Brief Communications

Polymorphism of the APOE Locus in the Azores Islands (Portugal)

CONCEIÇÃO BETTENCOURT,[1,2] RAFAEL MONTIEL,[1,2] CRISTINA SANTOS,[1,2] MARIA LEONOR PAVÃO,[1,3] ANA MARIA VIEGAS-CRESPO,[4] PAULA ALEXANDRA LOPES,[4] AND MANUELA LIMA[1,2]

Abstract Our aim in this study is to report on the polymorphism of the *APOE* gene in the Azores Islands (Portugal) to obtain a population baseline of the existing variation in this locus, known to be one of the genetic determinants of plasma lipid levels. One hundred twenty-six Azorean individuals were typed for the *APOE* polymorphism using standard PCR-RFLP. Allele frequencies obtained for *APOE*2*, *APOE*3*, and *APOE*4* were 6.75%, 83.73%, and 9.52%, respectively. The *APOE*3/*3* genotype presented the highest frequency (69.84%), and the *APOE*4/*4* genotype had the lowest frequency (0.79%). Genotype frequencies were in conformity with Hardy-Weinberg expectations. The observed genotype and allele frequencies were similar to those reported for other Iberian samples. Furthermore, Nei's gene diversity ($\hat{H} = 0.2864 \pm 0.0351$) was similar to that reported for samples from mainland Portugal. The data generated from this study will be of importance in the context of ongoing studies concerning the factors that influence lipid levels in the Azorean population.

Apolipoprotein E (ApoE) (MIM 107741) is a plasma glycoprotein with a molecular mass of about 34 kDa, formed by 299 amino acid residues. The *APOE* gene, which spans 3.6 kb, is located on chromosome 19q13.2 (Lin-Lee et al. 1985) and exhibits three major codominant alleles (*APOE*2*, *APOE*3*, and *APOE*4*) that codify for three isoforms of the protein (E2, E3, and E4) (Weisgraber et al. 1981). The *APOE* locus harbors one of the genes that is involved in the control of plasma lipid levels, accounting for about 10% of the total variation in cholesterol levels

[1]Center of Research in Natural Resources (CIRN), University of the Azores, Ponta Delgada, San Miguel, Azores, Portugal.
[2]Department of Biology, University of the Azores, Ponta Delgada, San Miguel, Azores, Portugal.
[3]Department of Technological Sciences and Development, University of the Azores, Ponta Delgada, San Miguel, Azores, Portugal.
[4]Center for Environmental Biology (CBA), Faculty of Sciences, University of Lisbon, Lisbon, Portugal.

Human Biology, August 2006, v. 78, no. 4, pp. 509–512.

KEY WORDS: APOLIPOPROTEINS, ISOFORMS, *APOE*, *APOE* POLYMORPHISM, LIPID LEVELS, AZORES ISLANDS, PORTUGAL, SPAIN.

(e.g., Sing and Davignon 1985). Studies that relate the lipid profile to genetic variants at the *APOE* locus have consistently shown that total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) are lowest in *APOE*2* carriers, intermediate in *APOE*3* carriers, and highest in *APOE*4* carriers (e.g., Tan et al. 2003).

A previous study (Pavão et al. 2006) of a sample of 118 apparently healthy subjects from the Azores Islands (Portugal) revealed that 34% of the subjects were highly hyperlipidemic; from these subjects more than 60% were hypercholesterolemic (TC > 250 mg/dL). Because the variation at the *APOE* locus has an important role in the control of lipid levels, and given the absence of population data for this locus in the Azores, our aim in this study is to report on *APOE* polymorphism in the Azorean population to obtain a population baseline for forthcoming studies, which will focus on the factors that influence lipid levels in this population.

Materials and Methods

Sampling and DNA Extraction. Blood samples were obtained from 126 healthy unrelated individuals born in the Azores, after obtaining their informed consent. For 84 samples, blood was dried on filter paper (FTA Classic Card, Whatman International Ltd., Maidstone, U.K.) and total DNA was extracted using Instragene Matrix (BioRad Laboratories, Amadora, Portugal), according to the manufacturer's specifications. For the remaining 42 samples, whole blood was collected in tubes containing EDTA and DNA was extracted using a salting-out procedure (Miller et al. 1988).

PCR Amplification and Typing. Oligonucleotide primers (apoE-F316: 5′-GAG ACG CGG GCA CGG CTG TCC-3′ and apoE-R625: 5′-GCA CGC GGC CCT GTT CCA CC-3′) were designed to amplify a 310-bp fragment of the *APOE* gene. The amplification products were subsequently digested with *Cfo*I (Roche Farmaceutica Quimica Lda., Amadora, Portugal) following the recommendations of the manufacturer. Genotypes were determined after electrophoresis of digestion products on 9% polyacrylamide gels, visualized by silver staining (Neilan et al. 1994). Samples previously typed in an independent laboratory were used as quality controls.

Statistical Analysis. Genotype frequencies were obtained from the banding patterns; allele frequencies were estimated using gene counting. Conformity with Hardy-Weinberg expectations was tested using the exact Hardy-Weinberg probability without bias. An exact test of population differentiation was conducted to evaluate the independence of the genotype and allele composition of the various populations. Nei's gene diversity was calculated and compared with values reported for other Portuguese samples. All the analyses were performed using the Arlequin package (Schneider et al. 2000), with a level of significance of 0.05.

Table 1. Genotype Frequencies, p Value for the Exact Test of Population Conformity with Hardy-Weinberg Equilibrium Expectations, and Allele Frequencies of *APOE* Polymorphism in the Azorean Population ($n = 126$)

Genotype or Allele	*Frequency*
Genotype	
*APOE*2/*2*	0
*APOE*2/*3*	0.1190
*APOE*2/*4*	0.0159
*APOE*3/*3*	0.6984
*APOE*3/*4*	0.1587
*APOE*4/*4*	0.0079
Hardy-Weinberg p value	0.9290
Allele	
*APOE*2*	0.0675
*APOE*3*	0.8373
*APOE*4*	0.0952

Results and Discussion

Observed genotype and allele frequencies of the *APOE* locus in the Azorean population are summarized in Table 1. The *APOE*3/*3* genotype presented the highest frequency (69.84%), and the *APOE*4/*4* genotype had the lowest frequency (0.79%); the *APOE*2/*2* genotype was absent. Genotype frequencies were in conformity with Hardy-Weinberg expectations (Table 1). The genotype and allele frequencies observed in this study were statistically similar to those reported for other Portuguese (Schiele et al. 2000; Rodrigues et al. 2005) and Spanish samples (Schiele et al. 2000). Nei's gene diversity calculated for the Azorean population ($\hat{H} = 0.2864 \pm 0.0351$) was similar to that reported for populations from mainland Portugal (Schiele et al. 2000; Rodrigues et al. 2005).

The results obtained provide information on the general population variation at the *APOE* locus, which is of importance in the context of forthcoming studies on the biological, environmental, and genetic factors that influence the lipid profile in the Azorean population.

Acknowledgments This study was supported by the Center of Research in Natural Resources (CIRN) through grant POCTI/ARRA Açores/172. C. Bettencourt is a recipient of a Ph.D. grant from Fundação para a Ciência e a Tecnologia (FCT) (grant SFRH/BD/21875/2005). R. Montiel (supported by grant SFRH/BPD/13256/2003) and C. Santos (supported by grant SFRH/BPD/20944/2004) are postdoctoral fellows of the FCT. We are indebted to all the participants in the study.

Received 18 November 2005; revision received 30 May 2006.

Literature Cited

Lin-Lee, Y. C., F. T. Kao, P. Cheung et al. 1985. Apolipoprotein E gene mapping and expression: Localization of the structural gene to human chromosome 19 and expression of ApoE mRNA in lipoprotein- and non-lipoprotein-producing tissues. *Biochemistry* 24:3751–3756.

Miller, S. A., D. D. Dykes, and H. F. Polesky. 1988. A simple salting-out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 16:1215.

Neilan, B. A., D. A. Leigh, E. Rapley et al. 1994. Microsatellite DNA screening: Rapid nondenaturing, nonisotopic dinucleotide repeat analysis. *Biotechniques* 17:708–711.

Pavão, M. L., T. Figueiredo, V. Santos et al. 2006. Whole blood glutathione peroxidase and erythrocyte superoxide dismutase activities, serum trace elements (Se, Cu, Zn), and cardiovascular risk factors in subjects from the city of Ponta Delgada (island of San Miguel, the Azores Archipelago, Portugal). *Biomarkers* 11:460–471.

Rodrigues, M. O., A. Fonseca, C. M. Dias et al. 2005. *APOE* genotypes and dyslipidemias in a sample of the Portuguese population. *Clin. Chem. Lab. Med.* 43:907–912.

Schiele, F., D. de Bacquer, M. Vincent-Viry et al. 2000. Apolipoprotein E serum concentration and polymorphism in six European countries: The ApoEurope Project. *Atherosclerosis* 152:475–488.

Schneider, S., D. Roessli, and L. Excoffier. 2000. *Arlequin: A Software for Population Genetics Data Analysis 2.000.* Geneva: Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva.

Sing, C. F., and J. Davignon. 1985. Role of the apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. *Am. J. Hum. Genet.* 37:268–285.

Tan, C. E., E. S. Tai, C. S. Tan et al. 2003. *APOE* polymorphism and lipid profile in three ethnic groups in the Singapore population. *Atherosclerosis* 170:253–260.

Weisgraber, K. H., S. C. Rall, and R. W. Mahley. 1981. Human E apoprotein heterogeneity: Cysteine-arginine interchanges in the amino acid sequence of the apo-E isoforms. *J. Biol. Chem.* 256:9077–9083.