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Polymorphisms of *APOE* and *APOB* genes and dyslipidemias in a South Brazilian Cohort

Caroline C. Gasparin[1], Luciane Viater Tureck[1], Lilian P. Ferrari[2], Ricardo L. Souza[1], Lupe Furtado-Alle[1]

ORIGINAL ARTICLE

ABSTRACT

Dyslipidemias are closely associated to the development of cardiovascular and cerebrovascular diseases, and therefore of great importance in public health. Many genes influence lipid levels, such as *APOE* and *APOB*. The present study aimed to investigate the effects of *APOE* gene alleles (rs7412: C>T and rs429358: T>C), and R3500Q mutation of *APOB* gene (rs5742904: G>A) in lipid levels in a South Brazilian cohort. 214 individuals were analyzed and the frequencies of *APOE ε2*, *ε3* and *ε4* alleles were found respectively as 9.25 ± 0.4625; 70.5 ± 3.525 and 20.25%± 1.401, while the frequency of R3500Q mutation of the *APOB* gene was 4.46 ± 1.00%. The ε4 allele was significantly more frequent in subjects with higher TC (Total Cholesterol) and LDL-C levels, while significantly higher frequency of the ε2 allele was found in individuals with higher HDL-C levels. The R3500Q mutation was suggested as a risk factor for higher TC levels, regardless gender and age (β = 21.326 ± 10377.78, p = 0.001).

Keywords: dyslipidemias, *APOE*, *APOB*, genetics.

Polimorfismos dos genes APOE e APOB e dislipidemias em uma Coorte do Sul do Brasil

RESUMO

As dislipidemias estão intimamente associadas ao desenvolvimento de doenças cardiovasculares e cerebrovasculares e, portanto, são de grande importância na saúde pública. Muitos genes influenciam os níveis lipídicos, como o *APOE* e o *APOB*. O presente estudo teve como objetivo investigar os efeitos dos alelos do gene *APOE* (rs7412: C>T e rs429358: T>C) e da mutação R3500Q do gene *APOB* (rs5742904: G>A) nos níveis lipídicos em uma coorte do Sul do Brasil. Foram analisados 214 indivíduos e as frequências econtradas dos alelos *ε2*, *ε3* e *ε4* do *APOE* foram, respectivamente, 9,25 ± 0,4625; 70,5 ± 3,525 e 20,25%± 1,401, enquanto a frequência da mutação *R3500Q* do gene *APOB* foi de 4,46 ± 1,00%. O alelo *ε4* foi significativamente mais frequente em indivíduos com níveis mais elevados de CT (colesterol total) e LDL-C, enquanto uma frequência significativamente maior do alelo ε2 foi encontrada em indivíduos com níveis mais elevados de HDL-C. Sugere-se que a mutação *R3500Q* constitua um fator de risco para níveis mais elevados de CT, independentemente do sexo e da idade (β = 21,326 ± 10.377,78, p = 0,001).

Palavras-chave: Dislipidemias, *APOE*, *APOB*, Genética.

Instituição afiliada – [1] Polymorphisms and Linkage Laboratory, Federal University of Paraná (UFPR), Department of Genetics, Curitiba - PR, Brazil. [2] Genetic and Immunology Laboratory, Autonomous University Center of Brazil (UniBrasil), Health School, Curitiba - PR, Brazil
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Autorcorrespondente*: Caroline Cardozo Gasparin - carolinecardozogasparin@gmail.com*

INTRODUCTION

Dyslipidemias constitute an important problem in public health, being related to the development of cardiovascular and cerebrovascular diseases (1, 2, 3). Around 53% of American adults have lipid abnormalities, including high levels of Low Density Lipoprotein Cholesterol (LDL-C) or Triglycerides (TG) and low concentrations of High Density Lipoprotein Cholesterol (HDL-C) (4). Coronary Arterial Disease (CAD) is considered the leading cause of death in Brazil and there is evidence that high cholesterol levels are the main modifiable risk factor for CAD (2, 5, 6).

Dyslipidemias are multifactorial traits and can be influenced by the environment, such as life habits and by genetic factors. Some studies in other populations have demonstrated that many genes can influence lipid levels, as the *APOB* and *APOE* genes (1, 7, 8, 9).

ApoB-100 is an important protein present in the surface of LDL-C, which is encoded by the *APOB* gene (2p24.1) and it is responsible by the linkage between this lipoprotein and its receptor (10, 11). The first and most frequent alteration in *APOB* gene is the R3500Q mutation (rs5742904: NM_000384.2:c.10580G>A; NP_000375.2:p.Arg3527Gln; R3527Q or R3500Q) and it results in a reduced affinity for LDL-c receptor (11, 12, 13, 14).

The APOE glycoprotein is encoded by the *APOE* gene (19q13.2) and plays an important role in the metabolism, transportation and redistribution of lipids, mediating the uptake of chylomicrons, Very Low Density Lipoprotein (VLDL) and Intermediate Density Lipoprotein (IDL) (15, 16). The ε2, ε3 and ε4 alleles (determined by the combination of the two following SNPs: rs7412:NC_000019.10:g.44908822C>T; NP_000032.1:p.Arg176Cys and rs429358:NC_000019.10:g.44908684T>C; NP_001289618.1:p.Cys130Arg) result in distinct forms of APOE, which are characterized by the combination of the amino acids present in two important positions. Therefore the three APOE major isoforms are E2 (rs7412T, rs429358T), E3 (rs7412C; rs429358T) and E4 (rs7412C; rs429358C) (14, 16).

Given the importance of dyslipidemias as a public health problem in Brazil, the aim of this study was to investigate if the R3500Q of *APOB* gene and ε2, ε3 and ε4 alleles of *APOE* gene influence the lipid profile in a Southern Brazilian cohort.

METHODOLOGY

Participants

The study sample consisted of 214 apparently healthy adults (41.90 years ± 9.7 years), 30 men and 184 women. Considering that the study participants constituted a population sample, no specific pathology was used as an inclusion criteria. All individuals in the sample were Euro-Brazilian residents in the city of Curitiba-PR (Southern Brazil). This project was approved by the Institutional Ethics Committee and informed consent was signed by all participants.

Lipidogram

Blood samples were collected in the morning after 12 hours of fasting, to perform measurements of total cholesterol (TC), high density lipoprotein (HDL-C), low density lipoprotein (LDL-C) and triglycerides (TG) by standard automated methods.

DNA analysis

DNA was extracted from peripheral blood samples by a salting-out method (17) and diluted to a final concentration of 20 ng/µL.

R3500Q mutation (rs5742904) of *APOB* gene and the alleles *ε2*, *ε3* and *ε4* of *APOE* gene (rs7412 - and rs429358) were identified by TaqMan SNP Genotyping Assay (Applied Biosystems). Each reaction had 3.0µL of Master Mix, 1.7 µL of MiliQ Water, 0.3 of µL of primer and 3.0 µL of DNA and the reactions were performed in Mastercycler realplex 2 according to the following steps: (1) 50°C / 2 min, (2) 95°C / 10 min, (3) repeated 50 times 95°C / 15 sec interspersed with 62ºC / min.

Statistical Analyses

To proceed with the statistical analysis, we classified the samples into groups above and below the median for the variables TC, LDL-C, HDL-C and TG. Each variable, stratified in two groups by its median, was plotted as a dependent variable in a logistic regression analysis. $\chi 2$ tests were performed using Clump (18) to test for Hardy-Weinberg equilibrium, and to compare allele proportions between groups above and below the median. Furthermore, the G^2–test was used to compare genotype proportions between groups. The probability value for the comparative tests were considered significant at $p < 0.05$ (5%).

RESULTS

Table 1 shows the descriptive statistics for the variables analyzed in the present study.

Regarding the R3500Q mutation of *APOB* gene, we observed that the genotype distribution was not in Hardy-Weinberg equilibrium in the whole sample (X^2 = 11.95 p = 0.0005) and for variables TC and LDL-C above the median (X^2 = 16.15 p = 0.0011; X^2 = 15.8 p = 0.012, respectively) and HDL-C below the median (X^2 = 8.39 and p = 0.039).

TC levels in the sample stratified by *APOB* gene R3500Q site genotypes are shown in Table 2. Allele frequencies between groups were significantly different, with the group above the median for TC having a higher frequency of the A allele (p = 0.029). Comparisons between carriers of the rarer allele (AA and GA) and homozygotes for the common allele (GG) revealed a tendency to a higher frequency of carriers of the rarer allele in the group above the median for the TC (p = 0.053). Logistic Regression Analysis, using TC stratified as below and above the median as a dependent variable, and R3500Q site genotypes (in the tested dominant model GG and GA are dominant over AA), gender and age as independent variables, identified the A allele of *APOB* gene as an independent risk factor to increased TC levels (± 10377.78 β = 21.326, p = 0.001).

Table 1. Descriptive statistics for age and lipid profile of 214 individuals analyzed in this study.

Variable	N	Mean±Standard Error	Median	Minimum	Maximum	Variance	Standard Deviation
Age	194	41.90±0.69	42	23	65	92.79	9.63
HDL-C	214	51.89±0.89	50	25	102	171.69	13.10
LDL-C	213	122.64±2.31	117.8	46.4	256.8	1136.90	33.72
TG	214	136.95±4.46	128	32	475	4254.75	65.23
TC	214	201.73±2.61	202.5	122	345	1456.67	38.17

Age is expressed in years. HDL-C, LDL-C, TG and TC are expressed in mg/dL. HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: triglycerides; TC: total cholesterol.

The distribution of allele and genotype frequencies of the *APOE* gene are shown in Table 3, for the whole sample and for the sample stratified as above and below the median of TC, LDL-C, HDL-C and TG. By analyzing the data in the groups above and below the median, significantly higher frequencies of the ε4 allele were observed in the stratums above the medians of TC ($X^2 = 5.29$, p=0.021) and LDL-C ($X^2 = 9.36$, p = 0.0022). Furthermore, the *ε2* allele was significantly more frequent in the group above the median of HDL-C ($X^2 = 4.10$, p = 0.0429). Regarding the genotypes, it was observed that ε3ε4 combination was more frequent in the group above the TC median (test-G = 17.33 and p = 0.0039); ε2ε4 had the highest frequency in the groups above the medians of LDL-C (Test-G = 18.09, p = 0.0028) and HDL-C (Test G = 17.08, p = 0.0044).

DISCUSSION

The purpose of the present study was to investigate if there is an association between the R3500Q (*APOB* gene) mutation and variants of *APOE* gene with lipid levels in a sample of a Southern Brazilian population of self-declared European ancestry. In our work the comparisons between individuals stratified by lipid profile markers levels, revealed significant differences in allele frequencies of genes involved in lipid metabolism, and thus possibly contributing to the normal variation in the lipid levels of this population.

Regarding the analyses of *APOB* gene, it was observed that the frequency of the rarer allele (A) was significantly higher in individuals with TC above the median and from the multiple logistic regression analysis, it was found that the *R3500Q* mutation is a risk factor, independent of age and gender, for increasing TC levels. The independence among the factors analyzed is very relevant, because the lipid levels are influenced by environmental, genetic factors and by gender, thus these factors could influence metabolic pathways and thus alter the cholesterol concentrations (6, 19, 20, 21).

Table 2. Distribution of allele and genotype frequencies of the R3500Q mutation of APOB gene in the groups above and below the median for the variable TC

R3500Q	Whole sample	Above the CT median	Below the CT median
GG	198 (92.96%)	95 (89,62%)	103 (96.26%)
GA	11 (5.16%)	8 (7,55%)	3 (2.8%)
AA	4 (1.88%)	3 (2,83%)	1 (0.94%)
G	95.54%±1.00	93,34%±0,02	97.66%±1.03
A	4.46%±1.00	6,60%±0,02	2.34%±1.03

Regarding the genotype lines are demonstrated the absolute frequencies (outside the parentheses) and the respective relative frequencies (between parentheses). Regarding the alleles lines are demonstrated the relative frequencies more or less the standard error of allelic frequencies.

Table 3. Distribution of allele and genotype frequencies of the APOE gene in the groups above and below the median for the variables TC, LDL-C, TG and HDL-C

Genotypes	Whole sample	Above the TC median	Below TC median	Above LDL-C median	Below LDL-C median	Above TG median	Below TG median	Above HDL-C median	Below HDL-C median
ε2ε2	3 (1.5%)	0 (0.0%)	3 (3.03%)	0 (0.0%)	3(3.125%)	3 (2.91%)	0 (0.0%)	3 (3.03%)	0 (0.0%)
ε2ε3	25 (12.5%)	10 (10.10%)	15 (15.15%)	13 (12.74%)	12 (12.5%)	13 (12.62%)	12 (12.63%)	13 (13.13%)	12 (12.12%)
ε2ε4	6 (3.0%)	4 (4.04%)	2 (2.02%)	5 (4.90%)	1 (1.04%)	1 (0.97%)	5 (5.26%)	6 (6.06%)	0 (0.0%)
ε3ε3	105 (52.5%)	44 (44.44%)	59 (59.59%)	43 (42.16%)	60 (62.5%)	54 (52.43%)	49 (51.58%)	54 (54.55%)	49 (49.50%)
ε3ε4	47 (23.5%)	34 (34.34%)	13 (13.13%)	30 (29.41%)	17 (17.71%)	19 (18.45%)	28 (29.48%)	19 (19.19%)	28 (28.28%)
ε4ε4	14 (7.0%)	7 (7.07%)	7 (7.07%)	11 (10.78%)	3 (3.125%)	13 (12.62%)	1 (1.05%)	4 (4.04%)	10 (10.10%)
ε2	9.25%±0.46	7.07±1.82	11.62±2.28	8.82±1.99	9.9±2.16	9.71±2.06	8.95±2.07	12.63±2.36	6.06±1.70
ε3	70.5%±3.52	66.67±3.35	73.74±3.13	63.24±3.38	77.6±3.01	67.96±3.25	72.63±3.23	70.71±3.23	69.7±3.27
ε4	20.25%±1.40	26.26±3.13	14.64±2.51	27.94±3.14	12.5±2.39	22.33±2.9	18.42±2.81	16.66±2.65	24.24±3.05

Regarding the genotype lines are demonstrated the absolute frequencies (outside the parentheses) and the respective relative frequencies (between parentheses). Regarding the alleles lines are demonstrated the relative frequencies more or less the standard error of allelic frequencies.

Our results are in agreement with the postulated effect of *R3500Q* mutation, which interferes with the conformation of the APOB 100 binding domain with the B / E receptor and thus reducing LDL-C affinity and consequently increasing serum levels of LDL-C and TC (12, 22, 23). Furthermore the R3500Q mutation is responsible for familial defective apolipoprotein B-100 (FDB) and its influence over lipid profile is studied worldwide (7, 24, 25). Therefore, our research contributes with data for a better understanding of genetic variants that influence changes on lipid levels in a Brazilian population sample.

According to a genome-wide association study (GWAS) there is a cluster of single nucleotide polymorphisms in the region of the *APOB* gene which is strongly associated with LDL-C levels (7). Furthermore they observed that the R3500Q mutation is strongly associated with LDL-C (7). Similarly to our study, Shen and cols. (7) also found an influence of this mutation over lipid profile and Nelken and cols. (8) observed the influence of this polymorphism on augmenting cholesterol levels in children and adolescents and thus suggesting that this genetic factor influences serum lipids levels early in life (7, 8).

The effect of the APOE polymorphisms in serum lipids levels varies in different populations and represents from 4 to 15% of LDL-C levels variation (26). In different populations the ε2 allele was associated with low levels of LDL-C, whereas the *ε4* was associated to high levels of LDL-C (1, 9). We observed that the *ε4* allele was more frequent in subjects with higher levels of TC and LDL-C, while the *ε2* allele is more frequent in subjects with higher concentrations of HDL-C, forming a protective effect, since one function of those lipoproteins is removing the excess of LDL-C from the circulation (19).

Considering the *ε4* allele influence over dyslipidemias and atherogenity its analyses is very important, specially because the consequences resulting from high lipid levels overwhelm the public health system. The atherogenity of the ε4 allele is probably due to its high affinity for apoE-binding receptors, leading to increased hepatic cholesterol pool which has the potential to down-regulate the LDLR, resulting in an accumulation of LDL-C and in an increased risk of atherosclerosis (27). ApoE3 and apoE4 bind to the LDLR with similarly high affinity, but the binding of apoE2 is somehow two orders of magnitude weaker (28).

Similarly to our findings, Han and cols. (29) found, in a case-control study of Vietnamese children, that ε4 carriers had the highest concentration of serum TC and LDL-C in dyslipidemia cases and in controls, while ε2 carriers had the lowest TC and LDL-C levels (29). Also in agreement with our results, Ferreira and cols observed a beneficial effect of the ε2 allele on lipid profile (30).

Furthermore, in the present research, we observed that the ε3ε4 combination was more frequent in the group above the TC median (test-G = 17.33 and p = 0.0039), agreeing with the results presented by Salazar and cols (31). We also found that the ε2ε4 combination was more frequent in the groups above the medians of LDL-C (Test-G = 18.09, p = 0.0028) and HDL-C (Test G = 17.08, p = 0.0044). The impact of the ε2ε4 genotype on lipid profile remains poorly documented and Freitas and cols. (32) observed that all subjects with *ε2ε4*genotype were in a group with one or more abnormal lipid profile markers at two evaluations (32). Villeneuve and cols. (33) also demonstrated that in the presence of abdominal obesity, the ε4 allele have a more pronounced effect than ε2 (33). Controversially to them, our findings allow us to suggest that in this sample in ε2ε4 genotypes the protective effect of the *ε2* allele is balanced with the damaging role of the ε4 allele. However, in ε3ε4 genotype, the damaging effect of e4 allele appears to be more pronounced.

We found that R3500Q site genotype distribution was out of Hardy-Weinberg equilibrium for the whole sample and for the groups above TC and LDL-C median and the stratum HDL-C below the median. Since in all plates, controls were used for both homozygotes, heterozygotes and negative controls, and genotyping were checked by two researchers independently, we excluded the possibility of technical error. We hypothesize that the R3500Q site genotype distribution is out of Hardy-Weinberg equilibrium probably due to the association between R3500Q allele and altered lipid profile. Considering that the total sample, as the strata above TC and LDL-C median and below HDL-C median, have a significant portion of dyslipidemic individuals, the above discussed association between R3500Q allele and altered lipid profile association may explain the absence of Hardy-Weinberg equilibrium for R3500Q site genotype distribution.

CONCLUSION

Considering that the *R3500Q* mutation of *APOB* gene was identified as an important risk factor for higher TC levels and the *ε4* allele of the *APOE* gene was significantly more frequent in subjects with higher TC and LDL-C levels, while the *ε2* allele was significantly more frequent in subjects with higher HDL-C levels, studies involving *APOB* and *APOE* genes have a great importance for public health. Thus, this work contributes for a better understanding of genetic variants that influence the lipid profile in a Brazilian population, aiming to identify population specific risk factors with potential use as biomarkers for prevention of related disorders such as cardiovascular and cerebrovascular diseases.

AUTHORSHIP:

Caroline C. Gasparin and Lupe Furtado-Alle participated in the conception and design of the study, and in the statistical analysis and writing of the manuscript. Luciane Viater Tureck, Lilian P. Ferrari and Ricardo L. Souza contributed reagents/materials/analysis tools and data analysis. All authors read, contributed with critical analyses and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicial to the impartiality of the reported research.

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ATTACHMENT

ETHICS COMMITTEE APPROVAL

Ministério da Educação
Universidade Federal do Paraná
Setor de Ciências da Saúde
Comitê de Ética em Pesquisa

UFPR

Curitiba, 13 de setembro de 2011.

Ilmo (a) Sr. (a)
Neiva Leite
Raul Osiecki
Ana claudia Vecchi Osiecki
Luciana da Silva timossi
Jean Fuzetti Cavazza

Nesta

Prezados Pesquisadores,

Comunicamos que o Projeto de Pesquisa intitulado **"Avaliação da saúde global, doenças crônicas e fatores associados em trabalhadores"** está de acordo com as normas éticas estabelecidas pela Resolução CNS 196/96, foi analisado pelo Comitê de Ética em Pesquisa do Setor de Ciências da Saúde da UFPR, em reunião realizada no dia 06 de julho de 2011 e apresentou pendência(s). Pendência(s) apresentada(s), documento(s) analisado(s) e projeto aprovado em 26 de agosto de 2011.

Registro **CEP/SD**: 1159.084.11.06 **CAAE**: 0082.0.091.000-11

Conforme a Resolução CNS 196/96, solicitamos que sejam apresentados a este CEP, relatórios sobre o andamento da pesquisa, bem como informações relativas às modificações do protocolo, cancelamento, encerramento e destino dos conhecimentos obtidos.

Data para entrega do 1º relatório parcial: 13/03/2012.

Atenciosamente

Profª. Drª. Cláudia Seely Rocco
Coordenadora do Comitê de Ética em
Pesquisa do Setor de Ciências da Saúde

Rua Padre Camargo, 280 – Alto da Glória – Curitiba-PR – C.EP 80060-240
Fone: (41)3360-7259 – e-mail: cometica.saude@ufpr.br