2

Jovana Nikolajević-Starčević,[1] Dražen Popović,[2] Marija Šantl Letonja,[3] Jana Makuc,[4] Maja Šeruga,[5] Andreja Cokan Vujkovac,[4] Zala Jenko Pražnikar,[6] Janez Stare,[7] Danijel Petrovič[1]

[1] *Univerza v Ljubljani, Medicinska fakulteta, Inštitut za histologijo in embriologijo*

[2] *Kirurški oddelek, Splošna bolnišnica Murska Sobota*

[3] *Radiološki oddelek, Splošna bolnišnica Murska Sobota*

[4] *Interni oddelek, Splošna bolnišnica Slovenj Gradec*

[5] *Interni oddelek, Splošna bolnišnica Murska Sobota*

[6] *Fakulteta za vede o zdravju, Univerza na Primorskem*

[7] *Inštitut za biostatistiko in medicinsko informatiko, Medicinska fakulteta, Univerza v Ljubljani*

Korespondenca/ Correspondence:
prof. dr. Danijel Petrovič
m: daniel.petrovic@mf.uni-lj.si

Izvleček

Izhodišča: Povečana serumska koncentracija lipoproteinov nizke gostote (LDL) je pomemben dejavnik tveganja za pojav in napredovanje ateroskleroze. Genetska variabilnost v genu za receptor LDL lahko vpliva na serumsko koncentracijo LDL in odgovor na zdravljenje s statini ter tako na pojav in napredovanje ateroskleroze. V raziskavi smo želeli ugotoviti, ali obstaja povezanost med polimorfizmom AvaII gena za receptor LDL (rs5925) s serumskimi ravnmi lipidov in debelino intime medije pri bolnikih s sladkorno boleznijo tipa 2 (SB2).

Metode: V raziskavo smo vključili 595 bolnikov s SB2 (399 zdravljenih s statini in 196 brez hipolipemika). Debelino intime medije smo izmerili s pomočjo B-načina ultrazvočnega prikaza. Biokemične analize smo opravili z uporabo standardnih metod. Genotipizacija je bila izvedena z metodo PCR v realnem času.

Rezultati: Razporeditev genotipov in alelne frekvence polimorfizma AvaII se niso statistično značilno razlikovale med bolniki s SB2, zdravljenimi s statini, in bolniki, ki niso jemali statinov. Pri bolnikih s SB2, zdravljenih s statini, smo najvišje serumske ravni celotnega holesterola in holesterola LDL ugotovili pri homozigotih A+A+. Po prilagoditvi na znane dejavnike tveganja za srčno-žilne bolezni so bili genotip A+A+ ($\beta = 0.441$ and $p = 0.04$), zdravljenje s statini kot tudi serumske ravni holesterola HDL, trigliceridov, visokoobčutljivega CRP in fibrinogena neodvisno povezani z debelino intime medije. Interakcije genotipov A-A+ in A+A+ polimorfizma AvaII in zdravljenja s statini niso bile statistično značilne.

Zaključki: Genotip A+A+ polimorfizma AvaII gena za LDL receptor je povezan z večjo debelino intime medije pri bolnikih s SB2.

Abstract

Introduction: Increased serum level of low-density lipoprotein (LDL) cholesterol is a well established risk factor for atherosclerosis development and progression. Genetic variation in the LDL receptor gene could modulate serum LDL level and response to statin treatment thus affecting atherosclerosis development and progression. The present study was designed to investigate the association between polymorphism AvaII (rs5925) of the LDL receptor gene with serum lipid levels and carotid intima-media thickness (CIMT) in patients with diabetes mellitus type 2 (DM2).

Methods: 595 patients with DM2 (399 with statin therapy and 196 without) were enrolled in the study. The carotid intima-media thickness was assessed ultrasonographically. Biochemical analyses were performed using standard biochemical

Ključne besede:
ateroskleroza; sladkorna bolezen tipa 2; debelina intime medije; LDL receptor; polimorfizem; farmakogenetika

Key words:
atherosclerosis; diabetes mellitus type 2; carotid intima-media thickness; LDL receptor polymorphism; pharmacogenetics

Citirajte kot/Cite as:
Zdrav Vestn 2014; 83 supl: I-5–12

Prispelo: 15. jul. 2013,
Sprejeto: 4. mar. 2014

methods. AvaII (rs5925) genotypes were determined by real-time PCR.

Results: Genotype distribution and allele frequencies were not statistically significantly different between DM2 patients with regard to statin therapy. In DM2 patients using statins the highest serum levels of total and LDL cholesterol were observed in homozygous carriers of the A+ allele. After adjustment for well established cardiovascular risk factors homozygosity for the A+ allele ($\beta = 0.441$ and $p = 0.04$), statin treatment as well as serum levels of HDL, triglycerides, hsCRP and fibrinogen were independently associated with CIMT. Interactions of AvaII genotypes A-A+ and A+A+ with statin treatment were not statistically significant.

Conclusion: Homozigosity for the A+ allele of the AvaII polymorphism is associated with greater CIMT in DM2 patients.

Introduction

Atherosclerotic cardiovascular disease (CVD) is now considered the major cause of chronic illness and early death in individuals with diabetes mellitus type 2 (DM2). The increased cardiovascular disease burden in patients with DM2 could, at least partially, be attributed to abnormalities in lipid and lipoprotein metabolism. Increased serum levels of cholesterol, especially low density lipoprotein (LDL), is one of the most important risk factors for atherosclerosis development and progression and its lowering remains the primary target in management of dislypidemia.[1]

LDL receptor (LDLR) plays an important role in cholesterol homeostasis as it regulates the uptake of LDL particles by the liver and delivers cholesterol to the adrenal glands and gonads for steroid hormone synthesis and to the liver for bile acid synthesis.[2] The human LDL receptor gene is located on chromosome 19 and contains 18 exons separated by 17 introns.[3] Until now, more than 770 mutations in the LDLR gene were reported.[4] Despite those causing familial hypercholesterolemia and consequently increased CVD risk, the impact of mutations of the LDL receptor gene on serum cholesterol levels and susceptibility to CVD is not well known. Previous studies reported an association between AvaII polymorphism and serum cholesterol level in both normolipidemic and hypercholesterolemic individuals[5-8], whereas Nakazone et al. reported no such association.[9] AvaII polymorphism has been also reported to be associated with variations in lipid lowering response to statin treatment in hypercholesterolemic patients.[10,11] Furthermore, previous studies reported an association between AvaII polymorphism with CAD.[7,12] Until now, there was no study investigating the association between AvaII polymorphism of the LDL receptor with carotid atherosclerosis.

High-resolution B-mode ultrasonography is a noninvasive method to assess vessel wall characteristics of the carotid arteries. It allows quantification of different surrogate markers of atherosclerosis such as carotid intima-media thickness (CIMT), presence of plaque and its structure as well as stenosis degree. CIMT is a good marker of early atherosclerosis and its progression.[13] It correlates well with cardiovascular risk factors and future cardiovascular events.[14,15]

The present study was designed to investigate the association between the polymorphism AvaII (rs5925) of the LDL receptor gene with serum lipid levels and carotid intima-media thickness in subjects with diabetes mellitus type 2.

Materials and Methods

Patients

In this cross-sectional study, 599 subjects with type 2 diabetes were enrolled. Among them 399 were on statin therapy: 234 (58.6 %) received atorvastatin 20 mg per day, 90 (22.5 %) received rosuvastatin 10 mg per day and 75 (18.9 %) received simvastatin 40 mg per day); 196 out of 599 subjects (33.4 %) with type 2 diabetes were without hypolipemic therapy. They were selected among patients admitted to the diabetes

outpatient clinics of the General Hospitals Murska Sobota and Slovenj Gradec, Slovenia. Patients were excluded if they had homozygous familial hypercholesterolaemia or a previous cardiovascular event such as myocardial infarction or a cerebral stroke. The research protocol was approved by the National Medical Ethics Committee. Clinical data, including smoking habits, duration and treatment of diabetes, arterial hypertension, hyperlipidemia and consuming any other drugs were obtained from medical records and questionnaires. Patients were asked if they were smokers at the time of recruitment (current smoker).

Ultrasonographic analysis

A high resolution B mode ultrasound analysis was performed using a portable ultrasound system, Toshiba Aplio SSA-700 (Toshiba Medical. System Corp., Tokyo, Japan) connected to a multi-frequency (7.5–10 MHz) linear array transducer. All examinations were performed by two radiologists, blinded to the participant's diabetes status. Patients were examined in the supine position with the head tilted backwards. The carotid arteries were examined from the supraclavicular fossa to the submandibular angle, including the common carotid artery (CCA), carotid bifurcations and the origins of the internal carotid arteries (ICA).

Table 1: Baseline characteristics of patients with DM2 with regard to statin therapy.

	Statin + n = 399	Statin - n = 196	p
Age (years)	62.1 ± 8.5	61.7 ± 10.5	0.74
Male gender (%)	222 (55.6)	117 (59.7)	0.35
Duration of DM2 (years)	12.5 ± 11.3	11.9 ± 6.5	0.48
Smoking (%)	43 (10.8)	10 (5.1)	0.02
Waist circumference (cm)	110 (102–118)	106 (98–113)	0.03
BMI (kg/m^2)	31.12 ± 4.62	30.34 ± 4.34	0.12
SBP (mm Hg)	148.7 ± 19.7	147.9 ± 21.6	0.74
DBP (mm Hg)	84.5 ± 11.3	86.9 ± 11.9	0.08
Fasting glucose (mmol/L)	8.24 ± 2.40	7.88 ± 2.78	0.44
HbA1c (%)	8.03 ± 4.51	7.54 ± 1.31	0.12
Total cholesterol (mmol/L)	4.55 ± 1.13	4.87 ± 1.31	0.03
HDL cholesterol (mmol/L)	1.1 (0.9–1.3)	1.2 (1.0–1.5)	0.51
LDL holesterol (mmol/L)	2.49 ± 0.92	2.90 ± 0.94	<0.001
Triglycerides (mmol/L)	2.2 (1.5–3.3)	2.2 (1.3–2.9)	0.64
hs-CRP (mg/L)	2.83 ± 2.65	2.74 ± 2.54	0.76
Fibrinogen (g/L)	3.97 ± 1.05	3.96 ± 1.18	0.93
ApoA1 (g/L)	1.68 ± 0.34	1.73 ± 0.34	0.42
ApoB (g/L)	0.87 ± 0.20	0.92 ± 0.21	0.19

Continuous variables were expressed as means ± standard deviations when normally distributed and as median (interquartile range) when asymmetrically distributed. Categorical variables were expressed as frequency (percentage).
BMI-body mass index; SBP-systolic blood pressure; DBP-diastolic blood pressure; HbA1c – glycated haemoglobin; hs-CRP-high sensitivity C-reactive protein.

The CIMT, defined as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface, was measured at 3 sites along the 10mm-long segment of the far wall of the CCA free of plaques, in agreement with the carotid intima-media thickness consensus.[16] The CIMT on the left and on the right were calculated as the mean of three readings, and the mean of the left and right CCA-CIMT measurements was used in the analysis. The interobserver reliability for CIMT measurements was found to be substantial ($\kappa = 0.74$, $p < 0.001$).

Biochemical analyses

Blood samples for biochemical analyses: total cholesterol, triglyceride levels, high-density lipoprotein (HDL), low-density lipoprotein (LDL) cholesterol level, fasting blood glucose and glycated haemoglobin (HbA1c), hsCRP and fibrinogen were collected after a 12-hour fasting period. All the blood biochemical analyses were determined by using standard biochemical methods in the hospital's accredited lab. Serum concentrations of apolipoprotein B (ApoB) and apoliporpotein A1 (ApoA1) were measured by a turbidimetric immunoassay on an AU 680 Chemistry System analyzer (Beckman Coulter, Nyon, Switzerland).

Genotyping

The genomic DNA was extracted from 100µL of whole blood using a FlexiGene DNA isolation kit, in accordance with the recommended protocol (Qiagene GmbH, Hilden, Germany). The AvaII (rs5925) polymorphism was determined with real-time PCR using StepOne™ (48-well) Real-Time PCR Systems (Applied Biosystems, Foster City, CA, USA). We used commercially available genotyping kit TagMan SNP Genotyping assay (C__2804279_10) (Applied Biosystem, Foster City, CA, USA) following the manufacturer's instructions.

Table 2: Genotype distribution and allele frequencies of the AvaII polymorphism in subgroups of patients with diabetes with regard to statin therapy.

AvaII	Statin + n = 399	Statin – n = 196	p
A-A-	67 (16.8)	26 (13.3)	0.41
A-A+	189 (47.4)	91 (46.4)	
A+A+	143 (35.8)	79 (40.3)	
Allele frequencies			
A-	323 (40.5)	143 (36.5)	0.18
A+	475 (59.5)	249 (63.5)	

Results were presented as frequency (percentage).

Statistical analysis

Continuous variables were expressed as means ± standard deviations, when normally distributed, and as median (interquartile range) when asymmetrically distributed. Normality of the continuous variables was examined by the Kolmogorov–Smirnov test. Continuous clinical data were compared using an unpaired Student's *t* test or analysis of variance (ANOVA) when normally distributed and the Mann-Whitney U-test or the Kruskal-Wallis H-test when asymmetrically distributed. The Pearson X^2 test was used to compare discrete variables and to test whether the genotypes distribution is in Hardy-Weinberg equilibrium. Pearson's correlation was performed to examine the association between independent variables. Due to the high correlation of LDL cholesterol with total cholesterol ($r = 0.86$, $p < 0.001$) and ApoB ($r = 0.82$, $p < 0.001$) they were not included together in the same statistical model. For the same reason, the body mass index (BMI) was not included in the model together with the waist circumference ($r = 0.45$, $p < 0.001$) while the systolic blood pressure was not included in the model together with the diastolic blood pressure ($r = 0.57$, $p < 0.001$).

To determine the association of the AvaII polymorphism with CIMT, a multiple linear regression analysis was performed. Linear regression model was adjusted for the presence of well established cardiovascular risk factors: age, gender, BMI, smoking, hypertension and systolic blood pressure. The results were presented as standardized β coefficients and P-values. Model fitness was evaluated by r-squared (coefficient of deter-

mination). A two-tailed P value less than 0.05 was considered statistically significant. A statistical analysis was performed using the SPSS program for Windows version 20 (SPSS Inc., Chicago, IL).

Results

Baseline characteristics of study participants

The baseline clinical and biochemical characteristics of the study participants with regard to statin therapy are shown in Table 1. There was no statistically significant difference regarding age, gender distribution and duration of DM2 between the two subgroups. Patients with DM2 using statins had higher smoking prevalence (p = 0.02) and greater waist circumference (p = 0.03) than those without statin therapy. As expected, serum levels of total and LDL cholesterol were statistically significantly lower in patients with DM2 using statins (p = 0.03 and < 0.001, respectively). No statistically significant difference in BMI, systolic and diastolic blood pressure, fasting glucose and HbA1c levels was observed between the two subgroups of patients with DM2. Furthermore, serum levels of ApoA1, ApoB and inflammatory markers (hsCRP and fibrinogen) were not statistically significantly different between the two subgroups.

The AvaII genotype distribution and allele frequencies in patients with DM2 with regard to statin therapy are shown in Table 2. The genotype distribution in both subgroups of patients with DM2 was compatible

Table 3: Plasma levels of lipid parameters and inflammatory markers and CIMT in patients with DM2 with regard to AvaII genotypes and statin treatment.

	Statin	AvaII			
		A-A-	A-A+	A+A+	p
Total chol. (mmol/L)	+	4.14 ± 0.81	4.53 ± 1.21	4.76 ± 1.13	0.03
	-	4.64 ± 1.39	4.67 ± 1.11	5.04 ± 1.51	0.27
LDL (mmol/L)	+	2.14 ± 0.74	2.46 ± 0.93	2.68 ± 0.95	0.02
	-	2.70 ± 0.68	2.78 ± 0.93	3.02 ± 1.01	0.28
HDL (mmol/L)	+	1.1 (1.0–1.35)	1.1 (1.0–1.35)	1.1 (1.0–1.5)	0.73
	-	1.1 (0.95–1.28)	1.2 (0.9–1.3)	1.1 (1.0–1.5)	0.75
Triglycerides (mmol/L)	+	1.9 (1.25–2.75)	1.84 (1.28–2.8)	1.9 (1.3–2.7)	0.99
	-	2.1 (1.0–2.7)	2.0 (1.4–3.1)	1.8 (1.2–2.3)	0.17
ApoA1 (g/L)	+	1.54 ± 0.21	1.65 ± 0.39	1.64 ± 0.33	0.54
	-	1.72 ± 0.30	1.68 ± 0.28	1.66 ± 0.33	0.92
ApoB (g/L)	+	0.82 ± 0.24	0.85 ± 0.20	0.92 ± 0.18	0.18
	-	0.97 ± 0.12	0.97 ± 0.17	0.89 ± 0.25	0.51
hs-CRP (mg/L)	+	2.98 ± 2.56	2.64 ± 2.86	2.78 ± 2.65	0.77
	-	2.19 ± 1.67	3.46 ± 3.81	2.99 ± 2.77	0.32
Fibrinogen (g/L)	+	4.98 ± 1.48	4.34 ± 1.10	4.17 ± 1.14	0.13
	-	4.72 ± 1.33	4.48 ± 1.12	4.16 ± 1.34	0.50
CIMT (µm)	+	870 ± 276	924 ± 212	979 ± 194	0.26
	-	967 ± 231	972 ± 267	999 ± 231	0.71

Continuous variables were expressed as means ± standard deviations when normally distributed and as median (interquartile range) when asymmetrically distributed.

with Hardy-Weinberg expectations. No statistically significant difference in either genotype distribution or allele frequencies was observed in patients with DM2 with regard to statin therapy (p = 0.41 and 0.18, respectively).

Biochemical parameters and CIMT with regard to AvaII genotypes

Table 3 outlines the serum levels of lipid parameters, ApoA1, ApoB and inflammatory markers (hsCRP and fibrinogen) in patients with DM2 with regard to AvaII genotypes and statin treatment. Serum levels of total and LDL cholesterol were statistically significantly different with regard to the AvaII genotypes in patients with DM2 using statins. Homozygous carriers of the A+ allele had the highest serum levels of total and LDL cholesterol (p = 0.03 and 0.02, respectively). In DM2 patients without statin therapy no statistically significant difference in biochemical parameters with regard to the AvaII genotypes was observed.

In both subgroups of DM2 patients the greatest CIMT values were observed in homozygous carriers of the A+ allele, but the differences were not statistically significant (p = 0.26 and 0.71) (Table 3).

Table 4: Linear regression analysis for variables independently associated with CIMT.

	CIMT	
	β	p
HDL (mmol/L)	-0.351	0.006
ApoB (g/L)	0.009	0.37
Triglycerides (mmol/L)	-0.327	0.007
hsCRP (mg/L)	0.271	0.04
Fibrinogen (g/L)	0.362	0.01
Statin treatment	-0.237	0.04
A-A+	0.242	0.26
A+A+	0.441	0.04

Model adjusted for age, gender, BMI, smoking, hypertension and systolic blood pressure.

Association of AvaII polymorphism and statin treatment with CIMT

The results of the multiple linear regression analysis for variables independently associated with CIMT are presented in Table 4. Interactions of AvaII genotypes A-A+ and A+A+ with statin treatment were not statistically significant (data not shown) so they have been excluded from the model. After adjustment for age, gender, BMI, smoking, hypertension and systolic blood pressure, variables independently associated with CIMT were: serum levels of HDL, triglycerides, hsCRP and fibrinogen as well as homozygosity for the A+ allele and statin treatment. Homozygosity for the A+ allele was associated with greater CIMT (β = 0.441; p = 0.04). Interestingly, serum triglyceride level was inversely associated with CIMT (β = 0.327, p = 0.007). Serum ApoB level was not independently associated with CIMT (p = 0.37). The model explained 44 % of variation of CIMT.

Replacing serum levels of ApoB in the model with LDL did not change the results of multivariate linear regression analysis to any meaningful extent.

Discussion

In the present study we observed an association between homozygosity for the A+ allele of the AvaII polymorphism and CIMT in patients with diabetes mellitus type 2.

To the best of our knowledge, this is the first study investigating the association between LDL receptor polymorphism and CIMT in patients with diabetes mellitus type 2. In the present study the distribution of AvaII genotypes and allele frequencies were not statistically significantly different between DM2 patients with regard to statin treatment. Frequency of the A+ allele and A+A+ polymorphism observed in our study were higher than those previously reported in healthy European populations.[17,18] Similar pattern of the AvaII genotypes distribution have been previously reported in patients with high risk CVD[7,10].

Common DNA polymorphisms in genes involved in lipid metabolism could be

responsible for normal variation in serum lipid profile and consequently to susceptibility to CVD. The evidence of an association between AvaII polymorphism and serum lipid levels is consistent, showing higher serum levels of total and LDL cholesterol in individuals carrying the A+ allele.[5-8] As some previous studies reported that AvaII polymorphism could affect lipid lowering response to treatment with fluvastatin and pravastatin[10,11] we analyzed lipid parameters in DM2 patients on statin therapy and those without statin separately. In both subgroups of DM2 patients serum levels of total and LDL cholesterol increased from A-A- to the A+A+ genotype. However, differences in serum total and LDL cholesterol levels were statistically significantly different only in those on statin therapy.

Limited number of studies investigating the association between the AvaII polymorphism and response to statin treatment yielded contradictory results.[10,11] However, in the present study interactions of both A-A+ and A+A+ genotype with statin treatment were not statistically significant. Further prospective studies are needed to elucidate whether the AvaII polymorphism of the LDL receptor gene affects response to statin treatment.

After adjustment for well established risk factors for atherosclerosis, homozygosity for the A+ allele was independently associated with increased CIMT. Due to the LDL particles' heterogeneity, a high concentration of small particles can occur despite a normal LDL cholesterol level, as often seen in patients with diabetes. As the total serum ApoB level may give a better estimate of the concentration of atherogenic particles than the LDL level,[19] we adjusted linear regression analysis for ApoB level instead for LDL.

Our results further support the evidence of association between A+A genotype with CVD.[7,12] This association is, at least partially, mediated by higher serum levels of total and LDL cholesterol observed in A+A+ homozygtes in both subgroups of DM2 patients. However, the mechanism underlying such association is still unknown. AvaII polymorphism represents a silent mutation which does not affect amino acid sequence, so the observed association probably results from a linkage disequilibrium with other functional variant.[5,17] Moreover, it has been suggested that the second domain of the LDL receptor (coded by exon 13) is 33 % homologous with a portion of the extracellular domain of the epidermal growth factor precursor, a peptide hormone involved in growth stimulation.[3,20] However, the importance of this finding needs to be further investigated.

Our study has possible limitations due to its cross-sectional design and relatively small sample size, providing limited power to study moderate genetic effects and to detect interactions with other, potentially relevant variables. Further studies are needed to definitely elucidate the impact of the genetic variability of the LDLR gene in modulating lipid and apolipoprotein levels as well as CVD risk. Further prospective studies are needed to elucidate whether the AvaII polymorphism of the LDL receptor gene affects response to statin treatment.

Conclusion

This is the first study investigating the association of the AvaII polymorphism with CIMT in patients with DM2. We observed an association between homozigosity for the A+ allele with greater CIMT in patients with diabetes.

References

1. Catapano AL, Reiner Z, De Backer G, Graham I, Taskinen MR, Wiklund O et al. ESC/EAS Guidelines for the management of dyslipidaemias The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). Atherosclerosis 2011; 217: 3-46.
2. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. Science 1986; 232: 34-47.
3. Südhof TC, Goldstein JL, Brown MS, Russell DW. The LDL receptor gene: a mosaic of exons shared with different proteins. Science 1985; 228: 815-22.
4. Sinha E, Walia GK, Gupta BP, Ghosh PK, Saraswathy KN. LDL-R AvaII and NcoI polymorphi-

sms: an indirect risk factor for coronary heart disease among a Mendelian population of Delhi, India. Biochem Genet 2010; 48: 807-15.
5. Ahn YI, Kamboh MI, Aston CE, Ferrell RE, Hamman RF. Role of common genetic polymorphisms in the LDL receptor gene in affecting plasma cholesterol levels in the general population. Arterioscler Thromb 1994; 14: 663-70.
6. Bertolini S, Cassanelli S, Garuti R, Ghisellini M, Simone ML, Rolleri M et al. Analysis of LDL receptor gene mutations in Italian patients with homozygous familial hypercholesterolemia. Arterioscler Thromb Vasc Biol 1999; 19: 408-18.
7. Salazar LA, Hirata MH, Giannini SD, Forti N, Diament J, Issa JS et al. Effects of Ava II and Hinc II polymorphisms at the LDL receptor gene on serum lipid levels of Brazilian individuals with high risk for coronary heart disease. J Clin Lab Anal 1999; 13: 251-8.
8. Wiseman SA, Powell JT, Humphries SE, Press M. The magnitude of the hypercholesterolemia of hypothyroidism is associated with variation in the low density lipoprotein receptor gene. J Clin Endocrinol Metab 1993; 77: 108-12.
9. Nakazone MA, De Marchi MA, Pinhel MA, Barros CF, Júlio MA, Pinheiro A et al. Effects of APOE, APOB and LDLR variants on serum lipids and lack of association with xanthelasma in individuals from Southeastern Brazil. Genet Mol Biol 2009; 32: 227-33.
10. Salazar LA, Hirata MH, Quintão EC, Hirata RD. Lipid-lowering response of the HMG-CoA reductase inhibitor fluvastatin is influenced by polymorphisms in the low-density lipoprotein receptor gene in Brazilian patients with primary hypercholesterolemia. J Clin Lab Anal 2000; 14: 125-31.
11. Lahoz C, Peña R, Mostaza JM, Laguna F, García--Iglesias MF, Taboada M et al. Baseline levels of low-density lipoprotein cholesterol and lipoprotein (a) and the AvaII polymorphism of the low--density lipoprotein receptor gene influence the response of low-density lipoprotein cholesterol to pravastatin treatment. Metabolism 2005; 54: 741-7.
12. Salazar LA, Hirata MH, Giannini SD, Forti N, Diament J, Lima TM et al. Seven DNA polymorphisms at the candidate genes of atherosclerosis in Brazilian women with angiographically documented coronary artery disease. Clin Chim Acta 2000; 300: 139-49.
13. Mookadam F, Moustafa SE, Lester SJ, Warsame T. Subclinical atherosclerosis: evolving role of carotid intima-media thickness. Prev Cardiol 2010; 13: 186-97
14. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 1999; 340: 14-22.
15. Polak JF, Szklo M, Kronmal RA, Burke GL, Shea S, Zavodni AE et al. The value of carotid artery plaque and intima-media thickness for incident cardiovascular disease: the multi-ethnic study of atherosclerosis. J Am Heart Assoc 2013; 2:e000087.
16. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N et al. Mannheim carotidintima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim,Germany, 2004, and Brussels, Belgium, 2006. Cerebrovasc Dis 2007; 23: 75-80.
17. Miserez AR, Schuster H, Chiodetti N, Keller U. Polymorphic haplotypes and recombination rates at the LDL receptor gene locus in subjects with and without familial hypercholesterolemia who are from different populations. Am J Hum Genet. 1993; 52: 808-26.
18. Humphries S, King-Underwood L, Gudnason V, Seed M, Delattre S, Clavey V et al. Six DNA polymorphisms in the low density lipoprotein receptor gene: their genetic relationship and an example of their use for identifying affected relatives of patients with familial hypercholesterolaemia. J Med Genet 1993; 30: 273-9.
19. Barter PJ, Ballantyne CM, Carmena R, Castro Cabezas M, Chapman MJ, Couture P et al. Apo B versus cholesterol in estimating cardiovascular risk and in guiding therapy: report of the thirty--person/ten-country panel. J Intern Med 2006; 259: 247-58.
20. Russell DW, Schneider WJ, Yamamoto T, Luskey KL, Brown MS, Goldstein JL. Domain map of the LDL receptor: sequence homology with the epidermal growth factor precursor. Cell 1984; 37: 577-85.