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Original article

Lack of association between carotid intima-media thickness and apolipoprotein (a) isoforms in a sample of Spanish general population

Pilar Calmarza (PhD)[a,*], Jose M. Trejo (MD, PhD)[b], Carlos Lapresta (MD)[a], Pilar Lopez (MD)[c]

[a] Hospital Universitario Miguel Servet, Zaragoza, Spain
[b] Neurology Department, Research Unit, Complejo Asistencial, Burgos, Spain
[c] Emergency Department, Research Unit, Complejo Asistencial, Burgos, Spain

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ABSTRACT

Objectives: The purpose of this study was to examine the relationship between apolipoprotein (a) isoforms and early atherosclerosis, assessed by carotid artery intima-media thickness in a sample of adults of the general population of Burgos, a city in the north of Spain.
Design and methods: Lipids, lipoprotein (a), number of carotid atherosclerotic plaques, if any, and the intima-media thickness in the far wall of both common carotid arteries by B-mode ultrasound were determined in a group of 171 adults from the general population of Burgos, Spain. Apolipoprotein (a) isoforms were determined in a random subset of 119 subjects.
Results: Increasing age, male sex, and past personal cardiovascular history were significantly associated with increased left, right, or average intima-media thickness of both carotid arteries in multivariate analysis.

No statistically significant association was found between apolipoprotein (a) isoforms and mean carotid intima-media thickness by bivariate or multivariate regression analysis.
Conclusions: In this sample of the general Spanish population, no association was found between apolipoprotein (a) isoforms and carotid artery intima-media thickness.

Introduction

Intima-media thickness (IMT) is an echographic measurement that estimates both inner arterial layers (intima and media) where atheroma begins, and therefore it is considered a good measure of atheroma in its initial stages. Both carotid atherosclerotic plaques and increased common carotid artery IMT are associated with a higher risk of ischemic stroke, presence and severity of coronary artery disease, and cardiovascular mortality [1–4]. A review of eight epidemiological studies showed that the IMT of the common carotid artery had independent predictive power with respect to cardiovascular events [5].

Lipoprotein (a) [Lp (a)], an emerging risk factor for cardiovascular disease, is a cholesterol rich lipoprotein composed of a low-density lipoprotein (LDL) particle and a highly polymorphic apolipoprotein (a) [Apo (a)], which is covalently linked to the apolipoprotein-B moiety of the LDL by a single disulphide bridge.

Apo (a) shares a striking structural similarity to plasminogen [6] and contains multiple repeated copies of a sequence that closely resembles plasminogen kringle 4 (KIV), followed by two sequences homologous to kringle V and serine protease domains of plasminogen, respectively.

The KIV-like sequences can be classified into 10 types, based on amino acid sequence. Each Apo (a) molecule contains a single copy of KIV types 1 and 3–10, but variable identical repeats (3 to more than 40) of kringle IV type 2, thus leading to different Apo (a) isoforms sizes [7,8], with molecular weights ranging from 280 to 800 kDa.

The concentration of Lp (a) is controlled by a single gene in the region 6q26-27 with multiple alleles, and each allele influences the concentration of Lp (a) differently [9], according to the number of expressed kringle IV repeats [10,11]. Other sequence variations of the Apo (a) locus may also influence Lp (a) plasma levels.

Lp (a) phenotypes have different functional properties as regards lysine binding affinity, the small Apo (a) isoforms showing the greatest affinity for fibrin [12], suggesting that the low molecular weight (LMW) Apo (a) isoform's size itself plays a role in atherogenesis [12].

Although a relationship has frequently been reported between Apo (a) isoforms with less than 22 KIV repeats and cardio- or cerebro-vascular diseases [13–15], few studies have investigated the relationship between phenotypes of Apo (a) and carotid artery IMT. In this study the association between Apo (a) isoforms and

* Corresponding author at: Avda. Cesáreo Alierta n° 38, Esc 1ª 5° A, Zaragoza, Spain. Tel.: +34 976 76 55 00/59 07 29; fax: +34 976 76 55 43.
E-mail address: mpcalmarza@salud.aragon.es (P. Calmarza).

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carotid IMT in a group of individuals from the general population of Burgos, northern Spain was studied.

Materials and methods

Population studied

This study was performed on 171 subjects randomly selected from the records of the 200,000 referral population of "Gamonal Antigua" Health Care Center of the city of Burgos, and their age and sex percentage distribution were those of the Spanish population in the 2004–2010 census. A sample size of 180 individuals was calculated in order to have an alpha error of 0.05, a power of 60% to detect a 0.2 mm difference from a normal IMT of 0.65 mm ± 0.15 mm and a 10% of expected losses of recruited participants. After data collection, losses were less than predicted, 9 participants, thus leaving 171 subjects valid for analysis.

Apo (a) isoforms were determined in a subset of 119 randomly selected subjects due to economic limitations. Lp (a) levels and established cardiovascular risk factors, such as blood pressure, total cholesterol, high-density lipoprotein (HDL)-cholesterol, LDL-cholesterol, and triglycerides were also determined.

Blood pressure was assessed by the mean of two systolic and diastolic readings after 10 min of rest in the supine position. Hypertension was defined as a systolic/diastolic blood pressure > 140/95 mm Hg or current use of antihypertensive drugs.

Body mass index (BMI), waist-to-hip ratio, and demographic data were also recorded. A standardized questionnaire on current and past personal or familial cardiovascular history, alcohol or tobacco consumption, sociodemographic variables, diabetes mellitus, or other previous disease was completed by each participant.

Informed consent for the procedures to be used was obtained from each subject. The study was approved by the Clinical Studies Committee of the referral hospital.

Analytical methods

Venous blood samples were obtained under standardized conditions and after 12 or more hours of fasting. Total cholesterol and triglycerides where determined enzymatically (Roche Diagnostics, Basel, Switzerland), LDL-cholesterol using the Friedewald formula, and very low-density lipoprotein (VLDL)-cholesterol by dividing the triglyceride concentration by 2.18. Both LDL- and VLDL-cholesterol were calculated in mmol/l.

Lp (a) in serum was quantified by a commercially available rate nephelometry method (Siemens, Marburg, Germany) with an analytical sensitivity of 0.002 g/l, no cross-reactivity with apolipoprotein B (<1%), a <5% cross-reactivity with plasminogen, and minimally affected by Apo (a) isoforms size heterogeneity [16].

Apo (a) phenotypes were determined by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE) and immunoblotting by a method modified from that described by Utermann et al. [17] according to Kraft et al. [18].

The samples were prepared by mixing 10 μl of serum with 85 μl of a buffered solution and 5 μl of β-mercaptoethanol. Aliquots of samples were then subjected to SDS PAGE in a 4–15% gradient polyacrylamide gel. The separated proteins were transferred to a nitrocellulose membrane and antigens were visualized on the nitrocellulose membrane by a double-antibody procedure. The first antibody was a polyclonal antihuman Lp (a), which is raised in sheep. The second antibody was an anti-sheep immunoglobulin G antibody conjugated to alkaline phosphatase. When the appropriate substrate was applied the bands became visible.

The different isoforms of Lp (a) were designated according to their electrophoretic mobility on SDS PAGE relative to a mix of five recombinant Apo (a) isoforms containing kringles 35, 27, 23, 19, and 14 KIV repeats that was used as standard reference.

The Apo (a) phenotypes were stratified into 2 subgroups according to the molecular weight of the smaller Apo (a) isoform, the LMW Apo (a) phenotype was determined by the occurrence of at least 1 Apo (a) isoform with 11–22 KIV repeats, and the high molecular weight (HMW) Apo (a) phenotype when all isoforms had >22 KIV repeats as they are conventionally designated [19].

Intima-media thickness determination

IMT was determined by B-mode ultrasound in the far wall of the left and right common carotid arteries, 1 cm proximal to its bifurcation. The ultrasound equipment was an HP Image Point (Hewlett-Packard, Palo Alto, CA, USA) with a 10 MHz linear probe that was placed on the neck of the subjects who lay supine, parallel to its longitudinal axis, in an anterolateral plane (60° angle, with 0° being the horizontal). Each IMT measurement was performed twice in both the left and right carotid of each subject, and the average right and left carotid IMT calculated. Measurements were conducted blind to the rest of the data, by the same investigator. An atheroma plaque was defined echographically as a hyperechogenicity or protrusion in the vascular lumen of the intima of at least twice the thickness of the adjacent intima media.

Statistical analysis

For each variable, the validity of assumption of normality was checked. This assumption held true for all variables, except for IMT, waist-to-hip ratio and Lp (a) concentration, in which results are expressed as medians and interquartile ranges (IR). In the rest, results are expressed as means ± standard deviation (SD).

To assess the relationship between IMT and Lp (a), a bivariate linear regression analysis was performed. The same analysis of IMT and secondary independent variables such as age, BMI, waist-to-hip ratio, systolic blood pressure, HDL-cholesterol, LDL-cholesterol, plasma triglycerides, and fasting glucose followed. To determine the association of Lp (a) and IMT after adjusting for secondary independent variables, a multivariate linear analysis was performed in which nominal variables such as gender and current or past personal or familial cardiovascular history were included as dummy variables.

IMT, age, Lp (a), and conventional analytical or anthropometric cardiovascular risk factors were compared between >22 k4 and ≤22 k4 repeats groups, using the Student's *t*-test when normally distributed or if not by the Mann–Whitney test as in the case of IMT, Lp (a), and waist-to-hip ratio.

We examined the association between IMT and Lp (a) carrying differently sized Apo (a) isoforms using multivariate linear regression analysis.

The Spearman's rank correlation coefficient was used to measure the statistical dependence between the molecular weight of the different isoforms of Apo (a) and the mean of the right and left IMT.

To determine the combined effect of increased Lp (a) concentrations and LMW, Apo (a) phenotypes were stratified into 4 groups according to Lp (a) concentrations (cut-off point 300 mg/l) and Apo (a) phenotypes (cut-off point 22 KIV repeats). All *p*-values less than 0.05 were considered to indicate statistical significance.

Statistics were calculated using the program SPSS 15 (SPSS Inc., Chicago, IL, USA).

Results

Anthropometric and lipoprotein parameters are shown in Table 1. Lp (a) ranged from 10 to 1060 mg/l (IR = 307.5) with a