Table 1. Apo E genotypes distribution and allele frequencies

	AD		Control	
	n	%	n	%
Genotype				
ε2/ε3	5	6.76	3	8.57
ε3/ε3	42	56.76	28	80.00
ε3/ε4	20	27.02	4	11.43
ε4/ε4	7	9.46	0	0
Allele				
ε2	5	3.37	3	4.29
ε3	109	73.67	63	90.00
ε4	34	22.96	4	5.71

Apo E genotype comparisons: (χ^2 = 8.0421, d.f. = 3, p = 0.0451; AD vs. Control).

Apo E allele frequency comparisons: (χ^2 = 9.8377, d.f. = 2, p = 0.0073; AD vs. Control).

n = Number of individuals; % = relative frequency.

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Apolipoprotein E ε4 Allele Is a Risk Factor for Alzheimer's Disease: The Central Region of Portugal (Coimbra) as a Case Study

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Alzheimer's disease (AD), an age-associated neurodegenerative disorder, is characterized by a progressive decline of the cognitive functions, in particular a loss of memory, learning and attention, and it does not have a simple etiology. Most of the cases are sporadic and the factors involved are unknown. A very small percentage of cases are hereditary and are due to mutations in one of the three genes: amyloid precursor protein (APP) on chromosome 21, presenilin-1 (PS1) on chromosome 14 or presenilin-2 (PS2) on chromosome 1. A fourth gene on chromosome 19 encoding the protein apolipoprotein E (Apo E) also appears to be implicated in the disease [1]. The *Apo E* gene is polymorphic with three major alleles – ε2, ε3, and ε4 – and results in six genotypes – ε2/ε2, ε2/ε3, ε3/ε3, ε3/ε4, ε4/ε4, and ε2/ε4. The ε3 allele is the most common in the general population. The ε4 allele increases the risk of AD and lowers the age of onset in a dose-dependent manner. Conversely, ε2 allele decreases the risk and delays AD onset [1]. In the present study we examined the *Apo E* genotype and allele frequencies as well as the relative risk of dementia in probable AD patients and control subjects of the central region of Portugal (Coimbra).

Seventy-four probable AD patients, 42 female and 32 male (age range 41–85 years; mean 68.243 ± 9.017 years) were recruited from the Neurological Unit of the University Hospital of Coimbra. Thirty-five age-matched healthy subjects free of cognitive impairment, 18 female and 17 male (age range 47–84 years; mean 64.971 ± 10.416 years) were recruited from the informants (spouses or nonkindred) for the cases with whom they share similar age and socioeconomic status. Patients with dementia met DSM IV [2] and NINCDS-ADRDA criteria [3] for the diagnosis of probable AD. They had at least a 1-year history of cognitive decline confirmed by neuropsychological examination. Neuroradiologic evaluation was also compatible with degenerative dementia. Laboratory studies, including thyroid gland function, lues serology, vitamin B_{12}, folate levels and CSF study were all normal. For Apo E genotyping, genomic DNA was extracted from peripheral blood, and allelic variants identified by polymerase chain reaction (PCR) [4]. The relative risk (OR) of AD was calculated by taking a ratio of probabilities of exposure given affection status, and the Fisher's exact test was used to calculate the 95% confidence intervals (CI) using the approximation of Woolf [5].

The main finding of the present study was that the relative risk of AD for individuals carrying the ε4 allele was significantly higher than for those not carrying the allele, and homozygosity for the ε4 allele increases the risk of AD in the central region of Portugal (Coimbra). The distribution of the *Apo E* genotypes and allele frequencies were significantly different between AD and control groups (table 1). The frequencies of the genotypes ε3/ε4 and ε4/ε4 as well as the ε4 allele frequency in the AD group were higher than those observed in the control group. The OR of AD associated with the presence of the ε4 allele versus its absence (OR = 4.4521 (95% CI = 1.4181–13.978), p = 0.0066) is statistically significant, indicating that the risk of AD for individuals carrying the ε4 allele is significantly higher than for those not carrying the ε4 allele. The OR of AD associated with one dosage of the ε4 allele (OR = 3.2979 (95% CI = 1.0280–10.580), p = 0.0490) versus zero dosage of the ε4 allele was slightly significant as compared with the control group. Although in reduced numbers all the individuals homozygous for the ε4 allele were associated with AD indicating that homozygosity of the ε4 allele increases the risk of AD.

In conclusion, the present study reinforces the idea that Apo E ε4 allele is a risk factor for AD [6, 7] and that homozygosity of the 4 allele increases the risk of AD [8]. Moreover, it confirms an earlier report [9] showing that the presence of the 4 allele increases the risk of AD in the Portuguese population.

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