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Relationships between genetic vascular risk polymorphism and aging. A case-control study in Venezuela.

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Keywords: aging; cardiovascular homeostasis; lipid metabolism; polymorphism; blood coagulation.

Abstract: Aging is an irreversible process that produces the progressive decline of physiological functions favoring the development of cardiovascular complications associated with genetic Risk Alleles (RA). A case-control study using a sample of 90 Venezuelan individuals was performed to determine the correlation between the incidence of accelerated aging for 14 polymorphisms in genes associated with blood coagulation, lipid, and cardiovascular homeostasis. Odds Ratio (OR) results showed a 41% increase in the risk of presenting accelerated aging in subjects with the rs1800790 RA in the FGB gene. The CC genotype for the rs1800775 in the CETP gene was associated with a 62%, and the TT genotype for the rs1801133 in the MTHFR gene increased risk by two times. However, none of these results were statistically significant. Only a significant association was determined between the presence of the homozygous deletion genotype for the rs4340 RA in the ACE gene with an increased risk up to ten times (OR: 10.6; CI: 1.23 - 90.67; $p<0.05$). Multivariable analyses showed that gender, obesity, hypercholesterolemia, hypertriglyceridemia, smoking, age, body mass index, systolic hypertension, the rs662 RA in the APOB, rs693 RA in the PON1 and rs1801133 RA in the MTHFR genes were the main environmental and genetic factors associated with accelerated aging.

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Relación entre polimorfismos de riesgo genético vascular y el envejecimiento. Un estudio caso-control en Venezuela.

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Palabras clave: envejecimiento; homeóstasis cardiovascular; metabolismo lipídico; polimorfismo; coagulación sanguínea.

Resumen: El envejecimiento es un proceso irreversible que produce el declive progresivo de las funciones fisiológicas favoreciendo el desarrollo de complicaciones cardiovasculares asociadas con alelos de riesgo (AR) genéticos. Se realizó un estudio caso-control empleando una muestra de 90 individuos venezolanos para determinar la correlación entre la incidencia de envejecimiento acelerado para 14 polimorfismos en genes asociados a coagulación sanguínea, lípidos y homeóstasis cardiovascular. Resultados de razón de probabilidades (RP) mostraron en un 41% de sujetos con el AR rs1800790 en el gen FGB un incremento en el riesgo de presentar envejecimiento acelerado. El genotipo CC para el rs1800775 en el gen CETP fue asociado con un incremento de riesgo de 62% y el genotipo TT para el rs1801133 en el gen MTHFR con un incremento en el riesgo de 2 veces. Sin embargo, ninguno de estos resultados fue estadísticamente significativo. Sólo se determinó una relación estadísticamente significativa entre la presencia del genotipo de deleción homocigota para el AR de rs4340 en el gen ACE con un riesgo incrementado de hasta 10 veces (RP: 10,6; CI: 1,23 – 90,67; $p<0,05$). Los análisis multivariable mostraron que el género, obesidad, hipercolesterolemia, hipertrigliceridemia, hábito tabáquico, edad, índice de masa corporal, hipertesión sistólica, el AR rs662 en el gen APOB, el AR rs693 en PON1 y el AR rs1801133 en el gen MTHFR eran los principales factores ambientales y genéticos asociados a envejecimiento acelerado.

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INTRODUCTION

Aging is a complex time-depending process that causes the progressive decline of the organism's physiological function, affecting the cells' adaptability and their ability to maintain homeostasis and leading to a general decline of all body systems. Therefore, aging increases the susceptibility of the organism to suffer various diseases, usually related to cellular senescence [1,2]. Aging is a multifactorial process in which the interaction of several genetic and environmental variables can determine the growing old rate among different individuals or between organs and tissues of one specific individual [3]. Specific genotypes appear to be associated with accelerating the depletion of the organism's metabolism leading to the premature presence of degenerative diseases and the stimulation and acceleration of the natural aging course [4].

Alterations in the coding genes of proteins associated with endothelial function, blood coagulation, and lipid metabolism have proven to be highly related to the appearance of cardiovascular diseases (CVDs), known pathologies related to aging. More-

over, several alleles for some polymorphic variants in different candidate genes related to vascular risk have been postulated as genetic markers of premature aging [5,6], and many studies have been dedicated to establishing genetic variants which might be associated with healthy aging and longevity [7-12]. As a result, multiple variants in genes involved in different cellular processes and metabolic pathways have been postulated [8,11] with special attention on genes related to CVDs due to the close relationship between these diseases and aging [13]. However, due to the complexity of the aging process, there are some inconsistencies in the relationships proposed regarding the genetic factors involved in aging [10,11,14], probably because the environmental factors also play an essential role in aging progression as they can modulate the influence of the genetic risk factors [13,15].

Here, we evaluated the relationship between accelerated aging and different genotypes of 13 polymorphisms in the following genes: Apolipoprotein B (APOB; rs693), Apolipoprotein E (APOE; rs429358 and rs7412), Cholesteryl Ester Transfer Protein (CETP; rs1800775), Paraoxonase 1 (PON1; rs662), Fibrinogen Beta Chain (FGB; rs1800790 and rs1800791), Coagulation Factor II (F2; rs1799963), Coagulation Factor V (F5; rs6025), Coagulation Factor VII (F7; rs6046), Methylenetetrahydrofolate Reductase (MTHFR; rs1801133), Angiotensin Converting Enzyme (ACE; rs4340) Angiotensinogen (AGT; rs699), and Nitric Oxide Synthase 3 (NOS3; rs1799983) in a selected sample of 90 subjects from Caracas, Venezuela.

These gene variants have been correlated to diseases linked to aging, such as CVDs [5,6], and some of these variants have also been associated with longevity [2,4,6]. Knowledge of the genetic factors that may influence the susceptibility of an individual to develop CVDs would help identify, prevent, or slow down the disease. Additionally, early diagnosis of these diseases can be used to plan personalized treatments to avoid the progression of these and other diseases and favor healthy aging.

MATERIALS AND METHODS

Subjects

The sample comprised 90 randomly selected individuals unrelated to the "Centro Médico Antienvejecimiento" (CMA, Caracas, Venezuela), whose biological age was determined. This sample was classified into two groups: i) 30 control individuals, in which the biological age was equal or under the chronological age, and ii) 60 patients, which were subdivided into 30 patients with aging grade 1 (G1), in which the biological age was between 1 and 14 years over their chronological age, and 30 patients with aging grade 2 (G2), in which the biological age was between 15 and 28 years over their chronological age.

Determination of the biological age

The biological age was estimated using various biological and anthropometrical parameters such as body weight, body mass index (BMI), body fat percentage, stimuli response time, accommodation reflex, static balance, skin elasticity, and blood pressure. By comparing the estimated biological age with the chronological age, which corresponds to the time that has passed since the individual´s birth, the subjects were classified into different aging grade groups [16].

Blood Sampling

Peripheral blood was collected from all subjects after obtaining their signed consent. A standard *proforma* was filled up with their personal information, having particular emphasis on age, gender, smoking habit (current smokers or non-smokers), presence of hypertension (defined as a systolic blood pressure of at least 140 mm Hg and/or diastolic blood pressure of at least 90 mm Hg), diabetes mellitus (defined by a blood glucose level of at least 6.93 mmol/L) and obesity (BMI over 30).

Genes and Polymorphisms Studied

We evaluated the relationship between accelerated aging and different genotypes of 13 polymorphisms in the following genes: Apolipoprotein B (APOB; rs693), Apolipoprotein E (APOE; rs429358 and rs7412), Cholesteryl Ester Transfer Protein (CETP; rs1800775), Paraoxonase 1 (PON1; rs662), Fibrinogen Beta Chain (FGB; rs1800790 and rs1800791), Coagulation Factor II (F2; rs1799963), Coagulation Factor V (F5; rs6025), Coagulation Factor VII (F7; rs6046), Methylenetetrahydrofolate Reductase (MTHFR; rs1801133), Angiotensin Converting Enzyme (ACE; rs4340), Angiotensinogen (AGT; rs699), and Nitric Oxide Synthase 3 (NOS3; rs1799983).

Genotyping

Genomic DNA was extracted from total peripheral blood as described by Bowen y Keeney [17]. Details regarding identifying the polymorphisms for every specific gene are presented in Table 1.

Thirty cycles were performed following a denaturation step at 94°C for 5 min. Each cycle consisted of incubations at 94°C for 1 minute, annealing temperature for 1 minute, and 72°C for 1 minute. A final extension step was carried out at 72°C for 10 min. PCR products were analyzed by electrophoresis on a 2.5% agarose gel containing SYBR Safe. Gel images were documented by using a digital camera equipped with ultraviolet filters.

The enzymatic digestions were carried out overnight at 37°C, and the digested samples were separated using an 8% polyacrylamide gel and visualized by silver staining [18].

Statistical Analysis

Values of continuous variables were expressed as means ± standard deviations (SD). The allelic frequency and the frequency of heterozygous and homozygous carriers of the studied polymorphisms were calculated in every subject group (control, G1, and G2). The number of cases and control subjects with a specific genotype was used to determine the risk, estimated as the Odds Ratio (OR) using the software PAST version 2.17c (2013) in both a recessive and dominant model. The OR represents the probability that the presence of accelerated aging occurs or not when we compared the patients with the control individuals, and it is defined as the ratio of occurrence of accelerated aging between the two groups [23]. Multivariable logistic curve regression analyses were used to monitor the risk of developing vascular disease as a result of accelerated aging under various conditions: genotype, age, gender, obesity, weight, body fat percentage, BMI, smoking, presence of hypertension, hypertriglyceridemia, hypercholesterolemia, and diabetes mellitus. The regression coefficients that were obtained represented the probability of suffering the disease because of the presence of the risk allele of the polymorphisms and the other variables studied. Statistical significance was set up at a $p \leq 0.05$.

RESULTS

General characteristics

The general and biological characteristics of the subjects conforming to the aging patient subgroups and control group are shown in Table 2. The patient subgroup 2 (G2) was mainly composed of young individuals, considering that the average chronological age (37.33 ± 11.8) was smaller than the other groups. The G2 group contained a higher percentage of individuals with smoking habits, diabetes, and obesity, as well as high blood pressure values. However, a higher percentage of individuals with hypercholesterolemia was observed in the control group (Control).

Genotyping

Except for the polymorphism in the APOB gene, all alleles and genotypes in the control group were within the Hardy-Weinberg equilibrium (data not shown).

Table 1
Detection techniques employed to determine genotype in the different polymorphism studied.

Gene	Polymorphism	Variant Type	Detection Technique	Primers Sequence	Ta	Possible Alleles	Reference
APOB	rs693	SNV	PCR-RFLP	3´-GATGAAACCAATGACAAAATCC-5´	58°C	G/A	19
				3´-AACAGTGAACCCTTGCTCTACC-5´			
APOE	rs429358	SNV	PCR-RFLP	3´-AGACGCGGGCACGGCTGTCCAAGGA-5´	62 °C	T/C	19
				3´-CCCRCG CGGGCCCCGGCCTGGTACAC-5´			
APOE	rs7412	SNV	PCR-RFLP	3´-AGACGCGGGCACGGCTGTCCAAGGA-5´	62°C	C/T	19
				3´-CCCRCGCGGGCCCCGGCCTGGTACAC-5´			
CETP	rs1800775	SNV	PCR-RFLP	3´- AGAATTGAAATGCCACAGACATTCC-5´	57°C	T/C	20
				3´-CCTTGATATGCATAAAATAACTCTGG-5´			
PON1	rs662	SNV	PCR-RFLP	3´- TTGAATGATATTGTTGCTGTGGGACCTGAG-5´	65°C	T/A/ C/G	19
				3´-CGACCACGCTAAACCCAAATACATCTCCCAGAA-5´			
FGB	rs1800790	SNV	PCR-RFLP	3´- GGTCTTTCTGATGTGTATT-5´	55°C	G/A	20
				3´-CTATTATTCTTTCTTGGTCTA-5´			
FGB	rs1800791	SNV	PCR-RFLP	3´-GTGTTCCTATTGATTCTTCTTGTAGG-5´	55°C	G/A	20
				3´-AATGAGGCCCATTTTCCTTGAAATT-5´			
AGT	rs699	SNV	PCR-RFLP	3´-GATGCGCACAAGGTCCTCTG-5´	61°C	T/C	19
				3´-CAGGGTGCTGTCCACACTGGCTCGC-5´			
F7	rs6046	SNV	PCR-RFLP	3´-CAGTCACGGMAGGTGGGAGAC-5´	56°C	G/A/ C/T	20
				3´-GGGGTAATTGACGTCTTCTT-5´			
MTHFR	rs1801133	SNV	PCR-RFLP	3´-GCCTCTCCTGACTGTCATCC-5´	61°C	C/T	21
				3´-CCCTTTTGGTGATGCTTGTT-5´			
NOS3	rs1799983	SNV	PCR-RFLP	3´-CATGAGGCTCAGCCCCAGAAC-5´	59°C	G/T	22
				3´-AGTCAATCCCTT TGGTGCTCAC-5´			
ACE	rs4340	Indel	PCR	3´-CTGGAGAGCCACTCCCATCCTTTCT-5´	58°C	Ins/ Del	19
				3´-GACGTGGCCATCACATTCGTCAGAT-5´			
				3´-TGGGACCACAGCGCCCGCCACTAC-5´ *	67°C		
				3´-TCGCCAGCCCTCCCATGCCCATAA-5´ *			
F2	rs1799963	SNV	ASPCR	3´-CACTGGGAGCATTGAGGCGC-5´	59°C	G/A	19
				3´-ATGAATAGCAATGGGAGCATTGAGGATT-5´			
				3´-ATGTGTTCCGCCTGAAGAAGTGGA-5´			
				3´-CCCACCTTCCCCTCTCTCCAGGCAAATGGG-5´ **			
				3´-GGGCCTCAGTCCCAACATGGCTAAGAGGTG-5´ **			
F5	rs6025	SNV	ASPCR	3´-CAAGGACAAAATACCTGTATTCAT-5´	58°C	C/A/T	19
				3´-CAAGGACAAAATACCTGTATTCTTT-5´			
				3´-GGCAGGAACAACACCATGAT-5´			
				3´-CCCACCTTCCCCTCTCTCCAGGCAAATGGG-5´ **			
				3´-GGGCCTCAGTCCCAACATGGCTAAGAGGTG-5´ **			

Abbreviations: PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism; PCR: Polymerase Chain Reaction; SNV: Single Nucleotide Variant; In/Del: Insertion-Deletion; ASPCR: Allele Specific Polymerase Chain Reaction; Ta: Annealing Temperature. *Used for genotype verification; **Used as internal control.

Table 2
General and biological characteristics of the population.

Variables	Control (*n*=30)	G1 (*n*=30)	G2 (*n*=30)
Chronological age (X ± SD)	55.07 ± 8.08	50.43 ± 8.52	37.33± 11.8
Differential age (Biological age-Chronological age) (X ± SD)	6.58 ± 4.89	6.87 ± 3.94	21± 10.8
Mode (years)	59	48	27
Female (%)	83.3	90	70
Presence of smoking habits (%)	30	33.3	43.3
Presence of diabetes (%)	0	0	6.7
Presence of obesity (%)	0	26.7	36.7
Presence of hypercholesterolemia (%)[1]	43.3	40	23.3
Presence of hypertriglyceridemia (%)[1]	16.7	26.7	23.3
Body weight (Kg)	58.95 ± 8.25 (*n*=29)	69.18 ± 13.62 (*n*=30)	74.27 ± 18.81 (*n*=30)
Body fat percentage (%)	28.84 ± 13.28 (*n*=29)	37.93 ± 9.99 (*n*=29)	36.27 ±11.67 (*n*=30)
Body Mass Index (kg/m^2)	22.91 ± 2.98 (*n*=29)	26.34 ± 4.04 (*n*=27)	27.21 ± 5.42 (*n*=29)
Stimuli response time* (cm)	17.93·20.14·22.54 (*n*=28)	15.40·19.43·17.80 (*n*=30)	15.48·16.24·19.21 (*n*=29)
Accommodation reflex** (cm)	20.76 ± 8.15 (*n*=29)	21.07 ± 10.26 (*n*=29)	17.48 ± 4.65 (*n*=29)
Static balance *** (s)	7.86 · 5.90 · 7.14 (*n*=29)	4.45 · 4.45 · 6.10 (*n*=29)	6.07 · 4.03 · 5.93 (*n*=29)
Skin elasticity (s)	29.02 ± 41.65 (*n*=29)	12.28 ± 23.32 (*n*=30)	6.24 ± 12.37 (*n*=30)
Blood pressure (mmHg) Systolic/ Diastolic	124.86/79.21 (*n*=29)	127.21/77.86 (*n*=29/28)	132.93/81.50 (*n*=30)

[1]Values came from answering "yes" in the questionnaire regarding the presence of hypercholesterolemia and hypertriglyceridemia. *n*<30 indicates that results couldn't be obtained for all individuals for a specific variable while all the others consider the 30 individuals' population. *Values resulted from three measurements of the response speed of the upper extremities due to visual stimuli. **Values represent the distance between the eye and the text when the text can still be focus correctly. ***Time in which the patient started to oscillate or swing with the eyes close.

The frequency of the risk allele of the rs1800790 polymorphism (f: 0.15; CI: 0.13 – 0.17) in the FGB gene, rs1799963 polymorphism (f: 0.02; CI: 0.03 – 0.01) in the F2 gene, rs6025 polymorphism (f: 0.03; CI: 0.05 – 0.02) in the F5 gene, rs1801133 polymorphism (f: 0.30; CI: 0.27 – 0.33) in the MTHFR gene, and rs4340 polymorphism (f: 0.45; CI: 0.42 – 0.48) in ACE gene was more significant in the G2 group than in the control group. The frequency of the risk allele of the rs1799983 polymorphism (f: 0.30; CI: 0.245 – 0.354) in the NOS3 gene and rs662 polymorphism (f: 0.37; 0.34 - 0.40) in the PON1 gene was higher in the G1 group than in the control group. Finally, the risk allele frequency of the rs429358 and rs7412 polymorphisms in the APOE gene was greater in

the control group (f: 0.21; CI: 0.16 - 0.25) than in the other groups.

Figura 1 shows schematically the OR value calculated for each polymorphic variant in the G1 and G2 subgroups. The associated risk calculated using the OR for the presence of the risk allele of the rs1800790 polymorphism in the FGB gene showed a 41% higher tendency to exhibit an accelerated aging (OR: 1.41; CI: 0.44-4.45) (Fig. 1), but these results were not statistically significant (p>0.05). Regarding the risk allele of the rs1799963 polymorphism in the F2 gene and of the rs6025 polymorphism in the F5 gene, we were unable to determine the OR value since the risk allele was only present in the G2 group. However, these two polymorphisms have been established as independent factors for vascular risk.

Moreover, the CC genotype for the rs1800775 polymorphism in the CETP gene was associated with a 62% increased risk of accelerated aging (OR: 1.62; CI: 0.40- 6.40; p>0.05). The calculated OR value for the rs662 polymorphism in the PON1 gene suggested that the occurrence of the risk allele is associated with a 15% increase in the risk of having accelerated aging (OR: 1.15; CI: 0.4-3.26; p>0.05) (Fig. 1). Also, the presence of the risk allele of the APOE gene was not associated with an increased risk of showing an accelerated aging (Fig. 1). All these results were not statistically significant (p>0.05).

Regarding the presence of the TT genotype for the rs1801133 polymorphism in the MTHFR gene, it was observed an increase by two times in the risk of exhibiting accelerated aging (OR: 2.07; CI: 0.18-24.15; p>0.05), however, this result was not statistically significant.

Similarly, the presence of the Del allele for the rs4340 polymorphism in the ACE gene was also associated with a two-time increase in the risk of exhibiting accelerated aging (OR: 2.07; CI: 0.18-24.15; p>0.05), showing no statistical significance. The risk was increased up to ten times (OR: 10.6; CI: 1.23-90.67; p<0.05) by the presence of the homozygote genotype for the risk allele displaying statistical significance.

The presence of the CC genotype for the rs699 polymorphism in the AGT gene was associated with a two time-increase in the risk of exhibiting accelerated aging (OR: 2.13; CI: 0.62-7.39; p>0.05) being this result not statistically significant.

Lastly, the presence of the T allele for the rs1799983 polymorphism in the NOS3 gene was associated with a 96% increase in

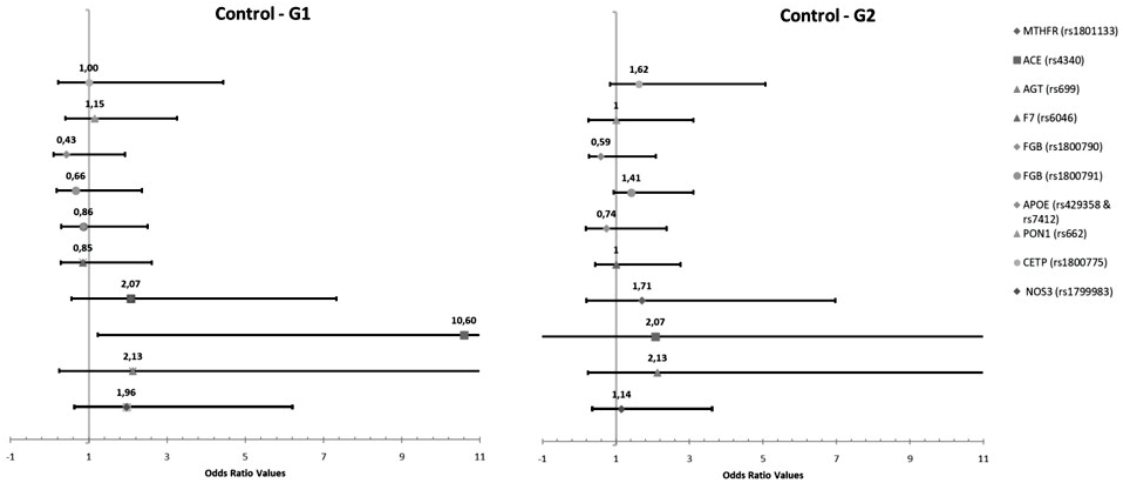

Fig. 1. OR values calculated comparing the control group with the G1 (Left) and G2 (Right) groups; with every value it shows the error bars which represent the confidence interval in each case.

the risk of exhibiting accelerated aging (OR: 2.13; CI: 0.62-7.39; p>0.05) being this result not statistically significant.

A multivariable statistical analysis was carried out to determine the association between the development of accelerated aging and each one of the genetic and environmental variables that define the sample. Table 3 shows the variables that were taken into consideration for the analysis. In the G1 subgroup, the environmental and genetic factors that proved to be associated with the development of an accelerated aging process were obesity, hypercholesterolemia and hypertriglyceridemia, age, BMI, APOE rs429358 and rs7412 polymorphisms, CETP rs1800775 polymorphism, FGB polymorphism rs1800790 and MTHFR polymorphism rs1801133. In the G2 subgroup, the environmental and genetic factors associated with accelerated aging were age, sex, body mass index, hypertriglyceridemia, smoking, systolic hypertension, F7 polymorphism rs6046, and MTHFR polymorphism rs1801133.

DISCUSSION

Recent studies in Latin American populations have shown the existence of differential ancestral contribution patterns between and within groups, which correlate with the indigenous population density before the conquest of America and with the current demographic growth patterns in these regions [24]. This agrees with genetic studies carried out in Venezuela, based on the analysis of blood group polymorphisms and DNA polymorphisms, which have revealed that, as in other Latin American countries, the conquest and colonization processes generated very heterogeneous populations. In general, the genetic component that prevails in these studies is the Mediterranean European, followed by the indigenous and, to a lesser extent, the African, and also with a marked inter- and intra-regional difference [25]. Specifically, for the population of Caracas, in a study carried out by Martínez *et al.* [26], who performed the analysis of five autosomal markers found in the high socioeconomic stratum, the European component (0.78) was found in a higher proportion than Sub-Saharan African, which was almost negligible (0.06); while for the low socioeconomic level, the Sub-Saharan, European, and Amerindian components were 0.21, 0.42 and 0.36, respectively. Therefore, to conduct genetic studies in a highly heterogeneous population like ours, it is imperative to understand the high degree of genetic variability of the different ethnic groups that inhabit the territory.

Attributable to the impact of the genetic constitution of any individual in the development of a particular phenotype, the sum of the genetic alterations or risk alleles of different genes can help us elucidate the effect of these in the evolution of the disease through molecular diagnosis. Likewise, the molecular diagnosis of risk alleles associated with vascular risk can help supplement the results of biochemical and clinical analyses and therefore provide an answer or an explanation to a disease or any given family history.

With the multivariable analysis we confirmed and demonstrated that age is directly related to accelerated aging, because as the years pass by diminishes the organism capacity to maintain homeostasis causing tissue failure and malfunction of the regulation systems, which in turn may produce an increase in the susceptibility to suffer various diseases. We also found that hypercholesterolemia and hypertriglyceridemia are risk factors associated with the development of accelerated aging, suggesting that the presence of high levels of cholesterol and triglycerides promote the rapid deterioration of the organism, mainly at the vascular level, given that high values of cholesterol and triglycerides are related to the formation of atheroma and the unfolding of atherosclerosis [27,28].

Other variables associated with an accelerated process of aging were obesity and

Table 3
Association between the different genetic and environmental factors and the development of vascular risk through an accelerated aging.

Variable	Association with G1 (n=60)	Association with G2 (n=60)
Sex	Not Associated	**Associated***
Diabetes *mellitus*	--	Not Associated
Obesity	**Associated***	Not Associated
Hypercholesterolemia	**Associated****	Not Associated
Hypertriglyceridemia	**Associated***	**Associated***
Smoking	Not Associated	**Associated***
Age	**Associated****	**Associated*****
Weight	**Associated****	Not Associated
Body fat percentage	Not Associated	Not Associated
BMI	Not Associated	**Associated*****
Systolic hypertension	Not Associated	**Associated*****
Diastolic hypertension	Not Associated	Not Associated
APOB (rs693) Allele 7545T	**Associated***	Not Associated
APOE (rs429358 y rs7412) Allele 388C y 526C	Not Associated	Not Associated
CETP (rs1800775) Allele -656C	Not Associated	Not Associated
PON1 (rs662) Allele 575G	**Associated***	Not Associated
FGB (rs1800790) Allele -455A	Not Associated	Not Associated
FGB (rs1800791) Allele -854[a]	Not Associated	Not Associated
F2 (rs1799963) Allele 20210A	--	Not Associated
F5 (rs6025) Allele 1691A	--	Not Associated
F7 (rs6046) Allele 10976G	Not Associated	Not Associated
MTHFR (rs1801133) Allele 655T	Not Associated	**Associated***
ACE (rs4340) Allele Del	Not Associated	Not Associated
AGT (rs699) Allele 803C	Not Associated	Not Associated
NOS3 (rs1799983) Allele 894T	Not Associated	Not Associated

* p< 0,05; ** p< 0,01; *** p< 0,001; -- excluded from the model.

BMI, being BMI a vascular risk indicator that is used universally to detect overweight and obesity. Obesity promotes the appearance of alterations in various mechanisms of hormonal regulation, being hyperinsulinemia and leptinemia, some of the most common hormonal alterations associated with obesity [29]. Moreover, obesity accelerates the aging of adipose cells increasing the formation of reactive oxygen species in fat cells, promoting inflammatory processes and insulin resistance. Aging and obesity not only favor the deregulation of the metabolism but also promote the development of hypertension, dyslipidemia, and cardiovascular complications [29,30].

Hypertension is known as a risk factor for the development of cardiovascular disease. In the present study, our results suggest that it is associated with accelerated aging, meaning that the deterioration of the vascular endothelium due to high blood pressure constitutes a significant risk factor for accelerating this process [31].

Regarding the allelic variant 20210A of the rs1799963 in the F2 gene, while an association could not be established in this investigation, this gene has been linked with premature aging, being reported with a decreased frequency in the middle to advanced-age individuals [32]. In turn, the allelic variant 1601A of the rs6025 polymorphism in the F5 gene has been associated as an independent factor that indicates blood hyper-coagulation.

The presence of this risk allele causes the production of a factor V protein that cannot be degraded by the activated protein C (APC), and consequently, an increase in the amount of factor V is obtained in the blood [33]. It is worth noting that similarly to rs1799963 in F2, the allelic frequency for the 1601A variant in F5 has been reported to decrease in individuals with advanced age (centenary individuals) [34]. Regarding the rs6046 polymorphism in the F7 gene, the presence of the mutant allele 1172A is beneficial for the prevention of cardiovascular diseases or the formation of vascular thrombus since this mutant allele produces a protein that has a deficient interaction with the tissue factor, causing a lower initiation of the secondary hemostasis [35].

Concerning the genes involved in lipid metabolism, it has been reported that the presence of the allele E4 in the APOE gene promotes the appearance of cardiovascular diseases, which is why many studies address the relationship between this gene and aging [7,8]. Although the OR values obtained here do not suggest that this risk allele is associated with accelerated aging, the multivariable analysis showed that the allele E4 is one of the risk factors that participate in the fast deterioration of the organism. This allele has been shown to decrease its frequency in advanced age centenary groups; in turn, the allele E2 which has been reported to have a protective effect, has been reported to increase in these groups, suggesting an association with the aging process [36]. The discrepancy might be because the multivariable analysis considers the interaction of various genetic variables in conjunction with environmental variables related to aging.

The risk allele -656C for the rs1800775 polymorphism in the CETP gene was found to be associated with accelerating aging. This polymorphism is linked to the transcription levels of the CETP gene, whose protein product is involved in the transference of cholesterol and other lipids from HDL to LDL and VLDL [37]. Thus, the risk allele -656C, which is the ancestral allele, has been associated with a higher transcription rate. The presence of this protein diminishes the cholesterol levels in the HDL [38], causing an increase in the risk of developing cardiovascular diseases and, therefore, increasing the risk of presenting an accelerated aging process.

Some studies have demonstrated that the antioxidant capacity of HDL and the enzymatic activity of PON1 decreases with age [38]. Likewise, the risk allele for the rs662 polymorphism in the PON1 gene has been associated with the reduction of the enzyme

arylesterase activity, which negatively affects the antioxidant capacity of HDL [39]. This could explain the relationship found in this study between the risk allele for the rs662 polymorphism and the accelerated process of aging. In this line of thought, the risk allele frequency has also been reported to decrease in nonagenarian and centennial individuals [14].

Concerning the genes involved in cardiovascular homeostasis, the allelic variant 655T of rs1801133 polymorphism in the MTHFR gene has been linked to a decrease in MTHFR enzymatic activity which in turn causes an increase in blood homocysteine [40], being reported a decrease of up to 70% in homozygotes individuals for the risk allele [41]. This homocysteine level change has been associated with a decline in physical functions. Proposed mechanisms regarding this outcome include direct endothelial damage caused by the generation of potent reactive oxygen species not only in endothelial tissues but also in proteins and DNA, along with an increase in the amount of telomere length loss[41]. The rs1801133 polymorphism has also been related to longevity with a decreased allelic frequency in centenary individuals for the risk allele 655T [42].

The allelic variant Del of rs4340 polymorphism in the ACE gene has been associated with an increase in plasmatic and cardiac ACE activity, causing an overexposition to high levels of Angiotensin II that on its own has been linked to a diverse repertoire of cardiovascular diseases such as hypertension and myocardial infarction[43]. It is worth noting that, while there has been a significant quantity of studies concerning this polymorphism, the mechanism by which it operates has not been elucidated completely; this due to the intronic nature of this polymorphic variant.

The allelic variant 803C of rs699 polymorphism in AGT gene has been reported to increase plasmatic AGT protein production up to 20% in homozygote individuals for the risk allele [44]; this increase has been adjudicated to a linkage disequilibrium between rs699 and rs5051 polymorphisms the last one located in the gene promoter imposing an augment in transcriptional activity of the AGT gene [45]. While this polymorphism has been linked with cardiovascular homeostasis alterations, few studies associate this variant with accelerated aging, some postulating it as a protection factor [46,47]. In contrast, others suggest there is no relationship at all. Similarly, to rs4340 polymorphism in ACE, the increase in AGT transcription rate is deeply linked to an increase in Angiotensin II levels, promoting the risk of developing cardiovascular diseases. However, in this case, the increased allelic frequency of the risk allele reported in advanced-age individuals suggest the existence of a protection element conferred by this variant; this protection has been postulated to exist due to the role as a skeletal muscle growth factor of angiotensin II [46–48].

Lastly, the allelic variant 894T of rs1799983 polymorphism in the NOS3 gene has been associated in previous studies with an increased probability of developing cardiovascular events and preeclampsia due to alterations in the NOS3 enzyme function [49]. In a previous case-control study we measured the nitric oxide concentration in serum through non-enzymatic colorimetric assays reporting that individuals carrying the 894T allelic variant showed a reduction of 46.47% in nitric oxide serum levels when compared with GG homozygote individuals. These results were statistically significant and showed that the presence of 894T can contribute to an increased risk of developing hypertension of up to four times in TT homozygote individuals (OR: 4.17; CI: 1.06-19.11; $p<0.05$) when compared to GG homozygote individuals [19].

In summary, according to the results of the OR analyses, the polymorphic variants considered in this study were not associated with the development of accelerated aging in a statistically significant way, except for the rs4340 polymorphism in the ACE gene

(recessive model). These results could be related to the sample size since it is essential to obtain statistically significant results. Nevertheless, the multivariable analysis showed a significant association between the variable's obesity, hypercholesterolemia and hypertriglyceridemia, age, body mass index, APOB rs693, and PON1 rs662 polymorphism with the development of accelerated aging in the G1 group. Also, the variables sex, hypertriglyceridemia, smoking, age, body mass index, systolic hypertension, and MTHFR rs1801133 polymorphism were linked with accelerated aging in the G2 group. Our findings showed that genetic and environmental factors are associated with an accelerated aging process.

The knowledge of the genetic profile is of great importance to complement the biochemical and clinical information of the individuals. The integral consideration of these parameters will allow the application of preventive antiaging medicine in an individualized way by making nutritional recommendations and modifications in lifestyle to reduce the incidence of diseases typically associated with age, such as cardiovascular diseases, and promote healthy aging.

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Author's Contributions

CC direction and designed research. CA, AB, MAM, AV and AA performed research and analyzed data. CC, AP and AC wrote the paper. JCM analyzed the clinical data. All authors read and approved the final manuscript.

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