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Original Article

R46L polymorphism in the PCSK9 gene: Relationship to lipid levels, subclinical vascular disease, and erectile dysfunction

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Ankle-brachial index;
Carotid atherosclerosis;
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LDL-cholesterol;
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BACKGROUND: The R46L variant of the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene has been related to lipid levels and cardiovascular disease.

OBJECTIVE: To evaluate the influence of this polymorphism on subclinical vascular disease and erectile dysfunction (ED).

METHODS: We analyzed the association of the PCSK9 rs11591147 single-nucleotide polymorphism with lipid levels, intima-media thickness (IMT), and the ankle-brachial index, in 1188 adults free of cardiovascular disease, randomly selected from the population. In 473 male participants, we also investigated its relationship with ED. The association of the R46L polymorphism with lipid levels was also assessed in 2 cohorts of 1103 prepuberal children and 830 adolescents.

RESULTS: The prevalence of the T allele was 2.9% in adults. Low-density lipoprotein cholesterol (LDL-cholesterol) levels did not vary according to this polymorphism (134 ± 32 vs 134 ± 31 mg/dL, for the TT + GT vs GG carriers, respectively, $P = .931$). Despite equal LDL-cholesterol levels, adults carrying the T allele had a lower mean common carotid IMT (0.685 ± 0.09 vs 0.723 ± 0.127 mm; $P = .035$), a lower maximum common carotid IMT (0.819 ± 0.11 vs 0.865 ± 0.159 mm; $P = .040$), and, in males, a lower prevalence of ED (36.8% vs 61%: $P = .036$), than GG carriers. Prevalence of the T allele was 3.2% in both cohorts of children. They had lower levels of LDL-cholesterol than GG subjects (100 vs 109 mg/dL; $P = .060$, for prepuberal children, and 85 vs 99 mg/dL; $P = .010$ for adolescents).

CONCLUSION: In our population, an association between the PCSK9 R46L variant and LDL-cholesterol levels is observed in children. In adults, although its association with lipid levels is not evident, there is a significant relationship between the PCSK9 R46L variant and markers of subclinical atherosclerosis, including IMT and ED.

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Introduction

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease involved in low-density lipoprotein (LDL) receptor degradation.[1] Increased plasma levels of PCSK9 as a consequence of gain of function mutations are associated with the development of familial hypercholesterolemia. Conversely, loss of function mutations is related to low levels of cholesterol and a reduced prevalence of coronary heart disease. A number of epidemiological studies from different populations have evaluated the effects of frequent PCSK9 genetic variants, on lipid and lipoprotein levels, subclinical vascular disease, and cardiovascular risk.[2-6] One of the single-nucleotide polymorphisms (SNPs) more consistently associated with lipid concentrations in white subjects of occidental origin is R46L (rs11591147). Carriers of the minor rs11591147 (T) allele have approximately 10% to 15% lower LDL-cholesterol levels and decreased cardiovascular risk.[3]

Different markers of subclinical vascular disease have been associated with an increased risk of future cardiovascular complications. Ankle-brachial index (ABI) has been previously related to a higher rate of incident cardiovascular disease,[7] and its measurement is recommended by different guidelines to improve risk stratification.[8] Carotid intima-media thickness (IMT) has also been associated with the development of coronary and cerebrovascular complications, although its potential to allow reclassification of subjects with different risk categories has been questioned.[8] Moreover, erectile dysfunction (ED) has been recognized as a marker of atherosclerosis,[9] and it has been independently associated with the rate of cardiovascular disorders.[10]

Despite these associations, only 2 previous studies, both using the atherosclerosis risk in communities cohort,[3,11] have assessed the relationship of the R46L polymorphism with markers of subclinical vascular disease. They have shown that carriers of the T allele have a reduced carotid IMT and a lower prevalence of peripheral artery disease (PAD).

The magnitude of the association of traditional risks factors with subclinical vascular disease and cardiovascular risk has been shown to be modified by race and ethnicity,[12] mainly due to interactions between the genetic background and environmental factors. Therefore, the prevalence of the R46L mutation and its relation with LDL-cholesterol levels and markers of subclinical vascular disease could differ among different populations, and in the present study, we have evaluated the association of the PCSK9 R46L variant with IMT, ABI, and ED, in adults free of cardiovascular disease. We have also evaluated the relation of this polymorphism with lipid and lipoprotein levels in adults and in 2 cohorts of children of different age.

Patients and methods

Three different samples were analyzed in our study: the SPREDIA-2 (**S**creening **PRE**-diabetes and type 2 **DIA**betes) cohort and 2 cohorts of healthy children.

SPREDIA-2: Study subjects, data collection, study variables, and methods

The SPREDIA-2 study is a population-based prospective cohort study with baseline screening, in the Region of Madrid (Spain).[13] A random sample of subjects, between 45 and 74 years, living in the north-west metropolitan area of Madrid (Spain) was selected for the study. In the reference population, there are approximately 183,000 persons of this age. Institutionalized subjects, those with severe chronic or terminal illnesses, or those chronically treated with steroids or antipsychotic drugs, were excluded.

Participants were scheduled in the outpatient clinic of the Hospital Carlos III after an overnight fast. On arrival, and after signing a consent form, a fasting blood analysis was obtained for measuring the blood levels of glucose, HBA1c, and lipids and lipoproteins.

Sociodemographic variables (date of birth, gender), cardiovascular risk factors (smoking habit, hypertension, alcohol ingestion), comorbidities, and current treatments were recorded in all individuals. All participants had a physical examination with the determination of height, weight, waist circumference, and blood pressure (the mean of the last 2 measurements after 3 determinations 5 minutes apart).

The 14-point Mediterranean adherence screener (MEDAS 14) was used to assess Mediterranean diet adherence. Prediabetes was defined as not having previous diabetes but having a fasting plasma glucose level between 100 and 125 mg/dL, A1C levels between 5.7% and 6.4%, or a 2h-OGTT plasma glucose between 140 and 199 mg/dL.

An echo-Doppler of both carotids was performed with a 7.5-mHz probe (Sonosite MicroMaxx Ultrasound; Sonosite Inc, Bothell, WA). Patients laid in the supine position with the neck rotated to the opposite site of the examination. One-centimeter images were obtained from the distal wall of the common carotid artery proximal to the bifurcation, in 3 different angle views. IMT was obtained using an automated software (Sonosite, Sonocalc IMT Software; Sonosite Inc), and the maximal region and the overall mean IMT values for each of the 6 segments analyzed (3 angles in 2 territories) were calculated. IMT values for the 3 different projections and for right and left carotid arteries were averaged to obtain the maximum CCA-IMT and the mean CCA-IMT. Carotid plaques were examined in all the carotid territory (common carotid, bulb, and internal and external carotid arteries) and were defined as a local thickening of the intima >1.5 mm or a thickening of >50% of the surrounding IMT value. Only 1 trained person made all IMT measurements. Replicate recordings were performed on 5% of the population, and the coefficient of variation was 4.6%.

The ABI measurements were performed using a bidirectional portable echo-Doppler of 8 MHz (Minidoppler HADECO ES-100, Kawasaki, Japan) and a calibrated mercury sphygmomanometer. The systolic blood pressure