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ORIGINAL ARTICLE

CME

Lipoprotein a: Extreme Elevation and Genetic Polymorphism Association with Cardiac and Vascular Lesions Evaluated by Computed Tomography

Lipoproteína "a": elevaciones extremas y polimorfismos genéticos, asociación con lesiones cardíacas y vasculares evaluadas por tomografía

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ABSTRACT

Background: Lipoprotein a [Lp(a)] has a complex structure, similar to low density lipoprotein, associated with one molecule of apolipoprotein a [apo(a)]. Elevated plasma levels of Lp(a) are related to greater risk of coronary artery disease and calcification of the aortic valve. This risk is mainly determined by genetic factors.
Objectives: The aim of this study was to analyze a group of patients with extreme Lp(a) levels [>100 mg/dL] and their association with calcifications of the aortic valve, coronary arteries and thoracic aorta detected by computed tomography scan, and to evaluate three genetic polymorphisms associated with Lp(a) levels and lesions in these three regions.
Methods: rs10455872 and rs2048327 polymorphisms were analyzed in 40 patients using high resolution melting and the number of KIV-2 repeats in the LPA gene was evaluated using quantitative PCR.
Patient mean age was 52.9 years (37% women) and mean Lp(a)was 170.4 mg/dL.
Results: Seventy-five percent of patients (30/40) presented at least one calcification in the computed tomography scan (valves, coronary arteries and/or thoracic aorta), and among them, 90% had at least one of the genetic factors associated with Lp(a) pathogenicity.
Conclusion: In a group of patients with elevated Lp(a) levels, we found a significant number of cardiovascular thoracic calcifications and genetic determinants associated with different Lp(a) isoforms that could be related with elevated Lp(a)levels and high risk of developing valve or vascular lesions.

Key words: Lipoprotein - Hyperlipidemia - Polymorphism, Genetic - Vascular Calcification

RESUMEN

Introducción: La lipoproteína a [Lp(a)] es una partícula compleja, similar a una lipoproteína de baja densidad, asociada con una molécula de apolipoproteína"a" [apo(a)]. La concentración elevada de Lp(a) plasmática se asocia con riesgo aumentado de enfermedad coronaria y de calcificación valvular aórtica. El riesgo inherente a esta relación está determinado principalmente por factores genéticos.
Objetivos: Analizar un grupo de pacientes con elevaciones extremas de Lp(a) [> 100 mg/dl], su asociación con calcificaciones cardiovasculares torácicas (valvulares, coronarias, aorta torácica) detectadas mediante tomografía axial computarizada y evaluar tres polimorfismos genéticos vinculados con los niveles de Lp(a) y lesiones en estas tres regiones.
Material y métodos: Se estudiaron 40 pacientes en los que se analizaron los polimorfismos rs10455872 y rs2048327 mediante high resolution melting y el número de repeticiones de la secuencia KIV del exón 2 del gen LPA mediante qPCR.
El promedio de edad de los pacientes fue de 52,9 años (37% mujeres) y el valor promedio de Lp(a) fue de 170,4 mg/dl.
Resultados: El 75% (30/40) de los pacientes presentó al menos una calcificación en la tomografía (valvular, coronaria y/o aorta torácica); de los pacientes con calcificaciones, el 90% presentaron al menos uno de los factores genéticos asociados con mayor patogenicidad de la Lp(a).
Conclusión: En un grupo de pacientes con niveles elevados de Lp(a) encontramos un número importante de calcificaciones cardiovasculares torácicas y determinantes genéticos asociados con diferentes isoformas de Lp(a), que podrían ocasionar los niveles elevados de Lp(a) y el riesgo de desarrollo de lesiones valvulares y/o vasculares.

Palabras clave: Lipoproteína - Hiperlipidemia - Polimorfismo genético-Calcificación vascular

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Abbreviations

DNA	Desoxyribonucleic acid	**HRM**	High resolution melting
HDL-C	High-density lipoprotein cholesterol	**LDL**	Low density lipoprotein
LDL-C	Low-density lipoprotein cholesterol	**Lp(a)**	Lipoprotein a
Non-HDL-C	Non-high density lipoprotein cholesterol	**PCR**	Polymerase chain reaction
TC	Total cholesterol	**SNP**	Single-nucleotide polymorphism
CVD	Cardiovascular disease	**TG**	Triglycerides

INTRODUCTION

Lipoprotein a [Lp(a)] is a lipoproteic fraction, similar to a low density lipoprotein, bound to a glycoprotein, apolipoprotein (a). (1) Elevated Lp(a) levels (> 30-50 mg/dL) are considered a causal and independent risk factor for cardiovascular disease. (2) Different lines of evidence have demonstrated that the double structure of Lp(a) and its elevated plasma levels are associated with calcification of the aortic root and atherosclerotic lesions, particularly in the coronary arteries. (3)

Levels of Lp(a) may vary up to a thousand-fold among individuals, and are mainly determined by variations in the gene encoding LPA [apolipoprotein(a)]; (4) in fact, Lp(a) is considered the protein with the strongest genetic control. (5) Patients with Lp(a) levels >100 mg/dL are considered as having extreme Lp(a) elevations and higher risk. (6)

There are different isofoms of Lp(a), and small isoforms are specifically harmful. (4) The LPA gene contains the kringle-IV type 2 (KIV-2)-like domain, which is the most influential factor in Lp(a) levels and Lp(a) isoforms; the repetition of this polimorphism determines the size of the apolipoprotein (a), and the number of repeats has an inverse correlation with Lp(a) concentrations. (5)

Additionally, single-nucleotide polymorphism (SNP) rs10455872 in the LPA gene is directly associated with high levels of Lp(a) and greater risk of aortic valve calcification/stenosis in different populations. (3-5, 7) At the beginning of 2016, the Genome Wide Association Study (GWAS) described a new type of SNP in the SLC22A3 gene (rs2048327) that could be associated with coronary artery disease and elevated Lp(a) levels. (8, 9)

Based on recent knowledge of the genetic background determining Lp(a) levels, using modern methods of molecular biology, it is possible to study the number of KIV repeats in the LPA gene and of SNPs associated with greater risk. (5)

Cardiac computed tomography scan and particularly the coronary artery calcium score have demonstrated to be the strongest predictors of coronary events, allowing the recategorization of cardiovascular risk. In addition, cardiac computed tomography scan has proved to be highly sensitive to evaluate calcification of the aortic root and thoracic aorta, allowing diagnosis with a non-invasive, accurate and highly reproducible method. (10, 11)

The aim of this study is to describe a group of patients with extreme LP(a) levels, its relationship with cardiac and vascular lesions evaluated by computed tomography scan, and its association with the genetic polymorphisms evaluated (rs10455872, rs2048327 and number of KIV-2 repeats).

METHODS

Criteria for patient selection

The cohort consisted of 40 consecutive unrelated adult patients with Lp(a) levels >100 mg/dL, measured by immunoturbidimetry. The following screening criteria were used: early ischemic heart disease (women <65 years and men <55 years), family history of early cardiovascular disease and/or elevated Lp(a) levels and finally recurrent cardiovascular disease despite maximal treatment with statins. Patients with history of family hypercholesterolemia were excluded from this analysis. Twelve patients were receiving secondary prevention at the moment of detection based on the criteria previously described.

Clinical data (age, sex, weight, height, blood pressure, smoking habits) was collected, as well as personal and family history of cardiovascular disease and the results of laboratory tests (TC, LDL-C, HDL-C, TG, non-HDL-C, BUN, creatinine, glomerular filtration rate, and blood glucose level). The information about the medications received by each patient was also recorded.

Computed tomography scan

A 16-detector row computed tomography scanner (Siemens Emotion 16 CT) was used to explore the thorax. Images were acquired using multislice scanning, resulting in a volume with section thickness of 0.675 mm and reconstructions with 3-mm collimation. The scan was acquired from the lung apex to the top of the diaphragm, with an average of 80 slices per study, a field of view ranging from 40 to 44 cm and dose of 110 kV and 15 mA. Radiation dose was 1.2 mSv. Calcifications in the coronary arteries, as well as in the aortic root (aortic annulus, fibrous trigone, sinotubular junction, aortic valve leaflets and sinuses of Valsalva), distal thoracic aorta and mitral annulus were explored using a threshold of 130 Hounsfield units as criterion to define calcification. The presence and the extent of the calcifications were evaluated qualitatively and were quantified using the Agatston index. (12-14)

Molecular genetic analysis

Blood samples were collected via venous puncture to obtain DNA using a commercially available kit (ADN High Pure PCR Template preparation Kit, Roche). The SNPs s10455872 and rs2048327 were analyzed using high-resolution melting (HRM). (15, 16) The SNP rs10455872 (A>G) was evaluated using the primes described by Santos et al. (7) and for the evaluation of SNP rs2048327 (A>G) we used primes that were specifically designed for this study with the Primer Premier software (PREMIER Biosoft International).

Real time-polymerase chain reaction (PCR) amplifications were performed with a Rotor Gene Q Thermocycler (Qiagen, Hilden, Germany) using a commercial PCR premix (Biodynamics). HRM analysis was performed after the amplifications. The different melting profiles were visualized using normalized graphs and genotypes were determined with the HRM analysis software Rotor Gene Q Series (Qiagen, Hilden, Germany).

The variance in the number of LPA KIV-2 copies was assessed by real-time PCR using the modified method described by Kamstrup et al. (6) The number of repeats was measured by relative quantification of single copy human gene beta-actine, calibrated with the DNA of patients with normal LP(a) levels, associating a value <1 with low number of KIV-2 repeats.

Statistical analysis

The allele frequencies of the SNPs rs10455872 and rs2048327 were determined. A genetic risk score was developed in a scale ranging from 0 to 5, depending on the number of genetic risk factors present in each patient. The presence of the G allele in the SNPs rs10455872 and rs2048327 or a cutoff value <1 in the analysis of the number of KIV-2 copies were considered risk factors. Continuous variables were expressed as mean ± standard deviation and categorical variables as percentages.

Ethical considerations

All the patients signed an informed consent form before participating in the study. Data was collected anonymously according to the Argentine personal data protection law Nº 25326.

RESULTS

Mean age was 53 years (62% were men). Mean TC level was 225 mg/dL. Among the 40 patients evaluated, 8 (20%) were taking medications for secondary prevention of cardiovascular disease.

Every calcification of the aortic root and thoracic aorta and a coronary calcium score >100 were considered abnormal calcifications and were taken into account for the analysis.

Thirty (75%) of the 40 patients evaluated had at least one calcification in the regions studied. Fifty-five calcifications were detected and distributed as follows: 11 patients presented one calcification and 19 had more than one (14 patients with two calcifications, 4 patients with three and one patient presented 4 calcifications in different regions). The calcifications were distributed according to the anatomic region: 49% (n=25) in the thoracic aorta, 35% (n=20) in the coronary arteries and 16% (n=9) in the aortic root. We did not detect calcification in the mitral annulus/mitral valve.

When the presence of genetic factors was evaluated, we found that the frequency of the allele G in the SNPs rs10455872 was 0.26. In 72% of the patients evaluated, the number of KIV-2 repeats was lower than that of patients with normal Lp(a) levels.

In our population, no patient presented with the SNP rs10455872 GG homozygous genotype, so the genetic score ranged from 0 to 4: 0 in 7%, 1 in 32%, 2 in 28%, 3 in 30% and 4 in 3%.

The 9 patients with calcification in the aortic root (100%) had at least one of the three genetic factors considered abnormal (score ≥1); 18 of the 20 patients with calcifications in the coronary arteries (90%) and 22 of the 25 with calcifications in the thoracic aorta (88%) also showed at least one of the risk factors associated with the molecular results.

Three patients had the SNP rs2048327 GG homozygous genotype and presented calcifications in two different territories (coronary arteries and thoracic aorta).

Figure 1 summarizes the genetic findings associated with the results of calcification.

Based on these results, the therapeutic management was changed in all the patients with a genetic score of 1 or greater and in all the patients that presented calcifications in the CT scan in any of the territories evaluated.

According to the criterion established, 12/40 (30%) were receiving statins at the time of inclusion and 37/40 (92%) after the analysis; 9/40 patients (22%) were taking aspirin and this number increased to 28/40 (70%) after the analysis (Figure 2). One patient was taking nicotinic acid at the beginning of the study and 7 patients after the evaluation.

DISCUSSION

When the presence of genetic factors was evaluated, we found that the frequency of the risk G allele in the SNP rs10455872 in our study group was higher than that of the general population described in international databases (0.26 vs. 0.07) but we did not find differences in the frequencies of the G allele in the polymorphism rs2048327 (0.35 vs. 0.35). (17)

Lp(a) is a highly atherogenic lipoproteic fraction that is mostly regulated by genetic factors. The genetic variants, including the great variation in the number of KIV repeats, confer Lp(a) different atherogenic properties. The method called “Mendelian randomization” was applied over two decades ago, demonstrating and supporting the causal relationship between this biomarker and CVD: (3) This method has demonstrated causality between LP(a) levels and specific genotypes and aortic valve stenosis. (18) The most explored genetic factor considered a determinant of Lp(a) levels and isoforms (5) is the kringle IV-type 2 domain (KIV-2) in the LPA gene, which has been studied for the first time in our country.

The present observational study has associated extreme Lp(a) levels (>100 mg/dL), genetic polymorphisms related with coronary artery disease, diseases of the aortic root and thoracic aorta and computed tomography scan of the chest to evaluate calcifications of these structures.

We have found that 75% of the patients presented calcifications and a clear relationship with the genetic factors and polymorphisms evaluated, specifically associated with cardiovascular diseases (coronary arter-

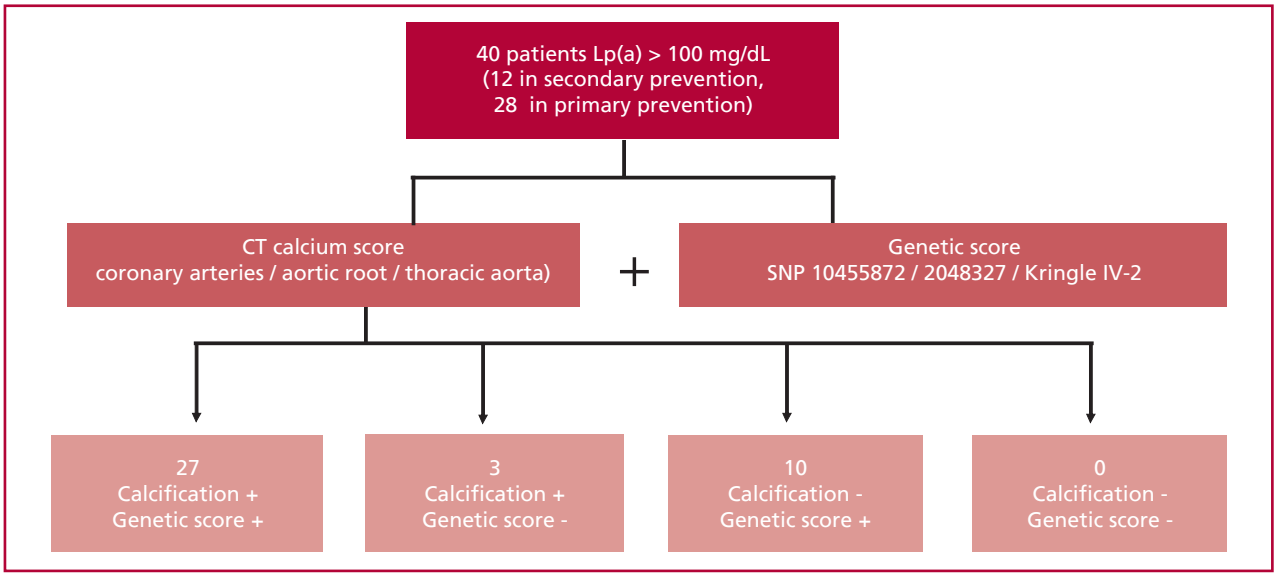

Fig. 1. Summary of the combinations found between calcifications and genetic score.

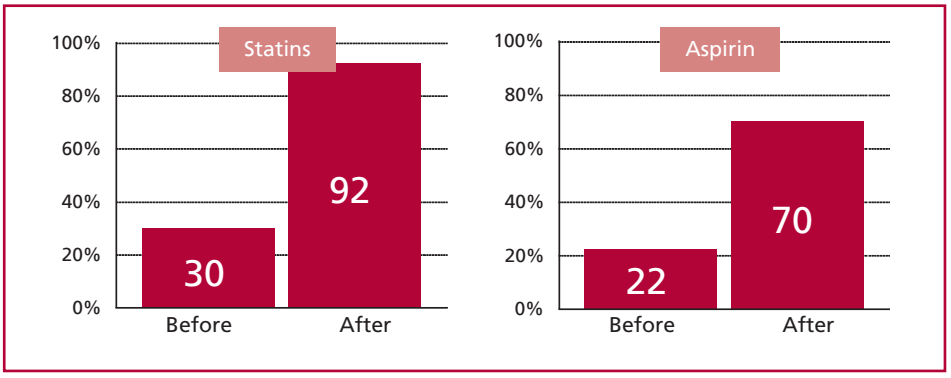

Fig. 2. Percent modification in the use of statins and aspirin before and after each patient evaluation.

ies, aortic valve and thoracic aorta).

In line with previous studies, we found that the frequency of the G risk allele in the SNP rs10455872 in our group of study is higher than that of the general population (0.26 vs. 0.07) which would indicate an association with elevated Lp(a) levels in these patients. (3, 5, 7) We did not find differences in the frequencies of the G allele in the polymorphism rs2048327 compared with the general population. Some interesting findings were observed regarding this SNP, as the presence of three GG homozygous patients with calcifications in two different territories (coronary arteries and thoracic aorta); yet, its association with the regulation of Lp(a) levels is just being evaluated, as it was discovered during 2016. (8, 9)

As expected, because the low number of repeats is associated with high Lp(a) levels, in 72% of the patients evaluated the number of KIV-2 repeats is lower than that of patients with normal Lp(a) levels.

Although the effect of the identified susceptibility variants is individually small, their effects are independent and additive. In the future, once the genetic variables associated with Lp(a) levels are identified, they can be incorporated into a genetic risk score consisting of the number of risk alleles adjusted for their individual weight. (9) In the present study, we used a risk score that could not be adjusted for the weight of each risk factor, as this information is still unknown; further studies would allow access to this type of tools for a better follow-up and management of these patients.

Regarding the association of genetic risk factors and calcification, it was seen that 27/40 patients (67%) with at least one genetic risk factor presented calcification. Two of the 40 patients (25%) with a genetic score≥1 did not present calcification, which would indicate that the presence of these markers does not have a full correlation with calcification; as we have previously mentioned, the influence that some of these markers have on Lp(a) levels and calcification is under evaluation. Three patients without any genetic risk factor presented calcification. However, as we have not analyzed all the genetic risk factors of the LPA gene, we cannot rule out that these calcifications are due to other polymorphisms related with Lp(a) or other cardiovascular risk factors. We did not find any patient

with genetic risk score 0 without calcifications.

The management and treatment of Lp(a) elevations has serious limitations as there are no direct pharmacological strategies to modify Lp(a) levels. Current recommendations establish that intensive therapy to reduce LDL-C level associated or not with aspirin is a logical and reasonable option due to the absence of drugs acting directly on Lp(a). (2)

From the point of view of the clinical applicability of our study, we were able to adjust and adequate the pharmacologic treatment, according to our findings. Thus, the use of statins and aspirin increased from 30% to 92% and from 22% to 70%, respectively.

Different Lp(a) levels and polymorphisms have been described in different parts of the world. (19) This is the first study that provides evidence of the close relationship between levels, specific polymorphisms and thoracic calcifications in a group of patients of our region.

The causal relation between Lp(a) and CVD seems clear and well established on the basis of different levels of evidence. However, the lack of an efficient andsafe therapeutic resource, with evidence of not only reducing Lp(a) levels, but also of preventing clinical events, seems to be the last missing link in this chain. (20) Therefore, the door is open, so that from genetics, and once we understand in detail the pathophysiology of the relationship between CVD and Lp(a), it may be possible to advance in the development of different strategies to reduce the risk of this association.

Limitations

Our study presents logic limitations; the lack of a control group weakens the analysis of causality. The small number of patients should not make us forget that extreme elevations of Lp(a) are extremely uncommon,a point which strengthens the study. (5, 6) Another limitation of the study from the viewpoint of molecular genetics is that we did not evaluate all the polymorphisms of the LPA gene, we do not have data of its allele frequencies in our population and that the interpretation of the number of KIV-2 repeats has not been validated. (4, 5) Finally, although computed tomography scan is considered the method of choice to detect subclinical atherosclerosis and valvular calcification, it could undervalue the presence of non-calcified lipid plaques. (11, 13).

CONCLUSIONS

We must emphasize that a significant group of patients with extreme Lp(a) elevations presented vascular calcifications and genetic polymorphisms related with the LPA gene. Based on molecular genetic testing and on a non-invasive method to detect vascular calcification and subclinical atherosclerosis, we have recategorized and adjusted the pharmacological management in a significant number of patients to reduce their cardiovascular risk.

Conflicts of interest

None declared. (See authors' conflicts of interest forms in the website/Supplementary material).

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