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Allele ε4 of *APOE* is a stronger predictor of Alzheimer risk in Sicily than in continental South Italy

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Abstract

The genotype of apolipoprotein E was examined in 173 sporadic Alzheimer's disease (AD) patients, 132 with late onset (LOAD) and 41 with early onset (EOAD), and in 174 healthy matched controls from Sicily. Despite a low frequency of the ε4 allele (6.3%, 95% CI: 4.2–9.4) in controls, ε4 allele was a stronger predictor of AD risk (odds ratio: 5.8, 95% CI: 3.5–9.4; $p < 0.0001$) than in most of the studies performed in other regions of Italy, and it has no influence on age at onset. ε4/ε4 and ε4/ε3 genotypes were similar predictors of AD risk. Conversely, a decreased risk was found in ε3 allele carriers (odds ratio: 0.3, 95% CI: 0.2–0.4; $p < 0.0001$), which remained significant when considering EOAD cases only (odds ratio: 0.2, 95% CI: 0.1–0.4, $p < 0.0001$). In conclusion, differences in association strength of ε4 allele with AD between Sicily and other regions of Italy suggest an influence of complex gene–gene and gene–environment interactions.

Apolipoprotein E (*APOE*) is a plasma protein, which mediates a high-affinity receptor binding of ApoB/E lipoproteins to the low-density lipoprotein (LDL) receptor [7]. Moreover *APOE* appears to be involved in other functions including differentiation, cell growth and immunoregulation and is synthesized in various organs, including spleen, kidney, liver and brain [23]. The human *APOE* gene is located on chromosome 19 and three alleles have been described (ε2, ε3 and ε4) which encode three major isoforms [12]. *APOE*3 is the most common isoform presenting a cysteine at amino acid residue 112 and an arginine at amino acid residue 158. *APOE*2 differs from *APOE*3 by a cysteine for arginine substitution at residue 158 and *APOE*4 differs from *APOE*3 by an arginine for cysteine substitution at residue 112. *APOE* genotype influences receptor-binding affinity and serum lipid levels [6,27,28,38]. The *APOE* polymorphism accounts for 6.5–8.8% of the variation in serum cholesterol levels [4]. The ε4 allele is associated with elevations in total cholesterol and with the risk of atherosclerotic diseases [24] while ε2 allele has the opposite effect [20].

The *APOE* ε4 allele is strongly associated with cerebrovascular and Alzheimer's disease [11,14,32]. Up to date, the ε4 allele is the strongest genetic predictor of sporadic Alzheimer's disease (sAD). Contradictory data have been published on the influence of ε4 allele on the age of AD onset [11,19,22,26,31,32,41,45]. On the other side, a protective effect of the *APOE* ε2 allele has been observed on familial AD and LOAD in different populations [10,30,43], while its association with EOAD appeared controversial [30,36,39,42]. In Western Europe, the frequency of *APOE* ε4 allele follows a geographic North–South decreasing trend both in sAD and in normal populations, and subsequently, the frequency of *APOE* ε3 allele increases from North-to-South in the same populations [9,17,29]. Whether the frequency of ε4 allele reflects the incidence and risk of AD is not known. In South Italy, an area with low ε4 allele frequency, a single study of

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risk association has been performed in the region of Apulia [31].

We have evaluated therefore the risk association of *APOE* genotype with occurrence and age at onset of sAD in Sicily, and we have compared it to that reported in other regions from Italy.

The 347 individuals included in this study were recruited at the IRCCS Oasi of Troina (Italy) and the diagnosis of probable AD was made following standard clinical criteria [1,25]. The 174 control subjects were individuals without symptoms of cognitive impairment and/or family history of dementia. Written informed consent was obtained from the participants or from their families. This study was approved by the ethical committee of the Oasi Institute. Patients and controls were all born in Sicily. We recruited 173 sAD patients (mean age 76.3 ± 8.1 S.D.), 132 of whom with LOAD (mean age 79.5 ± 5.5 S.D., 55 men and 77 women) and 41 with EOAD (mean age at onset 59.2 ± 5.1 S.D., 22 men and 19 women). The 174 control subjects were subdivided into two subgroups matched, respectively, with EOAD and LOAD: subjects aged 56–64 years (53 individuals, 29 men and 24 women, mean age 58.7 ± 4.5 S.D.) and over 64 years (121 individuals, 52 men and 69 women, mean age 78.7 ± 8.0 S.D.).

EDTA blood was obtained by venepuncture after overnight fasting and *APOE* genotypes were determined after isolating DNA from white blood cells using a method previously described in Anello et al. [2] modified from that described by Hixon and Vernier [18]. Two independent readers recorded the genotypes and retyped any discrepancies until resolved, without knowledge of the patient or control subject status. The χ^2-test was used to evaluate the eventual differences between *APOE* allele frequencies of AD subjects and healthy controls. The Fisher's exact probability test was preferred when at least one of the expected numbers was lower than five [37]. The genotype frequencies do not deviate significantly from those predicted by the Hardy–Weinberg equilibrium. The differences in the mean age at onset according to the number of *APOE* ε4 alleles were analysed statistically by means of the Mann–Whitney *U*-test. Age- and sex-adjusted odd ratio (OR) and the 95% confidence interval (CI) were computed between individuals with or without at least one ε2 or ε3 or ε4 allele using logistic regression. Statistical testing was always carried out at the conventional significance level of $p \leq 0.05$ and the Statview for Windows software (Microsoft 1998) was used to perform the statistical analyses.

The *APOE* allele frequencies in Sicilian AD patients and healthy controls are shown in Table 1. Compared to the control group, ε4 allele frequency was significantly higher in AD ($\chi^2 = 57.57$; $p < 0.0001$) and ε3 allele frequency significantly lower ($\chi^2 = 43.02$; $p < 0.0001$). These differences were also observed when splitting the AD patients into EOAD and LOAD and comparing them with the respective age- and sex-matched control groups (Table 1). In the entire population analysed, ε4 bearers were associated to AD by an odds ratio of 5.77 (95% CI: 3.53–9.43; $p < 0.0001$). The risk association was stronger for individuals at risk of EOAD (odds ratio: 9.59, CI: 2.91–31.56; $p = 0.0002$) than for individuals at risk of LOAD (odds ratio: 6.31, CI: 3.41–11.67; $p < 0.0001$). Reciprocally, a protective association of the ε3 allele with sAD was logically found for the entire population analysed (odds ratio: 0.27, 95% CI: 0.18–0.40; $p < 0.0001$), when considering the alleles ε2 and ε4 at baseline. In our population, no protective effect of the ε2 allele was observed, even in the group of EOAD. In contrast, a previous studies on the Italian population has reported a protective influence of the *APOE* ε2 allele in regard to sAD risk [31] but there is also a study showing an increased risk of EOAD [39].

Our study reported a frequency as low as 6.3% in a rural mountainous area of central Sicily, in agreement with that previously observed in other series from Sicily and Sardinia [5,9,13,15,16]. By comparison, it was estimated, respectively, to 8.5% and 9.4–9.8% in Central and North Italy [9] (Fig. 1), designing a North-to-South decreasing trend. The *APOE* ε4 allele was a stronger predictor of AD risk in Sicily than in any of the other Italian regions (Fig. 1). The association between the *APOE* ε4 allele and AD seems also to differ between North and South of Italy [15,31,39]. In Northern

Table 1
Statistical analysis by means of the χ^2-test of the *APOE* allele frequencies in early- (EOAD) and late-onset (LOAD) sporadic Alzheimer's disease (sAD) patients and in healthy control subjects in Sicily (Southern Italy)

	APOE alleles								
	ε2			ε3			ε4		
	n	Frequency	95% CI	*n*	Frequency	95% CI	*n*	Frequency	95% CI
AD patients	11	0.03	0.02–0.06	238	0.69[a]	0.64–0.73	97	0.28[a]	0.24–0.33
Controls (over 55)	16	0.05	0.03–0.07	310	0.89	0.85–0.92	22	0.06	0.04–0.09
EOAD patients	5	0.06	0.03–0.13	55	0.67[b]	0.56–0.76	22	0.27[b]	0.18–0.37
Controls (56–64)	3	0.03	0.01–0.08	98	0.92	0.86–0.96	5	0.05	0.02–0.12
LOAD patients	6	0.03	0.01–0.05	183	0.69[c]	0.63–0.75	75	0.28[c]	0.23–0.34
Controls (over 64)	13	0.05	0.03–0.09	212	0.88	0.83–0.91	17	0.07	0.04–0.11

CI: confidence interval.
[a] AD vs. controls (over 55 years), $p < 0.0001$.
[b] EOAD vs. controls (56–64 years), $p < 0.0001$.
[c] LOAD vs. controls (= or >65 years), $p < 0.0001$.

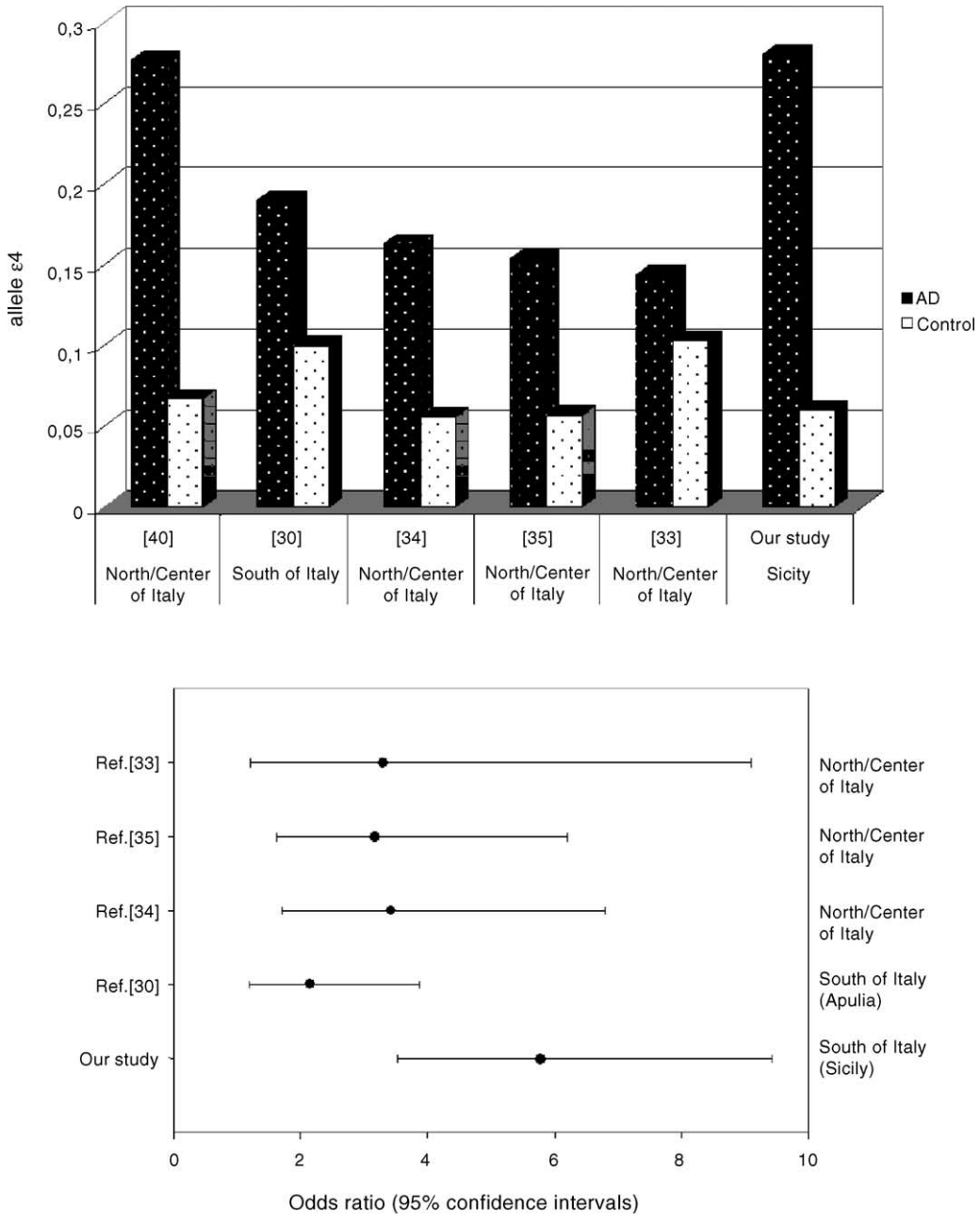

Fig. 1. Comparison of the relative frequency of *APOE* allele ε4 in Alzheimer patients (AD) and controls (top) and of the odds ratios of AD risk (bottom) in populations from North/Central and South Italy. Numbers within square brackets indicate references [30,33–35,40].

Italy, a three- to five-fold increase of *APOE* ε4 allele in sAD patients and an odds ratio in order of 3.1–3.4 have been reported [15,39], while a study on a Southern Italian population from Apulia found only a doubled frequency of *APOE* ε4 allele in sAD, with a risk association corresponding to an odds ratio of 2.1 [31]. In addition, this study failed to find a significant difference between LOAD and controls [31]. In contrast, we observed a four-fold-increased frequency of ε4 allele in sAD and an odds ratio of 5.8, despite the same low frequency in the control population than in continental South Italy (Table 1 and Fig. 1). In addition, another distinct feature between Apulia and Sicily concerned the association of alleles ε4 and ε2 with EOAD and LOAD. In Sicily, allele ε4 was at risk in the two subsets of the disease and allele ε2 was weakly protective only for EOAD while in Apulia the protective effect of allele ε2 is present in both subsets. Finally, our estimates of association of alleles ε4 and ε3 are closer to the situation observed in Northern Italy and France than in Apulia [21,39,40]. This difference may be due to the peculiar ethnical and geographic characteristics of Sicily and also to distinct gene–environment interactions. However, a much larger study with the same design of recruitment of patients from the distinct areas of Italy should be needed to reach a definitive conclusions.

The allele ε3 is the most frequent in all the human groups, especially in populations of the Mediterranean basin (0.849–0.898 in previous studies [8,9] and 0.890 in the present study). It has been previously suggested that the allele ε4, based on some functional properties it has and on its distribution among human populations, could be identified as a 'thrifty' allele and that its exposures to the contemporary environmental conditions (Western diet, longer lifespans) could have rendered it a susceptibility allele for AD [8]. The particularism of the allele ε4 association with AD in Sicily may result from complex gene–nutrient and gene–gene interactions. For example, the dietary habits of children from Sicily have a higher total caloric, monounsaturated fatty acid and fibre intake, and a lower saturated fatty acid and cholesterol intake, than an all-Italy sample of school-children [29]. Another epidemiological study conducted in 1989 in a rural village in the hilly hinterland 50 km from Palermo described a population, which had a very low incidence of early cardiovascular mortality, lower total cholesterol plasma concentration than the Italian average, and nutritional habits following the criteria of the Southern Italian Mediterranean diet [4]. Compared to other Italian regions, a higher allele frequency has also been reported in Sicily for MTHFR 677 C>T, a genetic determinant of homocysteine [44]. Homocysteine is associated with AD risk and this association is aggravated by carriage of ε4 and *MTHFR 677 T* alleles, respectively [3].

In conclusion, in contrast to what previously observed in another region of South Italy, we found that allele ε4, but not allele ε2, was strongly associated with the two subsets of AD, in Sicily. Ethnic background and genetic and environmental factors might be responsible for these peculiarities.

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