APOE and LDLR Gene Polymorphisms and Dyslipidemia Tracking. Rio de Janeiro Study

Rossana Ghessa Andrade de Freitas[1], Erika Maria Gonçalves Campana[1,2], Roberto Pozzan[1,2], Andréa Araujo Brandão[1,2], Ayrton Pires Brandão[1,2], Maria Eliane Campos Magalhães[1,2], Dayse Aparecida da Silva[1,3]

Universidade do Estado do Rio de Janeiro[1]; Hospital Universitário Pedro Ernesto[2]; Instituto de Biologia Roberto Alcântara Gomes[3], Rio de Janeiro, RJ – Brazil

Abstract

Background: Studies show an association between changes in apolipoprotein E (*ApoE*) and LDLR receptor with the occurrence of dyslipidemia.

Objectives: To investigate the association between polymorphisms of the *APOE* (ε2, ε3, ε4) and *LDLR* (A370T) genes with the persistence of abnormal serum lipid levels in young individuals followed up for 17 years in the Rio de Janeiro Study.

Methods: The study included 56 individuals (35 males) who underwent three assessments at different ages: A1 (mean age 13.30 ± 1.53 years), A2 (22.09 ± 1.91 years) and A3 (31.23 ± 1.99 years). Clinical evaluation with measurement of blood pressure (BP) and body mass index (BMI) was conducted at all three assessments. Measurement of waist circumference (WC) and serum lipids, and analysis of genetic polymorphisms by PCR-RFLP were performed at A2 and A3. Based on dyslipidemia tracking, three groups were established: 0 (no abnormal lipid value at A2 and A3), 1 (up to one abnormal lipid value at A2 or A3) and 2 (one or more abnormal lipid values at A2 and A3).

Results: Compared with groups 0 and 1, group 2 presented higher mean values of BP, BMI, WC, LDL-c and TG ($p < 0.01$) and lower mean values of HDL-c ($p = 0.001$). Across the assessments, all individuals with *APOE* genotypes ε2/ε4 and ε4/ε4 maintained at least one abnormal lipid variable, whereas those with genotype ε2/ε3 did not show abnormal values ($\chi 2 = 16.848$, $p = 0.032$). For the *LDLR* genotypes, there was no significant difference among the groups.

Conclusions: *APOE* gene polymorphisms were associated with dyslipidemia in young individuals followed up longitudinally from childhood. (Arq Bras Cardiol. 2015; 104(6):468-475)

Keywords: Polymorphism, Genetic; Dyslipidemias; Young Adult; Epidemiology; Apolipoproteins E.

Introduction

Cardiovascular diseases (CVDs) are the leading causes of death in adults worldwide, contributing to high rates of early morbidity and mortality[1,2]. In Brazil, CVDs concentrate annually 1/3 of the overall deaths[3,4].

Dyslipidemia is one of the risk factors (RF) for development of CVDs[5]. Given its importance, studies are being conducted to determine the abnormalities associated with plasma lipid changes and their implications on the occurrence of CVDs[6,7]. In genetics, several gene polymorphisms and mutations have been identified and associated with atherosclerosis and coronary artery disease (CAD)[8,9]. This is the case of the apolipoprotein E (*ApoE*), which is essential for the transport and metabolism of cholesterol and structural stability of lipoproteins[10,11], and whose gene has three polymorphic alleles (ε*2*, ε*3* and ε*4*)[12]. Population studies have shown higher plasma levels of low-density lipoprotein cholesterol (LDL-c) in carriers of the ε*4* allele[12], leading to an association of this allele with the occurrence of CVDs[13].

Mutations in the LDL receptor gene (*LDLR*) have also been implicated with dyslipidemias, particularly in primary forms of homozygous or heterozygous hypercholesterolemia such as familial hypercholesterolemia (FH), a condition associated with early severe atherosclerosis and CAD[14-16]. The *LDLR* gene encodes a protein with binding domains for apolipoproteins B and E. Among the different polymorphisms found in the *LDLR* gene, the A370T has been investigated for its association with increased lipid levels and cardiovascular risk[17,18].

Dyslipidemias may be present from an early age, and abnormal lipids tend to persist over time (tracking effect) until adulthood. The identification of genetic markers involved with abnormal lipid metabolism may contribute to the recognition at a young age of patterns of genetic susceptibility and guide interventions to correct these abnormalities.

Mailing address: Rossana Ghessa Andrade de Freitas •
Laboratório de Diagnósticos por DNA. Universidade do Estado do Rio de Janeiro. Instituto de Biologia Roberto Alcântara Gomes. Rua São Francisco Xavier, 524, Pavilhão Haroldo Lisboa da Cunha. Postal Code 20550-900, Rio de Janeiro, RJ – Brazil
E-mail: rghessa@yahoo.com.br
Manuscript received August 27, 2014; revised manuscript January 21, 2015; accepted January 28, 2015.

DOI: 10.5935/abc.20150036

Based on that, the aim of this study was to investigate the distribution pattern of polymorphisms of the *APOE* and *LDLR* genes and their associations with the dyslipidemia phenotype, notably on its tracking effect, in a young population followed up for 17 years.

Method

The sample of this study was retrieved from the Study of Rio de Janeiro (*Estudo do Rio de Janeiro*, ERJ). This longitudinal cohort study is part of a line of research on blood pressure (BP) and other cardiovascular RFs in young adults developed in 1983 at the Hypertension Unit of the *Hospital Universitário Pedro Ernesto* (HUPE) at *Universidade do Estado do Rio de Janeiro* (UERJ)[19].

The ERJ included three different assessments, named A1, A2 and A3. Assessment A1 was conducted between 1987 and 1988 in individuals aged 10-16 years (mean 13.30 ± 1.53 years), A2 was conducted between 1996 and 1999 in individuals aged 18-26 years (mean 22.09 ± 1.91 years) and A3 was conducted between 2004 and 2005 in individuals aged 27-35 years (mean 31.23 ± 1.99 years)[19].

We selected for genetic evaluation 75 individuals from the original ERJ cohort who had undergone all three assessments (A1, A2 and A3). However, laboratory evaluation was not performed in 19 individuals at A2 and these individuals were excluded from the analysis, yielding a study sample of 56 individuals with serum lipid evaluation at assessments A2 and A3, as well as genetic profile evaluation.

Dyslipidemia was considered present when one or more lipid values were increased (total cholesterol [Col-T], LDL-c and triglycerides [TG]) or decreased (high-density lipoprotein cholesterol [HDL-c]), alone or in combination, using as cutoff values those recommended by the V Brazilian Guideline on Dyslipidemias and Atherosclerosis Prevention[20]. The tracking effect consists of a repeat behavior of these abnormal variables (increase or decrease) at both assessments conducted during young adulthood (A2 and A3).

Three groups were established based on the occurrence of dyslipidemia tracking:

Group 0: No abnormal lipid variables in A2 and A3; n = 11 individuals (10 women) with a mean age of 30.89 ± 1.64 years;

Group 1: At least one abnormal lipid variable in one of the evaluations (A2 or A3); n = 12 individuals (10 men) with a mean age of 31.47 ± 2.35 years;

Group 2: One or more abnormal lipid variables in two evaluations (A2 and A3); n = 33 individuals (24 men) with a mean age of 31.25 ± 1.99 years.

Clinical, anthropometric, laboratory, and genetic evaluations

Both BP and BMI were evaluated at A1, A2 and A3. The A2 and A3 assessments also included measurements of Col-T, HDL-c and TG after a 12-hour fasting, and calculation of LDL-c. Blood collection for genetic testing and measurement of waist circumference (WC) were performed at A3.

Measurement of BP

Measurement of BP was carried out according to the recommendations of the VI Brazilian Guidelines of Hypertension[21]. The BP was measured on the right arm, with the individual lying down and then seated, using an aneroid mercury sphygmomanometer (Romed) fixed to the wall, and zeroed to the midaxillary line. We selected cuffs with size and width suitable for the circumference and length of the individuals' arms. We considered as systolic BP (SBP) the appearance of the first Korotkoff sound (Korotkoff phase I), and for diastolic BP (DBP), the disappearance of the sound (Korotkoff phase V). The BP was measured three times with 5-minute intervals between each measurement, and the last measurement was used for the analysis. We considered the BP to be increased in A1 when SBP and/or DBP was ≥ 95th percentile for gender and age, and in A2 and A3 when SBP was ≥ 140 mmHg and/or DBP ≥ 90 mmHg.

Anthropometric variables

Weight (W) and height (H) were measured on a platform scale (Filizola, São Paulo, Brazil) with a 150-kilogram (kg) capacity and 100-gram precision. Weight was expressed in kg and determined with the individual barefoot and wearing light clothes. Height was expressed in centimeters (cm) and determined from the distance between the vertex of the head to the soles of the feet with the individual in an upright position and barefoot[22].

From the measurements of weight and height, we calculated the BMI using the formula BMI = W/H^2 and expressed the results in kg/m^2.

The WC was measured parallel to the ground with a flexible and inelastic tape measure with precision of 0.1 cm, with the individual in an upright position and the abdomen relaxed. The measurement was determined horizontally on the shortest distance between the lower border of the last rib and the iliac crest, with the tape held firmly but without pressure against the skin[22].

Laboratory variables

Blood was collected by antecubital venipuncture under standard conditions in the morning (before 8:30 am) after a 12-hour fasting.

All samples were placed in siliconized vacuum tubes and processed in up to 30 minutes. Measurements were performed in the serum obtained after centrifugation at a speed of 3,500 rotations per minute for five minutes.

For measurement of serum cholesterol and HDL-c, we used the enzymatic colorimetric method CHOD/PAP and for measurement of TG, we used the enzymatic method GPD/BAP. To calculate the LDL-c levels, we used the Friedewald formula when TG levels were < 400 mg/dl.

Genetic analysis

DNA was extracted by the salting-out method using 2-ml aliquots of whole blood[23]. The analyses of the allelic variants of the *APOE* gene (ε*2*, ε*3*, ε*4*) were carried out with

the technique of polymerase chain reaction (PCR) with the primers *APOE* F 5'- TAA GCT TGG CAC GGC TGT CCA AGG A-3' and *APOE* R 5'- ACA GAA TTC GCC CCG GCC TGG TAC AC-3' in 35 PCR cycles (95ºC for 60 sec, 63ºC for 60 sec and 72ºC for 120 sec) to amplify a product of 244 base pairs (bp)[24]. The products of PCR amplification were digested with the enzyme *Hha*I (Fermentas) and the fragments were visualized in 12% polyacrylamide gel by silver nitrate staining. The fragments representing each genotype are as follows: ε*2*ε*3* (91, 83 and 48 bp), ε*3*ε*4* (91, 72 and 48 bp), ε*2*ε*4* (91, 83, 72 and 48 bp) and ε*3*ε*3* (91 and 48 bp).

To genotype the A370T polymorphism, a region of 150 bp was amplified by PCR using the primers P1: 5'-GAG TGT CAG GAT CCC GAC ACC TGC GCC-3' and P2: 5 '-AAG TCG ACC CAC CCG CCT GCC TCC CGT-3' in 35 cycles (95°C for 60 sec, 68°C for 60 sec and 72° C for 120 sec)[25]. To determine the polymorphism, PCR products were digested with the enzyme *Hae*III (Biotech), and fragments were separated on 3.5% agarose gel and visualized by ethidium bromide staining. The fragments representing A allele were 77, 47 and 26 bp, and those representing the T allele were 124 and 26 bp.

To demonstrate the random genetic distribution of the ERJ cohort, the genotype and allelic frequencies of the polymorphisms analyzed in the study were compared with those observed in a cohort of 75 non-hospitalized individuals (41 men and 34 women) randomly selected from a DNA database of more than 10,000 individuals who underwent parental testing, provided for this study by the *Laboratório de Diagnósticos por DNA* (LDD) at UERJ. This sample was identified as LDD. It is worth noting that the populations are not required to be phenotypically homogeneous for comparison of genetic distribution.

The study was approved by the Ethics Committee for Research of HUPE under the number 2130-CEP/HUPE, and all participants signed an Informed Consent Form.

Statistical Analysis

For statistical treatment of the data, we used the software SPSS for Windows version 12.0 (Chicago, Illinois, USA). Gene and haplotype frequencies were estimated according to Saitou and Nei[26], using the program Arlequin, version 3.0[27]. Chi-square test (χ^2) and analysis of variance (F) were used to compare the pattern of distribution of the polymorphisms with clinical anthropometric and lipid variables, with $p < 0.05$ results considered significant. The test of homogeneity of variances was applied to evaluate the normal distribution of the studied variables.

Results

The sample consisted of 56 individuals, 35 (62.5%) of whom were males and 21 (37.5%) females, aged between 27-35 years (mean 31.23 ± 1.99 years). Table 1 presents the clinical, anthropometric and laboratory variables of the studied population at all three assessments (A1, A2 and A3).

Table 2 shows the results of clinical (SBP and DBP), anthropometric (WC and BMI) and laboratory (Col-T, HDL-c, LDL-c and TG) variables at A3 for all three groups (groups 0, 1 and 2) stratified by dyslipidemia tracking. When compared with groups 0 and 1, group 2 presented higher mean values of SBP, BMI, WC, TG and LDL-c and lower mean values of HDL-c.

The genetic analysis of the 56 individuals identified the following genotypes for *APOE*: ε*3*ε*3* (62.5%), ε*3*ε*4* (25.0%), ε*2*ε*3* (5.4%), ε*2*ε*4* (5.4%) and ε*4*ε*4* (1.8%) (Table 3).

As for the distribution of the *APOE* genotypes in the groups according to the occurrence of dyslipidemia tracking, we observed that in group 0, genotype ε*3*ε*3* affected 45.5% of the individuals, followed by ε*2*ε*3* and ε*3*ε*4*, each affecting 27.3% of the individuals. In group 1, the ε*3*ε*3* genotype was present in 83.3% of the individuals and the ε*3*ε*4* genotype in 16.7%. Genotypes ε*2*ε*3*, ε*2*ε*4* and ε*4*ε*4* were not observed in group 1. In group 2, which

Table 1 – Clinical, anthropometric and laboratory variables of the studied population at three assessments*

Variables	Evaluations		
	A1 (n = 56)	A2 (n = 56)	A3 (n = 75)
Age (years)	13.30 ± 1.53	22.09 ± 1.91	31.23 ± 1.99
SBP (mmHg)	115.28 ± 14.83	124.35 ± 13.79	125.43 ± 16.67
DBP (mmHg)	63.81 ± 12.84	79.86 ± 10.79	83.20 ± 13.72
BMI (kg/m^2)	20.26 ± 3.05	24.04 ± 3.64	26.79 ± 5.53
WC (cm)	-	-	92.96 ± 14.66
Col-T (mg/dL)	-	175.37 ± 34.34	181.44 ± 31.72
TG (mg/dL)	-	88.37 ± 42.34	103.71 ± 56.14
HDL-c (mg/dL)	-	45.87 ± 13.16	49.05 ± 15.87
LDL-C (mg/dL)	-	111.82 ± 27.58	111.23 ± 27.95

** Values are expressed as mean ± standard deviation; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; WC: waist circumference; Col-T: total cholesterol; TG: triglycerides; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol.*

Table 2 – Clinical, anthropometric and laboratory variables at A3 in the studied groups according to dyslipidemia tracking*

Variables	Groups of dyslipidemia			Test F	p
	0 (n = 11)	1 (n = 12)	2 (n = 33)		
Age (years)	30.89 ± 1.64	31.47 ± 2.35	31.25 ± 1.99	0.239	0.788
SBP (mmHg)	114.36 ± 14.58	120.17 ± 13.05	129.76 ± 15.79	5.01	0.01
DBP (mmHg)	76.36 ± 8.66	78.17 ± 13.89	85.27 ± 13.44	2.69	0.07
BMI (kg/m^2)	23.81 ± 3.72	24.34 ± 4.02	28.67 ± 5.81	5.43	0.007
WC (cm)	80.50 ± 6.74	86.91 ± 8.72	98.63 ± 14.26	10.86	< 0.001
Col-T (mg/dL)	164.45 ± 18.35	179.58 ± 29.12	187.78 ± 34.50	2.37	0.10
HDL-c (mg/dL)	62.09 ± 9.63	53.0 ± 11.55	43.51 ± 15.99	7.50	0.001
LDL-C (mg/dL)	89.47 ± 13.59	106.56 ± 28.60	119.90 ± 27.55	6.01	0.004
TG (mg/dL)	64.18 ± 31.44	81.33 ± 41.37	125.03 ± 57.68	7.48	0.001
Gender	10 F / 1 M	2 F/ 10 M	9 F/ 24 M	-	-

** Values expressed as mean ± standard deviation; group 0: no abnormal lipid variable at A2 and A3; group 1: one or more abnormal lipid variables at A2 or A3; group 2: one or more abnormal lipid variables at A2 and A3; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; WC: waist circumference; Col-T: total cholesterol; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; TG: triglycerides; F: female; M: male; F-test: analysis of variance.*

Table 3 – *APOE* genotypes according to dyslipidemia group*

APOE genotypes	Dyslipidemia group			Total
	0 n = 11 (19.6%)	1 n = 12 (21.4%)	2 n = 33 (59.0%)	n = 56 (100.0%)
ε2ε3 n(%)	3 (27.3%)	-	-	3 (5.4%)
ε3ε3 n(%)	5 (45.5%)	10 (83.3%)	20 (60.6%)	35 (62.5%)
ε3ε4 n(%)	3 (27.3%)	2 (16.7%)	9 (27.3%)	14 (25%)
ε2ε4 n(%)	-	-	3 (9.1%)	3 (5.4%)
ε4ε4 n(%)	-	-	1 (3.0%)	1 (1.8%)

** Values are expressed as n (%) χ^2 = 16.848; p = 0.032; APOE: apolipoprotein E gene; group 0: no abnormal lipid variable at A2 and A3; group 1: one or more abnormal lipid variables at A2 or A3; group 2: one or more abnormal lipid variables at A2 and A3.*

featured the greatest genotype diversity in the sample, the distribution was 60.6% for *ε3ε3*, 27.3% for *ε3ε4*, 9.1% for *ε2ε4* and 3% for *ε4ε4*. Genotype *ε2ε3* was not observed in this group. This distribution had statistical significance (χ^2 = 16.848, p = 0.0032) and showed that 39.4% of the individuals in group 2 had genotypes containing the *ε4* allele. It should be noted that all subjects with genotypes *ε2ε4* and *ε4ε4* maintained at least one abnormal lipid value in A2 and A3 (group 2 – one or more abnormal lipid variables in A2 and A3), whereas none of the subjects with genotype *ε2ε3* had abnormal lipids (group 0 – no abnormal lipid values in A2 and A3) (Table 3).

We also analyzed the polymorphisms of the *LDLR* gene and identified the following genotypes: AA in 85.7%, AT in 12.5% and TT in 1.8% of the individuals (Table 4).

The analysis of the distribution of *LDLR* genotypes according to dyslipidemia tracking showed no statistically significant difference between the groups (Table 4).

For comparison purposes, the genotype and allele frequencies of *APOE* and *LDLR* gene polymorphisms of the 56 individuals from the ERJ cohort and 75 individuals from the LDD cohort are shown in Table 5. Fisher's exact test showed no significant differences between the allele distributions of the ERJ and LDD cohorts.

Discussion

Studies have shown an association between *APOE* and *LDLR* genotypes with increased levels of lipid macromolecules such as Col-T, TG and LDL-c, decreased levels of HDL-c, and cardiovascular disease, especially CAD[11,28].

In the present study, we investigated the distribution pattern of *APOE* and *LDLR* gene polymorphisms in a population of adolescents followed up for 17 years, considering the occurrence of dyslipidemia based on change (increase or decrease) of one or more lipid variables and their repetition (tracking) at two different moments (A2 and A3) during young adulthood.

Table 4 – *LDLR* genotypes according to dyslipidemia group*

LDLR genotypes	Dyslipidemia group			Total
	0 n = 11 (19.6%)	1 n = 12 (21.4%)	2 n = 33 (59.0%)	n = 56 (100.0%)
AA n (%)	9 (81.8%)	10 (83.3%)	29 (87.9%)	48 (85.7%)
AT n (%)	2 (18.2%)	2 (16.7%)	3 (9.1%)	7 (12.5%)
TT n (%)	-	-	1 (3.0%)	1 (1.8%)

** Values expressed as n (%) χ^2 = 1.500; p = 0.827; LDLR: low-density lipoprotein receptor gene; group 0: no abnormal lipid variable at A2 and A3; group 1: one or more abnormal lipid variable at A2 or A3; group 2: one or more abnormal lipid variable at A2 and A3.*

Table 5 – Genotype and allele frequencies of *APOE* and *LDLR* gene polymorphisms in the ERJ and LDD cohorts

APOE			*LDLR*		
Genotypes	**Frequency (%)**		**Genotypes**	**Frequency (%)**	
	ERJ(n = 56)	LDD(n = 75)		ERJ(n = 56)	LDD(n = 75)
ε2ε3	5.3	4.2	AA	88.0	86.5
ε3ε3	66.7	75.0	AT	10.6	13.5
ε3ε4	22.7	18.0	TT	1.3	-
ε2ε4	4.0	2.8			
ε4ε4	1.3	-			
Alleles	**Frequency (%)**		**Alleles**	**Frequency (%)**	
ε2	6.6	3.5	A	93.5	93.3
ε3	79.4	86.0	T	6.5	6.7
ε4	14.0	10.5			
Ho	34.6	25.0	Ho	10.5	13.5
He	34.8	24.8	He	12.3	12.6
HWE	p = 0.5098 DP = 0.0005	p = 0.2128 DP = 0.0004	HWE	p = 0.2715 DP = 0.0004	p = 1.000 DP = 0.00
Fisher's exact test	p = 0.51528 DP = 0.001	-	Fisher's exact test	p = 0.990, SD = 0.003	-

ERJ: participants of the Rio de Janeiro Study; LDD: cohort from the Laboratório de Diagnósticos por DNA; APO: apolipoprotein E gene; LDLR: low-density lipoprotein receptor gene; He: expected heterozygosity, Ho: observed heterozygosity; HWE: Hardy-Weinberg equilibrium; SD: standard deviation.

In the analysis of *APOE* polymorphisms based on dyslipidemia tracking, our study showed that individuals with genotype ε2ε3 were concentrated in group 0, that is, the group in which lipid variables (Col-T, TG, HDL-c and LDL-c) were normal at two evaluations (A2 and A3). These findings are in agreement with those by Ferreira et al.[29] who demonstrated in a study with 216 individuals (109 with dyslipidemia and 107 without dyslipidemia) a similar frequency of allele distribution for *APOE* polymorphisms in both groups. However, in individuals with normal lipid levels in that study, the presence of the ε2 allele was strongly associated with low serum levels of Col-T and LDL-c, which may suggest a possible protective role associated with this allele[29,30].

Similarly, Bazzaz et al.[31], in a cohort study with 320 individuals in Iran, investigated the association between *APOE* gene polymorphisms, lipid profile and BMI. The authors observed that the ε2 allele was more frequent in individuals with Col-T < 200 mg/dl (p = 0.01), and found an even greater association of the individuals with normal serum Col-T levels with genotype ε2ε3 when compared with individuals with abnormal levels of Col-T (p = 0.003)[31].

As for the ε4 allele, studies in the general population and hypertensive patients have shown an association of this allele with an increase in levels of Col-T and LDL-c. Due to that, the ε4 allele has been associated with higher risk of CAD even in healthy individuals[28,32]. Fuzikawa et al.[30], in a study with 1,406 adults of both genders, observed a high prevalence of hypertension (61.3%) and higher average values of LDL-c in patients with the ε4 allele (p = 0.036) when compared with patients with the ε2 allele (p < 0.001)[30]. Similarly, Salazar et al.[33] showed an association of the ε4 allele with dyslipidemias in a study that investigated *APOE* gene polymorphism in

150 women with and without CAD. Compared with the control group in that cohort, women with CAD showed significantly higher levels of Col-T, TG and LDL-c and a higher frequency of the ε*4* allele and the ε*3*ε*4* genotype[33].

In the present study, 18 individuals (32.1%) were carriers of the ε*4* allele in different genotype combinations, 13 (72.2%) of whom were in group 2 which aggregated higher values of SBP, BMI and WC and therefore, worse cardiovascular risk profile. It is worth noting that the only individual in the cohort with genotype ε*4*ε*4* was in group 2.

Thus, the evidence seems to point to a possible damaging role for the ε*4* allele and a protective role for the ε*2* allele. However, the combination of these alleles in different genotypes can render these roles less clear. In this study for example, all subjects with genotype ε*2*ε*4* were in group 2 which had the worse risk profile and one or more abnormal lipid variables at two evaluations. This result seems to suggest that when the ε*4* allele is present, the protective role of the ε*2* allele is either lost or decreased.

In the present study, we also observed a predominance of males in group 2 (n = 24; 72.72%) (Table 2). This group presented the worst cardiovascular risk profile, which is in line with the premise that the male gender is associated with increased cardiovascular risk[21].

We found no association between *LDLR* gene polymorphisms and dyslipidemia tracking in the young individuals of this cohort. Frikke-Schmidt et al.[25] also found no significant association between plasma levels of Col-T, LDL-c and individuals with AA genotype compared with those with a TT genotype. However, these authors reported increased risk (3.6 times) of ischemic stroke in homozygous individuals with a TT genotype compared with those with an AA genotype[25].

We found five genotypes for the *APOE* polymorphism in the ERJ cohort. As for the *LDLR* polymorphism, all three were found in this cohort. The most frequent *APOE* genotypes were ε*3*ε*3* and the most frequent *LDLR* genotype was AA.

It is worth noting that the genotype and allele frequencies found in the ERJ cohort for *APOE* gene polymorphisms were similar to those found in cohorts from other states in Brazil, such as the cohort from Rio Grande do Sul (Porto Alegre)[12] and in other cohorts worldwide[34]. The genotype and allele frequencies of the ERJ cohort for the *LDLR* polymorphisms were similar to those found in other populations, such as the one from the study of Frikke-Schmidt et al.[25]. Similarly, the comparison of the genetic distribution in the ERJ cohort with that from a representative sample group randomly selected from the state of Rio de Janeiro (LDD), and the confirmation of the homogeneity of their distributions, suggests that the ERJ cohort has a random profile, suitable for the development of the proposed study.

Despite the limitations of the study, the association between a specific genetic profile with the presence of dyslipidemia in young individuals over time in a small population brings a novel and relatively unknown perspective to the currently available medical literature. Studies with more than 20 years of follow-up, such as the ERJ, have losses associated with the longitudinal tracking of the participants, but have unequivocally contributed to a better understanding of the behavior of cardiovascular risk factors in the Brazilian population.

Conclusion

A study of *APOE* gene polymorphisms in participants of the ERJ showed that the presence of the ε*4* allele was more prevalent in group 2, which consisted of young individuals with repeatedly abnormal lipid variables during longitudinal follow-up (tracking effect). This group also showed aggregation of worse anthropometric variables (higher BMI and WC) and increased BP, rendering a worse cardiovascular risk profile to these individuals.

This study was partially funded by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) and *Fundação Carlos Chagas de Amparo à Pesquisa do Estado do Rio de Janeiro* (FAPERJ).

Author contributions

Conception and design of the research:Freitas RGA, Campana EMG, Brandão AA, Brandão AP, Magalhães MEC, Silva DA. Acquisition of data: Freitas RGA, Campana EMG, Brandão AP, Silva DA. Analysis and interpretation of the data: Freitas RGA, Pozzan R. Statistical analysis: Pozzan R. Writing of the manuscript: Brandão AA, Magalhães MEC, Silva DA. Critical revision of the manuscript for intellectual content: Freitas RGA. Supervision / as the major investigador:Magalhães MEC, Silva DA.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Study Association

This article is part of the thesis of master submitted by Rossana Ghessa Andrade de Freitas, from Universidade do Estado do Rio de Janeiro.

Sources of Funding

This study was funded by CAPES E FAPERJ.

References

1. World Health Organization (WHO). [Internet]. Programmes. Cardiovascular disease. About cardiovascular diseases. Definition. [cited 2013 Oct 16]. Available from: <http://www.who.int/cardiovascular_diseases/about_cvd/en>

2. World Health Organization (WHO). [Internet]. Media Centre. Fact Sheets. Noncommunicable diseases. Updated March 2013. [cited 2013 Oct 16]. Available from: <http://www.who.int/mediacentre/factsheets/fs355/en/index.html>

3. Ministério da Saúde. [Internet]. Rede Interagencial de Informações para a Saúde (RIPSA). Indicadores e dados básicos (IDB). Indicadores de mortalidade. [atualizada em 2012] [acesso em 2014 dez. 03]. Disponível em: <http://tabnet.datasus.gov.br/cgi/idb2012/matriz.htm>

4. Ministério da Saúde. Secretaria de Vigilância em Saúde. [Internet]. 6a ed. Brasília; 2009. [atualizada em 2010 dez. 14]. [acesso em 2011 jun. 20]. Disponível em: <http://portal.saude.gov.br/portal/aplicacoes/noticias/default.cfm?pg=dspDetalheNoticia&id_area=124&CO_NOTICIA=11994>

5. World Health Organization (WHO). [Internet]. Prevention of cardiovascular disease: guidelines for assessment and management of cardiovascular risk. Part 1: The total risk approach to prevention of cardiovascular disease. Washington DC; 2007. [cited 2013 Oct 16]. Available from:<http://whqlibdoc.who.int/publications/2007/9789241547178_eng.pdf>

6. Berenson GS, Srinivasan SR, Bao W, Newman WP 3rd, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med. 1998;338(23):1650-6.

7. Quezada A, Fajardo MA, Rodríguez MA, Ponce G. Análisis de los factores de riesgo vinculados con el síndrome metabólico en niños de Comodoro Rivadavia. Bioquímica y Patología Clínica. 2010;74(1):30-5.

8. López-Reyes A, Rodríguez-Pérez JM, Fernández-Torres J, Martínez-Rodríguez N, Pérez-Hernández N, Fuentes-Gómez AJ, et al. The HIF1A rs 2057482 polymorphism is associated with risk of developing premature coronary artery disease and with some metabolic and cardiovascular risk factors. The Genetics of Atherosclerotic Disease (GEA) Mexican Study. Exp Mol Pathol. 2014;96(3):405-10.

9. Keenan TE, Rader DJ. Genetics of lipid traits and relationship to coronary artery disease. Curr Cardiol Rep. 2013;15(9):396.

10. Costa PM. Polimorfismo da apolipoproteína E e perfil de distribuição de subfrações de lipoproteínas. [Dissertação]. Porto Alegre: Pontifícia Universidade Católica do Rio Grande do Sul; 2004.

11. Martinelli N, Olivieri O, Shen GQ, Trabetti E, Pizzolo F, Busti F, et al. Additive effect of LRP8/APOER2 R952Q variant to *APOE* ε2/ε3/ε4 genotype in modulating apolipoprotein E concentration and the risk of myocardial infarction: a case-control study. BMC Med Genet. 2009;10:41.

12. Schwanke CHA, Cruz IBM, Leal NF, Scheibe R, Moriguchi Y, Moriguchi EH. Análise da associação entre polimorfismo do gene da apolipoproteína E e fatores de risco cardiovasculares em idosos longevos. Arq Bras Cardiol. 2002;78(6):561-70.

13. Smith JD. Apolipoprotein E4: an allele associated with many diseases. Ann Med. 2000;32(2):118-27.

14. Marques e Sá AC. O papel dos polimorfismos genéticos na doença cardíaca isquêmica [Dissertação]. Porto: Universidade do Porto; 2010.

15. Guardamagna O, Restagno G, Rolfo E, Pederiva C, Martini S, Abello F, et al. The type of *LDLR* gene mutation predicts cardiovascular risk in children with familial hypercholesterolemia. J Pediatr. 2009; 155(2):199-204.e2.

16. Oliveira PR. Dislipidemia: hipercolesterolemia familiar. [monografia]. Santa Barbara d'Oeste(SP): Anhanguera Educacional S.A; 2010.

17. Vieira JR, Whittall RA, Cooper JA, Miller GJ, Humphries SE. The A370T variant (StuI Polymorphism) in the LDL receptor gene is not associated with plasma lipid levels or cardiovascular risk in UK men. Ann Hum Genet. 2006; 70(Pt 6):697-704.

18. Wang J, Huff E, Janecka L, Hegele RA. Low density lipoprotein receptor (*LDLR*) gene mutations in Canadian subjects with familial hypercholesterolemia, but not of French descent. Hum Mutat. 2001;18(4):359.

19. Campana EM, Brandão AA, Pozzan R, França MF, Fonseca FL, Pizzi OL, et al. Pressão arterial em jovens como marcador de risco cardiovascular. Estudo do Rio de Janeiro. Arq Bras Cardiol. 2009;93(6):657-65.

20. Xavier HT, Izar MC, Faria Neto JR, Assad MH, Rocha VZ, Sposito AC, et al; Sociedade Brasileira de Cardiologia. V Diretriz Brasileira de Dislipidemias e Prevenção da Aterosclerose. Rio de Janeiro. Arq Bras Cardiol. 2013; 101(4 supl. 1):1-22.

21. Sociedade Brasileira de Cardiologia; Sociedade Brasileira de Hipertensão; Sociedade Brasileira de Nefrologia. VI Diretrizes Brasileiras de Hipertensão. Arq Bras Cardiol. 2010;95(1 supl. 1):1-51.

22. Callaway CW, Chumlea WC, Bouchard C, Himes JH, Lohman TG, Martin AD, et al. Circumferences. In: Lohman TG, Roche AF, Martorell R, eds. Anthropometric standardization reference manual. Champaign: Human Kinetics; 1991. p.44-5.

23. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16(3):1215.

24. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res. 1990; 31(3):545-8.

25. Frikke-Schmidt R, Nordestgaard BG, Schnohr P, Tybjaerg-Hansen A. Single nucleotide polymorphism in the low-density lipoprotein receptor is associated with a threefold risk of stroke. A case-control and prospective study. Eur Heart J. 2004; 25(11):943-51.

26. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4(4):406-25.

27. Excoffier L, Laval G, Schneider S. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online. 2007:1:47-50.

28. Moreno JA, López-Miranda J, Pérez-Jiménez F. Influencia de los factores genéticos y ambientales en el metabolismo lipídico y riesgo cardiovascular asociado al gen apõe. Med Clin (Barc). 2006;127(9):343-51.

29. Ferreira CN, Carvalho MG, Fernandes AP, Lima LM, Loures-Valle AA, Dantas J, et al. Comparative study of apolipoprotein-E polymorphism and plasma lipid levels in dyslipidemic and asymptomatic subjects, and their implication in cardio/cerebro-vascular disorders. Neurochem Int. 2010;56(1):177-82.

30. Fuzikawa AK, Peixoto SV, Taufer M, Moriguchi EH, Lima-Costa MF. Association of *ApoE* polymorphisms with prevalent hypertension in 1406 older adults: the Bambuí Health Aging Study (BHAS). Braz J Med Biol Res. 2008; 41(2):89-94.

31. Bazzaz JT, Nazari M, Nazem H, Amiri P, Fakhrzadeh H, Heshmat R, et al. Apolipoprotein E gene polymorphism and total serum cholesterol level in Iranian population. J Postgrad Med. 2010; 56(3):173-5.

32. Beydoun MA, Beydoun HA, Kaufman JS, An Y, Resnick SM, O'Brien R, et al. Apolipoprotein E *ε4* allele interacts with sex and cognitive status to influence all-cause and cause-specific mortality in U.S. older adults. J Am Geriatr Soc. 2013;61(4):525-34.

33. 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 Chem Acta. 2000; 300(1-2):139-49.

34. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arterioesclerosis.1988; 8(1):1-21.