APOE genotypes and dyslipidemias in a sample of the Portuguese population

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Abstract

The objective of this work was to study the distribution of apolipoprotein E (*APOE*) genotypes in a sample of the Portuguese population, and its association with the dyslipidemias observed. Study participants were healthy users of local Public Health Laboratories in six regions of mainland Portugal (Porto, Vila Real, Viseu, Lisboa, Portalegre and Faro). A total of 779 men and 1153 women aged 15–74 years agreed to participate. Fasting lipid levels and *APOE* genotypes were determined centrally at the National Institute of Health in Lisboa. The frequency distribution of *APOE* alleles was: $\varepsilon2=5.3\%$, $\varepsilon3=84.9\%$ and $\varepsilon4=9.8\%$. Dyslipidemias were present in 66.6% of men and 60.7% of women. Comparison of *APOE* genotypes and relative allele frequencies showed that in dyslipidemic compared to normolipidemic subjects, the $\varepsilon4$ allele was more frequent in both sexes, although in a more pronounced way in men than in women due to higher frequencies of $\varepsilon3/\varepsilon4$ and $\varepsilon4/\varepsilon4$ genotypes. The known association of the $\varepsilon4$ allele with high cholesterol levels, the association of the $\varepsilon2$ allele with low cholesterol levels, and the association of the $\varepsilon2$ allele with high levels of triglycerides and low levels of high-density lipoprotein-cholesterol were confirmed in this study.

Keywords: *APOE* genotypes; cardiovascular diseases; dyslipidemias; Portuguese regions.

Introduction

The apolipopotein E (*APOE*) gene locus is polymorphic and three common alleles, $\varepsilon2$, $\varepsilon3$, $\varepsilon4$, are inherited in a codominant way. These encode isoforms of the corresponding plasma proteins, E2, E3, and E4, which can occur in six different phenotypes. The E3/3 phenotype is the commonest in different populations.

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These isoforms differ by a single amino acid substitution and, consequently, have different binding affinities for the four known apoE receptors. Specific literature on the complex biochemical aspects of the mechanisms involved is abundant (1–3).

The isoforms are related to amino acid substitutions in E2, E3, and E4 that influence their ionic binding to specific receptor domains and kinetics (4). Through this principal mechanism, apoE influences the metabolism of atherogenic lipoproteins – apoB-containing lipoproteins – and the reverse cholesterol transport (5).

Compared with $\varepsilon3$, the $\varepsilon2$ allele is frequently associated with low levels of total and low-density lipoprotein-cholesterol (LDL-C) and the opposite typically occurs with the $\varepsilon4$ allele. Nevertheless, the $\varepsilon2$ allele is also associated with a tendency towards higher triglyceride levels (6). *APOE* genotypes may thus influence mean plasma lipid values and the frequency of various types of dyslipidemias, which are considered important risk factors for the development of atherosclerosis and cardiovascular diseases (CVD) (7, 8).

The Portuguese population is characterized by high rates of dyslipidemias and CVD, but not much is known about the influence of *APOE* genotypes on these situations (9–12).

The aim of this work was to study the distribution of *APOE* genotypes in the population of six regions of mainland Portugal and to relate them to the dyslipidemias observed.

Materials and methods

Sampling locations and subjects

Six regions across mainland Portugal were selected as sampling locations: Porto, Vila Real and Viseu in the north; Lisboa and Portalegre in the center; and Faro in the South.

Blood samples were collected from users of the six local Public Health Laboratories attending for health control checks, who were randomly invited to participate every day during 6 months.

The sample population distribution was: 24.9% in Porto, 27.6% in Lisbon, the biggest cities, 12.8% in Vila Real, 12.7% in Viseu, 11.7% in Portalegre and 10.3% in Faro, totaling 779 men and 1153 women, aged 15–74 years. None of them reported taking hypolipidemic drugs or oral contraceptives. All subjects gave their written informed consent to participate and the Ethics Committee at the National Institute of Health (NIH) approved the study.

Blood samples

Blood samples were collected by venepuncture, with subjects in a sitting position after overnight fasting. Two vacutainer tubes were used, one with EDTA for DNA extraction

Table 1 Lipid characteristics of normolipidemic and dyslipidemic participants.

Normolipidemic subjects	Male n = 260 (33.38%)	Female n = 453 (39.29%)	p
Age, years	38.7 ± 17.6	37.5 ± 14.5	NS
Cholesterol, mmol/L	4.08 ± 0.58	4.20 ± 0.55	<0.01
Triglycerides, mmol/L	0.96 ± 0.29	0.95 ± 0.32	NS
HDL-C, mmol/L	1.27 ± 0.19	1.46 ± 0.27	<0.001
LDL-C, mmol/L	2.39 ± 0.52	2.33 ± 0.50	NS
ApoA1, g/L	1.39 ± 0.21	1.53 ± 0.26	<0.001
ApoB, g/L	0.90 ± 0.18	0.89 ± 0.17	NS
Dyslipidemic subjects	Male n = 519 (66.62%)	Female n = 700 (60.71%)	p
Age, years	48.8 ± 14.4	49.6 ± 15.4	NS
Cholesterol, mmol/L	5.58 ± 1.26	5.81 ± 1.07	0.001
Triglycerides, mmol/L	2.10 ± 1.41	1.62 ± 0.91	<0.001
HDL-C, mmol/L	1.13 ± 0.33	1.45 ± 0.39	<0.001
LDL-C, mmol/L	3.57 ± 1.13	3.66 ± 0.98	NS
ApoA1, g/L	1.39 ± 0.29	1.62 ± 0.34	<0.001
ApoB, g/L	1.28 ± 0.30	1.30 ± 0.29	NS

Values are mean ± SD. NS, not significant.

and the other for serum separation. Samples were transported at 4°C, when possible by airmail, to the Central Clinical Chemistry Laboratory at the NIH, where lipids were immediately quantified and EDTA samples were stored at −80°C. *APOE* genotyping was performed within 1 month.

DNA extraction and *APOE* genotyping

DNA extraction was performed according to the method of Miller et al. (13). *APOE* genotyping was performed by PCR amplification and subsequent digestion with restriction enzyme *Hha*I as described by Hixson and Vernier (14).

Lipid analysis

Serum total cholesterol, high-density lipoprotein-cholesterol (HDL-C) and triglycerides were measured using an enzymatic colorimetric method. Apoliproteins A1 and B were determined by immunoturbidimetry using a Hitachi 911 automated clinical chemistry analyzer (Boehringer Mannheim, Kobe, Japan). The same manufacturer supplied the reagents. The intra- and inter-assay coefficients of variation for these assays were 2.6% and 2.5% for total cholesterol, 4.0% and 4.5% for HDL-C, 2.0% and 2.5% for triglycerides, 1.7% and 6.6% for apoA1, and 3.2% and 8.9% for apoB, respectively.

LDL-C was calculated according to the Friedwald-Fredrickson equation for individuals whose serum triglyceride levels were lower than 4.54 mmol/L.

Different dyslipidemic types were defined based on total cholesterol, LDL-C, HDL-C, and triglyceride levels according to cut-off values specified by the American Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in adults (15).

Statistical analysis

Student's t-test for independent samples, or the non-parametric Mann-Whitney U-test, when appropriate, was used for comparing the mean values of age and lipid parameters. The assumption of equality of variances was tested using Levene's test. A simple gene-counting method was used to estimate allelic frequencies. Hardy-Weinberg equilibrium and statistical comparisons between populations were performed with a χ^2 test.

The association between *APOE* genotypes and blood lipid levels was determined by computation of specific odds ratios (ORs) for the association of each blood lipid with each of the three *APOE* alleles (present vs. absent) in both men and women. The different ORs were adjusted for the different age structure of study subpopulations using logistic regression.

Statistical analyses were performed using SPSS software version 11.0 (SPSS Inc., Chicago, IL, USA) and STATA version 8 for Windows (STATA Corporation, College Station, TX, USA). p-Values less than 0.05 were considered statistically significant.

Results

Lipid results

Gender differences in blood lipid levels were observed in normolipidemic subjects: women had higher cholesterol than men, but HDL-C and apoA1 were lower in men than in women.

Dyslipidemic subjects, 66.6% men and 60.7% women, showed the same differences as normolipidemic participants: higher cholesterol in women, and higher triglycerides, lower HDL-C and apoA1 in men (Table 1).

Dyslipidemia characterized by hypercholesterolemia was more prevalent in women than in men. On the other hand, isolated hypertriglyceridemia or in association with low HDL-C was more frequent in men.

Dyslipidemic subjects were on average 10 years older than normolipidemics and this was taken into account when calculating the ORs for the association between *APOE* alleles and lipid concentrations, adjusting for age using logistic regression methods.

Distribution of *APOE* genotypes

Table 2 shows the distribution of *APOE* genotypes in Hardy-Weinberg equilibrium in all participating regions. A borderline deviation was observed only when *APOE* genotypes were considered together ($\chi^2 = 6.01$; p = 0.049).

Table 2   Frequency of *APOE* genotypes and alleles in six Portuguese regions.

	Porto	Vila Real	Viseu	Lisboa	Portalegre	Faro	Total
Genotype							
ε2/ε2	1	1	1	2	3	0	8 (0.4%)
ε2/ε3	35	25	20	49	18	15	162 (8.9%)
ε2/ε4	1	2	3	5	3	1	15 (0.8%)
ε3/ε3	381	169	154	361	134	126	1325 (72.4%)
ε3/ε4	69	34	36	90	31	34	294 (16.1%)
ε4/ε4	7	1	6	4	3	4	25 (1.4%)
Total	494	232	220	511	192	180	1829
χ^2 test	5.24	0.34	4.399	0.60	5.92	1.94	6.01
p	0.073	0.844	0.111	0.741	0.052	0.379	0.049
Allele							
ε2	0.038	0.058	0.060	0.057	0.070	0.045	0.053
ε3	0.877	0.791	0.825	0.842	0.826	0.838	0.849
ε4	0.085	0.151	0.115	0.101	0.104	0.117	0.098

The results showed a high *ε3* allele frequency and relatively low *ε4* frequency, with *ε2* the least common allele.

To evaluate the importance of *APOE* polymorphisms for dyslipidemias, we compared *APOE* genotypes and relative allele frequencies in normolipidemic and dyslipidemic subjects stratified by sex.

In general terms we observed that genotypes with the *ε4* allele were more frequent in dyslipidemic participants. This difference was more pronounced in men than in women (7.1% vs. 12.0% in men; 7.6% vs. 10.4% in women).

APOE genotypes and lipids

In men, the presence of the *ε2* allele was significantly associated with a lower probability of having LDL-C above 4.14 mmol/L (age-adjusted OR, 0.34; 95% CI, 0.15–0.75). All other associations were not statistically significant. The presence of the *ε3* allele was not associated with either a lower or higher probability of having any of the dyslipidemias studied.

On the other hand the presence of the *ε4* allele was significantly associated with a higher probability of having HDL-C <1.04 mmol/L and triglycerides ≥1.71 mmol/L (age-adjusted OR, 2.26; 95% CI, 1.41–3.62), and a higher probability of having LDL-C above 4.14 mmol/L (age-adjusted OR, 1.68; 95% CI, 1.09–2.57) and HDL-C below 1.04 mmol/L (age-adjusted OR, 1.67; 95% CI, 1.11–2.49).

In women, the presence of the *ε2* allele was associated with a significantly lower probability of having cholesterol ≥5.17 and ≤6.18 mmol/L (age-adjusted OR, 0.62; 95% CI, 0.39–0.99), LDL-C ≥3.37 and ≤4.14 mmol/L (age-adjusted OR, 0.49; 95% CI, 0.27–0.87) and LDL-C above 4.14 mmol/L (age-adjusted OR, 0.38; 95% CI, 0.19–0.78). All other associations were not statistically significant for allele *ε2*. The presence of allele *ε3* was not associated with either a lower or higher probability of having any of the dyslipidemias studied.

The presence of the *ε4* allele was significantly associated with a higher probability of having LDL-C above 4.14 mmol/L (age-adjusted OR, 1.79; 95% CI, 1.19–2.69) and cholesterol >6.21 mmol/L (OR, 1.85: 95% CI, 1.25–2.74). All other associations were not statistically significant for allele *ε4*.

Table 3 presents mean values and standard deviation for lipid parameters by sex and by the most prevalent *APOE* genotypes.

Discussion

The allelic frequencies observed in this study are in agreement with those we obtained in another study of the Portuguese population conducted in the Lisbon area under the ApoEurope Project (16). As in almost all populations, the *ε3/ε3* genotype was by far the most frequent (72.4%), and the *ε3* allele accounted for the greatest majority of the *APOE* gene pool (84.9%) while *ε4* accounted for 9.8% and *ε2* for 5.3%. As a result of the lower frequencies of the *ε4* and *ε2* alleles, the genotypes *ε4/ε4*, *ε2/ε4* and *ε2/ε2* were relatively rare. We did not find significant differences in

Table 3   Mean values and standard deviation for lipids, apoA1, apoB and age, according to *APOE* genotype and sex.

	Men			Women		
	ε2/ε3	*ε3/ε3*	*ε3/ε4*	*ε2/ε3*	*ε3/ε3*	*ε3/ε4*
Number	62	515	128	96	781	157
Total cholesterol, mmol/L	4.95±1.49	5.00±1.20	5.31±1.18	4.64±1.09	5.21±1.13	5.36±1.19
HDL-C, mmol/L	1.24±0.35	1.19±0.28	1.11±0.29	1.47±0.36	1.46±0.35	1.45±0.34
LDL-C, mmol/L	3.63±4.14	3.78±3.91	4.44±4.68	2.76±2.42	3.28±1.85	3.46±2.02
Triglycerides, mmol/L	1.74±1.13	1.72±2.06	1.88±1.33	1.46±1.16	1.34±0.74	1.33±0.72
ApoA1, g/L	1.45±0.27	1.39±0.26	1.32±0.26	1.62±0.33	1.59±0.31	1.59±0.28
ApoB, g/L	1.03±0.26	1.14±0.32	1.25±0.31	0.95±0.26	1.15±0.31	1.20±0.32
Age, years	45.8±17.2	44.9±15.9	46.2±16.6	43.5±15.2	45.1±16.3	43.7±15.9

ε2/ε2, *ε2/ε4* and *ε4/ε4* genotypes were not included in this analysis on account of their low frequencies.

the distribution of the *APOE* alleles in the six participating regions of this study.

Worldwide *APOE* allele frequencies differ in various geographic regions. In Europe the $\varepsilon 2$ allele shows an increasing gradient from north to south, and the opposite is observed for the $\varepsilon 4$ allele. The prevalence of cardiac atherosclerotic diseases, and cholesterol and triglyceride concentrations also show the latter distribution. The Finnish population has the highest prevalence of $\varepsilon 4$ (17.8%), while the Greek (Crete) (8.5%) and Portuguese populations (9.7%) have the lowest prevalence (16).

In a comparative study organized by Mahley and Rall (17) on *APOE* allele frequencies in 16 distinct countries/world regions/ethnic groups, the $\varepsilon 2$ frequency ranged from 4.1% to 13% and that of $\varepsilon 4$ from 7.4% to 29.1%. Our results are closer to regions with the lowest frequencies of $\varepsilon 4$ and $\varepsilon 2$, such as Japan and Singapore. A study in the Moroccan population also found values rather similar to ours: $\varepsilon 4 = 11\%$, $\varepsilon 3 = 84\%$, and $\varepsilon 2 = 5\%$ (18).

When comparing *APOE* genotypes and relative allele frequencies in normo- and dyslipidemic subjects, we observed that the frequency of the $\varepsilon 4$ allele was higher in dyslipidemic subjects of both sexes. This was more pronounced in men due to higher frequencies of the $\varepsilon 3/\varepsilon 4$ and $\varepsilon 4/\varepsilon 4$ genotypes than in women, whom, on the other hand, had a low frequency of the $\varepsilon 2$ allele due to a much lower number of $\varepsilon 2/\varepsilon 3$ genotypes.

More detailed analysis of the relationship between lipids and genotypes (Table 3) shows that genotypes with the $\varepsilon 2$ and $\varepsilon 4$ alleles have respectively lower and higher plasma cholesterol levels compared with the $\varepsilon 3/\varepsilon 3$ subset.

The genotype associated with higher triglyceride and lower HDL-C levels was $\varepsilon 3/\varepsilon 4$ in men. According to Dallongeville et al. (6), cardiovascular risk due to the $\varepsilon 4$ allele is increased in individuals with the $\varepsilon 3/\varepsilon 4$ genotype due to elevated levels of triglycerides and lower HDL-C concentrations – this is the characteristic dyslipidemia of the metabolic syndrome. Moreover, as mentioned by Assmann and co-workers, this $\varepsilon 3/\varepsilon 4$ heterozygosity compared with $\varepsilon 3/\varepsilon 2$ heterozygosity and $\varepsilon 3/\varepsilon 3$ homozygosity is accompanied by an earlier age at myocardial infarction (19).

Considering the associations between lipids and *APOE* alleles through the relative ORs, we can again confirm the non-association of $\varepsilon 2$ with cholesterol and the association between $\varepsilon 2$ and high triglycerides/low HDL-C, as well as the association between $\varepsilon 4$ and dyslipidemias rich in cholesterol. The numbers expressing these ORs are relatively low, approximately 1 and 2, which is usual in this type of work [see the Framingham Offspring Study (7)].

Dyslipidemias were observed in a high percentage of the subjects studied (over 60%). This finding is relevant, as dyslipidemias are an important risk factor for atherosclerotic disorders, which in Portugal result in high mortality and morbidity rates [39% of verified deaths in 1999, 52% by stroke and 22% by cardiac heart disease (12, 20)].

Moreover the relationship that dyslipidemias have with the pathogenesis of atherosclerosis is extensively documented and its environmental determinants and clinical consequences are recognized (21–34).

According to this scientific evidence, Portuguese health authorities and scientific societies have widely and repeatedly recommended interventions for earlier diagnosis and control of dyslipidemias in the population, recognizing their importance throughout the country (35–37).

In our study, age and sex were associated with increased serum concentrations of the lipids and apolipoproteins quantified, with the exception of HDL-C, which was decreased.

Regarding age, the usual explanations expressed in the literature cannot be excluded: weight gain during adult life, heredity, selective mortality, and probably a decrease in receptor activity (38, 39).

In relation to higher triglyceride values in men than in women and low levels of HDL-C, either in isolation or combined, it is possible that these reflect the influence of obesity, hypertension, a sedentary life and other risk factors associated with metabolic syndrome, which are becoming increasingly prevalent in our population, especially in men (20). The increasing prevalence of type 2 diabetes may also be an important cause. In fact, hyperinsulinemia is associated with increased very low-density lipoprotein (VLDL) secretion by the liver, decreased activity of lipoprotein lipase and decreased HDL-C (40).

Hormonal status as a gender-specific factor could also explain some of the lipid differences observed between men and women. The higher cholesterol values observed in women are probably the result of multiple effects of sex hormones on lipoprotein metabolism. The high number of women aged over 45 years in the sample population may explain some of the differences observed in lipid concentrations, due to the importance of menopause.

Returning to the *APOE* genotypes/allelic frequencies for normo- vs. dyslipidemic subjects, we observed that $\varepsilon 4$, the allele considered the most pathogenic, is more frequent in dyslipidemic than normolipidemic subjects (4.9% excess in males; 2.8% in females). In our opinion, this small excess of $\varepsilon 4$ alleles in the dyslipidemic population studied, together with the low OR values, suggests that $\varepsilon 4$ is not a major factor in explaining the occurrence of dyslipidemias in Portugal.

In fact, we cannot exclude the possibility that important genetic abnormalities may be present that influence dyslipidemias, such as familial hypercholesterolemia, familial defective apoB-100, and type III hyperlipoproteinemia, although these show a low prevalence in populations studied so far. Nevertheless, it is most probable that lipid variations are polygenic and also depend on environmental factors such as lifestyle.

Major changes in lifestyle have taken place in Portugal since the 1960s, in part associated with an inversion of the age pyramid, approaching what is observed in EU countries.

According to the few nutrition surveys and official aggregate statistics based on commerce and production, during the last four decades, patterns of food intake have changed progressively from a healthy Mediterranean diet to an atherogenic diet, with a considerable increase in the supply and use of meat, dairy products, oils and fats (except olive oil) and added sugars (41). On the other hand, the supply of fish, cereals and vegetables has decreased. Energy supply from fats, which represented only approximately 24.7% of the total energy intake in the 1960s, was 34.3% of the total energy intake in the late 1990s. Animal fat intake increased from 10% to 14% of the total energy intake during the same period (41).

It is thought that different dietary patterns in Portuguese regions that may have existed in the past have become attenuated and cannot be easily differentiated today. To the best of our knowledge, only a report from 1988 based on the 1980 national food consumption survey identified two different nutritional profiles in the country. That in the northern regions, north from the Tejo River (Lisbon area excluded) had traditional food intake habits, with an excess of salt and alcoholic beverages. In the other regions, in the south, a higher intake of proteins and animal fats and a lower intake of carbohydrates and alcoholic beverages were observed (42).

Consequently, standardized morbidity and mortality rates for nutrition-related diseases were higher in the north (liver cirrhosis, esophageal cancer, gastric cancer, CVD and stroke), whereas southern regions had lower CVD, colon cancer and breast cancer rates.

Regions considered in this study included Porto, Vila Real and Viseu in the north, where the frequency of CVD is higher than the national average (191.7/100,000 per year) and Lisboa, Portalegre and Faro, where CVD is less frequent than the national average (79.9/100,000 per year).

Other lifestyle-related risk factors deserve mention due to their impact on the lipid profile of the population. According to the World Drink Trends Statistics, alcohol consumption is high in Portugal (14 L/capita per year), clearly above the mean of EU countries. The three available National Health Interview Surveys show that approximately 50% of the population report alcohol consumption. The contribution of wine, beer and spirits is changing, with wine being more frequently used by middle-aged and older people, whereas beer and spirits are increasingly being used by the young (43).

Portugal has the lowest smoking prevalence in Europe. Data from the three National Health Interview Surveys show that the prevalence of daily smoking in men is slowly decreasing (33.3%, 29.2%, 29.3% respectively), but increasing in women (5.0%, 6.5%, 7.9%). The increased number of female smokers is particularly relevant in younger age groups, while the decrease in men is observed in all age groups except 35–44 years (43).

In Portugal, as in other countries, there is a trend to a progressive increase in the prevalence of obesity and hypertension (44, 45).

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