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APOE-ε4 POLYMORPHISM AND COGNITIVE DEFICIT AMONG THE ELDERLY POPULATION OF FERNANDO DE NORONHA

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Abstract – Background: Polymorphism of the gene for apolipoprotein E (APOE) is an important risk factor for the development of Alzheimer's disease. The ε4 allele of the APOE gene has been linked with a number of neuropsychiatric illnesses, and also with stress and depression among geriatric populations. **Objective:** To identify APOE-ε4 polymorphism and correlate this with cognitive deficit among the elderly population of the island of Fernando de Noronha. **Method:** Neuropsychiatric tests (mini-mental state examination, verbal fluency test and clock drawing test) were applied to 52 elderly people without Alzheimer's disease. DNA was isolated from peripheral blood and genotyping of APOE was done by the PCR-RFLP method. **Results:** 87% of the elderly population (mean age 69.6±7.0) had cognitive deficit. **Conclusion:** The observed frequency of the ε4 allele was 10%, but the correlation between the presence of ε4 and cognitive deficit in this population was not statistically significant.

KEY WORDS: APOE, polymorphism, elderly people, cognitive deficit.

Polimorfismo de APOE-ε4 e déficit cognitivo na população idosa de Fernando de Noronha

Resumo – Introdução: Polimorfismos no gene da apoliproteína E (APOE) são importantes fatores de risco para o desenvolvimento da doença de Alzheimer (DA). O alelo ε4 do gene APOE tem sido relacionado com declínio cognitivo e algumas doenças neuropsiquiátricas, primariamente a doença de Alzheimer. **Objetivo:** Identificar os polimorfismos de APOE-ε4 e relacionar com deficit cognitivo na população idosa da ilha de Fernando de Noronha. **Método:** Foram aplicados testes neuropsiquiátricos (mini exame do estado mental, teste de fluência verbal e teste do relógio) em 52 idosos sem DA. O DNA foi isolado do sangue periférico e a genotipagem de APOE foi realizada por PCR-RFLP. **Resultados:** 87% da população idosa com idade média de 69.6±7.0 apresentou déficit cognitivo. Foi observada uma freqüência de 10% do alelo ε4. **Conclusão:** Não foi encontrada significância estatística quando relacionada a presença deste alelo e déficit cognitivo nos idosos avaliados.

PALAVRAS-CHAVE: APOE, polimorfismos, idosos, déficit cognitivo.

Changes in the shape of the world's population pyramid over the past century have demonstrated increases in the numbers of elderly people. Neurodegenerative illnesses most commonly appear at such ages. Alzheimer's disease (AD) is the most frequent of these, and this indicates that there is a need to use diagnostic techniques to identify risk factors for cognitive decline among elderly populations. Individuals who ultimately develop degenerative dementia such as AD are likely to transition through a period of mild impairment. Mild cognitive impairment (MCI) is a term used to describe this transitional zone between normal aging and very early dementia. It represents a condition in which individuals present memory impairment that is greater than expected for their age[1]. Over the last decade, many studies have been conducted to investigate the relationship between family history, genetic markers and cognitive profile among elderly populations. Apolipoprotein E was identified as a risk factor for

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AD[2]. The APOE gene is inherited as one of three alleles, termed ε2 (Arg148→Cys), ε3 (Cys112) and ε4 (Cys112→Arg), with mean frequencies in the general population of about 8%, 78% and 14%, respectively[3]. The degree of AD risk conferred by ε4 rises in a "gene dose" manner, increasing with the number of ε4 alleles inherited, from zero (noncarriers) to one (heterozygote) and two (homozygote)[4]. Because AD usually appears late in life and progresses slowly, the ε4 allele may exert its effects prior to the clinical diagnosis of AD. Consistent with this view, studies have shown that older carriers of the ε4 allele who do not have dementia nevertheless do show cognitive deficits[5]. New research has indicated that the ε4 allele is a weak predictor of Alzheimer dementia among elderly individuals[6].

AD patients carrying the ε4 allele have also shown pronounced medial temporal lobe atrophy[7,8]. Furthermore, the APOE gene has been linked with several cognitive processes, such as spatial attention[9] and working memory[5], for which ε4 carriers have shown deficits in relation to ε4 non-carriers. Cognitive deficits associated with ε4 are not confined to older adults. They may also occur as early as midlife, a decade or more before the likely onset of symptoms of AD in those destined to acquire the disease. Such findings suggest that a cognitive phenotype of APOE could exist independently of the possible development of AD. Possession of the ε4 allele is far from being a guarantee of subsequent dementia, given that only about 50% of ε4 homozygotes have developed AD by age 80[10] or 90 years[11].

The purpose of the present study was to investigate the relationship between the APOE gene and neuropsychological characters of the elderly residents of the island of Fernando de Noronha, State of Pernambuco, a population without previous records, in order to obtain data that might contribute towards promoting actions to improve the lives of this elderly population.

METHOD

Participants

In April 2006, we evaluated 52 elderly individuals, representing 59% of this population segment on the island of Fernando de Noronha. This island is a Maritime National Park located in the Atlantic Ocean, around 220 miles east of the Brazilian coast. It has just over 2,800 inhabitants, who are descendents of prisoners, military personnel or people who have come in to develop services. The elderly people in our sample had lived there for at least thirty years.

Ethics

The research protocol was approved by the Scientific and Ethics Committee of the University of Pernambuco (UPE). All subjects signed the informed consent statement.

Laboratory methods

Genomic DNA was extracted by means of the salting-out method[12], from peripheral blood, anticoagulated with ethylenediaminetetracetic acid (EDTA). The APOE genotyping was done by the PCR method (polymerase chain reaction). The forward primer sequence was 5'-TCCAAGGAGCTGCAGGCGGCGCA-3' and the reverse sequence was 5'-ACAGAATTCGCCCCGGCCT GGTACACTGCCA-3'. The polymorphism was investigated using enzymatic cleavage using the restriction enzyme HhaI (RFLP)[13]. Fragments of 72 bp, 48 bp and 33 bp were produced from APOE-ε4, fragments of 91 bp and 81 bp from APOE-ε2 and fragments of 91 bp, 48 bp and 33 bp from APOE-ε3.

Cognitive tests

The elderly people were examined using the clinical dementia rating (CDR) scale[14] and the NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association) criteria[15]. They also underwent the following neuropsychological tests to assess their memory, executive functions, orientation and language: mini-mental state examination (MMSE)[16] , verbal fluency test (VFT)[17] and clock drawing test (CDT)[18]. The data are presented as means, standard deviations and quartiles. They were also tested using the Hachinski ischaemia score[19], Yesavage's geriatric depression scale[20] and the Lewy body dementia test[21]. For the tests in which cognitive function was considered in terms of education level, this was taken to be the number of years of schooling that the individual had had. The sample was stratified into three groups: illiterates, individuals with one to eight years of schooling and individuals with more than eight years. The cutoff scores for MMSE cognitive deficit were 13 for illiterates, 18 for individuals with 1-8 years of schooling and 26 for individuals with more than 8 years. The cutoffs for VFT were 9 and 13 for below and above eight years of schooling, respectively. For CDT, the cutoff point was 7 for all groups.

Data analysis

The descriptive analysis of the sample is presented as means and standard deviations (SD) for quantitative variables, and as relative frequencies (%) for qualitative variables. The APOE allele frequencies were estimated by a gene-counting method and genotype distributions were compared with those expected according to the Hardy-Weinberg hypothesis of genetic equilibrium, by means of a chi-square test for goodness of fit. The mean scores from the MMSE and VFT according to gender, age and education level were compared by means of the analysis of variance test (ANOVA). Associations between qualitative variables and comparisons between proportions were estimated using chi-square tests, corrected by the Yates factor as appropriate and using Fisher's exact test.

RESULTS

We evaluated 52 individuals (26 men and 26 women)

Table 1. Cognitive performances of elderly people on Fernando de Noronha according to gender, age and schooling level.

Cognitive test			Mean±SD	Q1	Median	Q3	Q4
MMSE			**19.4±4.1**	**17**	**19**	**22**	**29**
	Gender	Male	19.5±5.0	16.5	19	23	29
		Female	19.5±3.0	17.2	19	21	26
	Age group	60-74	19.8±3.9	17	19	22	29
		75-95	17.1±4.1	16.5	18.5	19.25	21
	Schooling	Illiterate	15.5±3.0	15	16	17	21
		1-8	19.4±3.4	18	19	21.2	26
		>8	23.7±3.5	21.2	24.5	26	29
	APOE	ε4	17.28±4.1				
		Non-ε4	18.90±3.9				
FVT			**12.04±3.30**	**9**	**12**	**14.5**	**19**
	Gender	Male	12.81±3.21	10.25	13	15	18
		Female	11.36±3.29	9	12	13	19
	Age group	60-74	12.42±3.33	10	13	15	19
		75-95	9.86±2.19	8.5	9	11.5	13
	Schooling	Illiterate	9.0±0.75	8.75	9	9.25	10
		1-8	12.06±3.26	10.5	12	14	18
		>8	15.0±2.20	12.75	15	15.5	19
	APOE	ε4	17.28±4.1				
		Non-ε4	18.9±3.9				

between 60 and 95 years old, with a mean age of 69.6 years (SD 7.0). They were subdivided into two age groups: 60 to 74 years (78.5%) and over 74 years (21.5%). According to schooling level, this sample was stratified as illiterate (17.3%), 1–8 years of formal education (67.3%) and over eight years of formal education (15.4%). No statistical difference in age and education level was observed between the genders. The older group of elderly people was totally (99%) functionally illiterate (i.e. not more than 0 to 3 years of schooling), whereas only 61% of the younger group were functionally illiterate (p=0.030).

The APOE genotype frequencies in our sample were: ε3/3 – 71.7%; ε4/4 – 6.10%; ε2/2 - 0%; ε3/4 – 4.1%; ε2/4 – 4.1%; and ε2/3 – 14.3%. The allele frequencies were ε2 – 0.09; ε3 – 0.81; and ε4 – 0.10. The proportion of carriers of ε4 allele (homozygous and heterozygous) was 14.3% and the frequencies were as expected according to Hardy-Weinberg genetic equilibrium (p=0.25). The APOE genotype distributions were homogeneous in both genders.

Three male individuals presented clinical dementia (5.7%). One of these cases was diagnosed as probable AD (aged 77; schooling <4 years; genotype ε2/3) and the other two as possible AD (one aged 82; schooling 4-8 years; genotype ε2/3; and the other aged 77; schooling <4 years; genotype ε3/3). None of these elderly men with dementia had the ε4 allele, since they had the ε3/3 and ε2/3 genotypes.

Table 1 shows the mean scores and quartile values from the MMSE and VFT cognitive tests, according to gender, age and schooling. For MMSE, the mean was 19.4 (SD 4.1) and the quartiles were 17, 19. 22 and 29; and for VFT, the mean was 12.04 (SD 3.30) and the quartiles were 9, 12, 14.5 and 19. Neither test indicated any statistical differences regarding gender or age, after adjustment for schooling and ε4 presence. However, when stratified for educational level, the subjects differed such that performance increased with increasing number of years of schooling (p=0.0002).

Table 2. Proportions of whole sample and APOE-ε4 carriers who were positive for cognitive deficit, among dementia-free subjects from Fernando de Noronha, Pernambuco, according to cognitive test.

Positive for cognitive deficit				
	Whole sample		APOE-ε4 carriers	
	n	%	n	%
MMSE	21	42.8	5	23.8
FVT	15	31.9	2	13.3
CDT	25	53.2	6	24.0

The sample was also stratified to analyze cognitive deficits, into groups with and without deficit. These data are in Table 2 and indicate that 42.8%, 31.9% and 53.2% of the whole dementia-free sample of elderly people presented cognitive deficit, as revealed by the MMES, VFT and CDT cognitive tests, respectively. The frequencies of

individuals positive for cognitive deficit who were ε4 carriers were 23.8%, 13,3% and 24.0% from the MMSE, VFT and CDT tests, respectively.

DISCUSSION

The prevalence of cognitive deficit on the island of Fernando de Noronha (5.7%) was lower than what was observed by Herrera et al. (2002)[22] in Catanduva, State of São Paulo (7.1%), among which 55.1% was AD; 14.4% was AD with vascular cerebral disease and 9.3% was vascular dementia. They observed that the prevalence increased with age and female gender and had an inverse association with education level.

Among the few researchers taking a community focus have been Corrêa and Veras. Corrêa[23] reported prevalence of dementia of 21.4 %. On the other hand, Veras[24] detected mean prevalence of cognitive impairment of 15.04%, with a range from 5.95% to 29.75%, probably as a function of education and socioeconomic levels in stratified subsamples.

Few studies have compared normal elderly Brazilians with those presenting pathological conditions of cognitive nature. Different criteria have been used in order to obtain data that can standardize the rules for applying and evaluating cognitive tests adjusted for large and culturally diverse populations. Thus, data have been expressed as means, medians or quartiles and different cutoff points have been suggested for separating normal and anomalous performances in the tests, as a function of schooling and age.

There are significant differences in cognition between elderly individuals. Their cognition is characterized by declines in higher functions, affecting mainly the capacity to learn declarative content and executive functions[25]. Many studies have shown an inverse relation between cognitive performance in answering cognitive tests and the subjects' ages and schooling levels, among Brazilian populations[26-28]. Our results confirm that cognitive performance correlates with education level, but not with age after adjustment for schooling level.

Comparing our mean scores from the cognitive tests with others in the literature, the cognitive performance from the MMSE in our data was lower than in other studies[29,30], both for the whole sample and according to age and education level. The differences between our study and others cannot be attributed to greater proportions of illiterates and few years of schooling in our sample. From the VFT, the mean score obtained was not the same as in other studies[31,32], but the results seem compatible with the mean number of years of schooling.

The differences between data from different studies may reflect the heterogeneity of elementary-level teaching in different regions of Brazil. They may constitute a warning that the appropriateness of the test application parameters needs to be reviewed in order to determine what the normal variations are and to facilitate the diagnosis of cognitive impairment.

Although correction factors need to be used in cognitive tests because of motor-sensory dysfunctions and age, differentiation of the cutoff points for education levels may lead to profiles for cognitive impairment cases that express our reality better than do standard cutoff points[33]. Taking the criteria of scores of 13, 18 and 23 as the cutoff points for MMSE, 9 and 13 for VFT[34,35] and 7 for CDT made it possible to create subgroups of elderly individuals and to consider that subjects with values below these cutoffs might present cognitive deficit. In this way, we obtained cognitive deficits of 42.8% to MMSE, 31.9% to VFT and 53.2% to CDT.

Individuals presenting marked cognitive deficits provide a large field of study for evaluating and investigating genetic variants that influence cognitive function, since there is the possibility that neuropsychological impairment can partially be attributed to genotype manifestations. Modern large-scale techniques can now be applied to vast populations of genes, and such techniques may explain the contributions of genetic factors towards the development of illnesses. This provides the basis for understanding their pathogenesis and makes it possible to design new and more efficient approaches for early diagnosis and treatment.

One of the loci that has been studied most is the APOE locus, which has three alleles (ε2, ε3 and ε4). The influence of the apoε4 allele on the acceleration of cognitive deficit and development of AD seems to vary with ethnicity, age and gender. The frequency of the apoε4 allele among our elderly sample from Fernando de Noronha was 10%. This was lower than values obtained among individuals without dementia of Caucasian (11.5%) and African descent (22.5%) in the State of Rio Grande do Sul[36] and Caucasians (12%) in São Paulo, but was greater than in another study in São Paulo (8.9%)[37]. All of these were case-control studies and they showed an association between ε4 and AD in Brazilian populations. We did not make inferences regarding dementia because of our small sample, but a brief analysis among the non-demented elderly individuals showed us that the frequency of cognitive deficit among carriers in relation to other genotypes was not statistically significant.

It is important to put the island of Fernando de Noronha into its particular historical context. Its small and special population results from a historical process that started with its status as the first Hereditary Administra-

tive Division (Capitania) of Brazil, in the sixteenth century, with subsequent invasions by many European peoples, until its definitive occupation by Portugal. The first permanent settlement was founded around 1770, but the island became uninhabited and completely abandoned again several times. It was a Federal Territory during World War II; it has been a prison for political and ordinary prisoners; and today it is a district within the State of Pernambuco. Thus, the allele frequencies in this elderly people may represent more a sample coming from different parts of Brazil, especially the Northeast of Brazil, than a generation coming from a local past generation.

In conclusion, this study showed a significant relationship between cognitive performance and schooling among this elderly sample on the island of Fernando de Noronha, but not in relation to age and gender. The MMSE, VFT and CDT cognitive tests revealed different proportions of cognitive deficit, but only CDT presented a significant difference according to education among this sample. We did not find any association between the ε4 allele and cognitive deficit.

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