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Original Article

APOE ε2 and ε4 influence the susceptibility for Alzheimer's disease but not other dementias

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Abstract: Apolipoprotein E (*APOE*) genotype was determined in a population of patients with dementia, including 735 patients with Alzheimer's disease (AD), 75 with Frontotemporal Lobar Degeneration (FTLD), 97 with Vascular Dementia (VaD) and 40 with Lewy Body Dementia (LBD), as well as in 506 age- and gender-matched controls (CON). *APOE* ε2 allele frequency was lower in patients with AD (2.8%) than in CON (6.4%, $P \leq 0.001$, OR: 0.41). Similar results were obtained comparing AD with FTLD (6.7%, $P \leq 0.01$, OR: 0.37), at difference from VaD (5.6%, $P > 0.05$) or LBD (5.0%, $P > 0.05$). The frequency of the *APOE* ε4 allele was increased in patients with AD (25.1%) as compared with CON (8.2%, $P \leq 0.001$, OR: 4.24), FTLD (11.3%, $P \leq 0.001$, OR: 2.67), VaD (11.8%, $P \leq 0.001$, OR: 3.02), or LBD (13.8%, $P = 0.048$, OR: 2.07). The frequency of the ε4/ε4 genotype was increased in AD patients compared with controls (6.3 versus 0.8%, $P \leq 0.001$, OR: 8.38). The presence of the ε2 allele is a protective factor for AD, whereas the ε4 allele acts as a risk factor for the disease. Both alleles do not influence the susceptibility to FTLD, LBD and VaD.

Keywords: Apolipoprotein E (APOE), Alzheimer's disease (AD), Frontotemporal Lobar Degeneraton (FTLD), Vascular dementia (VaD), Lewy body dementia (LBD), risk factor

Introduction

Current research on neurodegenerative diseases is focused on two main and inter-related objectives: identification of pathogenic mechanisms and therapeutic approaches. Although a cause for most neurodegenerative disease is not known, a multifactorial origin is often the case, involving mainly genetic, environmental, and nutritional factors. As concerns Alzheimer's disease (AD, OMIM #104300), the most common degenerative dementia, the known predisposing role of a particular genotype for the gene that encodes apolipoprotein E (*APOE,* OMIM #107741) makes the presence of *APOE* ε4 allele a candidate marker for the disease [1]. APOE is involved in the uptake of lipids generated after neuronal degeneration and in their redistribution to proliferating and repairing cells [2,3]. Enhanced synthesis of APOE E3, but not APOE E4, was demonstrated to stimulate repair of local hippocampal damage [4].

Whereas the presence of *APOE ε4* allele is associated with a significantly greater risk of developing AD [5-11], the ε2 allele seems to have a somewhat protective role towards the disease, even if some discordant data exist [12-15]. In this regard, the paucity of data is probably due, at least in part, to the very low frequency of ε2 allele. A neuropathological study on 296 AD brains [16] evidenced that, whereas the *APOE* ε4/ε4 genotype was associated with a higher tissue load of neuritic plaques and neurofibrillary tangles, the presence of the ε2 allele was protective against the formation of neuritic

plaques.

In vitro studies demonstrated that APOE E3 stimulates, but APOE E4 inhibits neuronal sprouting in murine hippocampal cultures [17]. In addition, APOE has been shown to bind beta amyloid (Aβ) and can therefore be important in the clearance of Aβ [17]. The binding capacity for Aβ is much greater for APOE E2 than for the other isoforms, and this could explain the protection exerted against AD [17]. Other experimental evidence strongly suggests that both protection by APOE E2 and pathogenetic role of APOE E4 are in strict relationship with amyloid processing [18].

To date, the effect of the *APOE* genotype on the development of other types of dementia is still controversial. A number of studies suggested an association between Frontotemporal Lobar Degeneration (FTLD, OMIM #600274) and *APOE* ε4 allele [19, 20]. Other Authors [21, 22] however, did not replicate these data, possibly due to the small sample size analyzed in their study. Recent findings demonstrated an association between the *APOE ε4* allele and FTLD in males, but not females [23], possibly explaining the discrepancies previously reported. Concerning the e*2* allele in the development of FTLD, heterogeneous data have been obtained in different populations. Bernardi et al. [19] showed a protective effect of this allele towards FTLD, whereas other Authors failed to do so [22-24]. Despite these results, a recent meta-analysis comprising a total of 364 FTD patients and 2671 controls (CON) demonstrated an increased susceptibility to FTD in ε*2* carriers [25].

Additional studies demonstrated an increased *APOE ε4* frequency in Vascular Dementia (VaD), similar to that found in AD [26-28], whereas more recent findings did not replicate such association [29-31].

To further study the role of *APOE* in the development of AD and other forms of dementia, we genotyped for *APOE* a large population of CON and patients with different types of dementia (AD, FTLD, LBD – OMIM #127750 - and VaD) and compared the *APOE* distribution in the different subgroups in order to assess the possible effect of ε*2* and ε*4* alleles upon the susceptibility to develop these dementias.

Materials and methods

Subjects

Nine-hundred forty-seven patients were consecutively recruited at the Alzheimer Units of Ospedale Maggiore Policlinico-IRCCS and Ospedale Sacco (Milan) and at the Presidio Ospedaliero di Magenta (Milan). All patients underwent a standard battery of examinations, including medical history, physical and neurological examination, screening laboratory tests, neurocognitive evaluation (to assess memory, language and constructional praxis), brain Magnetic Resonance Imaging (MRI) or Computed Tomography (CT) and, if indicated, Positron Emission Computed Tomography (PET). Dementia severity was assessed by the Clinical Dementia Rating (CDR) and the Mini Mental State Examination (MMSE) score. Seven-hundred thirty-five patients (206 males and 529 females, mean age at disease onset ±S.E.M.: 73.8± 0.36 years) were diagnosed by exclusion as affected by probable AD, according to NINCDS-ADRDA criteria [32]. One hundred-seven AD patients had an onset of the disease before age 65 (EOAD). For this subjects the possible presence of familial AD was excluded as no first-degree relative was affected by AD. Seventy-five patients (34 males and 41 females, mean age at onset ±S.E.M.: 68.7±1.97 years) were diagnosed as sporadic FTLD in accordance with consensus criteria proposed by Neary et al. [33]. These consensus criteria identify three clinical syndromes: Frontotemporal dementia (FTD), Progressive nonfluent Aphasia (PA) and Semantic Dementia (SD), which reflect the clinical heterogeneity of FTLD. Five out of 75 patients with FTLD had PA, 2 had SD and the remainder were diagnosed as FTD.

Forty patients (19 males and 21 females, mean age at onset ±S.E.M.: 74.5±1.18 years) were diagnosed as LBD according to McKeith et al. criteria [34]. Diagnosis of VaD was formulated in 97 patients (52 males and 45 females, mean age at onset ±S.E.M.: 73.0±1.03 years) according to NINDS-AIREN criteria [35]. The control group consisted of 506 subjects matched to patients for ethnic background and age (175 males and 331 females; mean age±S.E.M: 68.7±0.59 years, *P*>0.05 versus patients), without memory complaints (MMSE score ranging from 28 to 30), including healthy age-matched volunteers recruited either at nursing homes or

Table 1. *APO E* allele and genotype frequencies (%) in patients and CON

APOE	CON n=506	AD n=735	Mean age at onset (years± SD)	OTHER DEMENTIAS n=212
Allele				
ε2	65 (6.4)	41 (2.8)*	-	25 (5.9)
ε3	864 (85.4)	1060 (72.1)	-	348 (82.0)
ε4	83 (8.2)	369 (25.1)**	-	51 (12.1)
Genotype				
ε2/e2	3 (0.6)	1 (0.1)	NA	1 (0.5)
ε2/e3	54 (10.7)	29 (3.9)	78.0±3.5#	22 (10.4)
ε2/e4	5 (1.0)	10 (1.4)	81.3±5.2#	1 (0.5)
ε3/e3	370 (73.1)	382 (52.0)	74.5±5.0	141 (66.5)
ε3/e4	70 (13.8)	267 (36.3)	73.4±7.0	44 (20.7)
ε4/e4	4 (0.8)	46 (6.3)***	72.9±5.2	3 (1.4)

The genotypic distributions of CON and AD respected Hardy-Weinberg equilibrium ($p>0.05$ for the χ^2 test between observed and expected values). *$P\leq0.001$; OR: 0.41, CI: 0.27-0.62, AD versus controls; $P\leq0.01$; OR: 0.45, CI: 0.26-0.77, AD versus other dementias; **$P\leq0.001$; OR: 4.24, CI: 3.20-5.61, AD versus controls; $P\leq0.01$; OR: 2.64, CI: 1.88-3.81, AD versus other dementias; ***$P\leq0.001$; OR: 8.38, CI: 3.00-23.43, AD versus controls; $P\leq0.01$; OR: 4.65, CI: 1.43-15.10, AD verus other dementias; #$P\leq0.01$ versus ε3/ε3 genotype, one-way ANOVA followed by Dunnett's *post hoc* test. SD: standard deviation

at the Centers involved in this study (non-consanguineous patients' kindreds). An informed consent to participate in this study, performed in accordance with the Helsinki Declaration of 1975, was given by all individuals or their caregivers.

APOE genotyping

High-molecular weight DNA was isolated from whole blood using a Flexigene Kit (Qiagen, Hildren, Gemany), as described by the manufacturer. The amount of DNA for each sample was determined by measuring the optical density at 260 nm wavelengths using a spectrophotometer (Eppendorf AG, Germany). *APOE* genotype was determined by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay. DNA was amplified using specific primers and then digested with *Hha*I, as previously described [36].

Statistical analysis

Allelic and genotypic frequencies were obtained by direct counting. χ^2 test was used to test for Hardy-Weinberg equilibrium and for differences in allele distribution among groups. The odds ratio (OR) was calculated along with its 95% confidence interval (CI).

Results

APOE ε2 distribution

APOE allelic and genotypic frequencies in patients and controls are summarized in **Table 1**. The allelic frequency of the ε2 allele was significantly decreased in patients with AD (2.8%) as compared with either controls (6.4%, $P\leq0.001$, OR: 0.41, CI: 0.27-0.62) or patients with other types of dementia (5.9%, $P\leq0.01$, OR: 0.45, CI: 0.26-0.77). When comparing AD patients with FTLD, a statistically significant difference was found (2.8 versus 6.7%, $P\leq0.01$, OR: 0.37, CI: 0.18-0.77), while the same comparison between AD and VaD or LBD was not significant (5.6 and 5.0%, respectively, $P>0.05$, **Table 2**). None of patients with PA and SD was a carrier of the ε2 allele. No significant differences in ε2 distribution were found between controls and FTLD, LBD or VaD. The calculation of the mean

Table 2. *APO E* allele and genotype frequencies (%) in AD compared with FTLD, LBD and VaD

Allele	CON n=506		AD n=735		OTHER DEMENTIAS n=212	
	ε4⁻	ε4⁺	ε4⁻	ε4⁺	ε4⁻	ε4⁺
ε2⁻	370 (73.1)	74 (14.6)	382 (52.0)	313 (42.6)**	141 (66.5)	47 (22.2)
ε2⁺	57 (11.3)	5 (1.0)	30 (4.1)*	10 (1.3)	23 (10.8)	1 (0.5)

The genotypic distributions of AD, FTLD, LBD and VaD respected Hardy-Weinberg equilibrium ($p>0.05$ for the χ^2 test between observed and expected values); *$P \leq 0.01$; OR: 0.37, CI: 0.18-0.77, AD versus FTLD; **$P \leq 0.001$; OR: 2.67, CI: 1.53-4.67, AD versus FTLD; P=0.048; OR: 2.07, CI: 1.02-4.21, AD versus LBD; $P \leq 0.001$; OR: 3.02 CI: 1.81-5.04, AD versus VaD

age at onset according to *APOE* genotype in AD patients highlighted a significant increase of age at onset for cases with ε2/ε4 or ε2/ε3 genotypes in comparison to age at onset for the ε3/ε3 group. No differences in allelic and genotypic frequencies of ε*2* were observed after stratifying patients according to gender (data not shown).

APOE ε4 distribution

The allelic frequency of the ε*4* allele was significantly higher in patients with AD (25.1%) than in controls (8.2%, *P*≤0.001, OR: 4.24, CI: 3.20-5.61) or patients with other kinds of dementia (12.1%, *P*≤0.01, OR: 2.64, CI: 1.88-3.81; **Table 1**). Comparing AD patients with the different disorders included in the last group, a statistically significant difference was found between AD and FTLD (25.1 versus 11.3%, *P*≤0.001, OR: 2.67, CI: 1.53-4.67), as well as between AD and VaD (25.1 versus 11.8%, *P*≤0.001, OR: 3.02, CI: 1.81-5.04). Concerning LBD, a borderline significance was found when comparing with AD (25.1 versus 13.8%, *P*=0.048, OR: 2.07, CI: 1.02-4.21, **Table 2**). No significant differences in e*4* distribution were observed between controls and FTLD or LBD or VaD. The frequency of the *APOE* ε4/ε4 genotype was significantly increased in AD patients as compared with either controls or other forms of dementia (6.3 versus 0.8%, *P*≤0.001, OR: 8.38, CI: 3.00-23.43 or versus 1.4%, *P*≤0.01, OR: 4.65, CI: 1.43-15.10, respectively). No differences in allelic and genotypic frequencies of ε*4* were observed stratifying patients according to gender (data not shown). However, the frequency of the ε4/ε4 genotype was markedly high in EOAD (12.1%) compared to controls, with an over 15-fold increased risk to develop AD (*P*≤0.001, OR: 17.46, CI: 5.57-54.71). The calculation of the mean age at onset according to *APOE* genotype in AD patients did not find a decrease of age at onset for cases with ε3/ε4 or ε4/ε4 genotypes in comparison to age at onset for the ε3/ε3 group.

APOE ε2 and ε4 combination

The lowest OR was found in patients carrying the protective ε2 allele but not the risk ε4 allele as compared with either controls or patients with other types of dementia (4.1 versus 11.3%, *P*≤0.001, OR: 0.34, CI: 0.22-0.54 or versus 10.8%, *P*≤0.001, OR: 0.35, CI: 0.20-0.62, respectively, **Table 3**). Conversely, the highest OR was found comparing patients carrying the ε4 but not the ε2 allele (42.6 versus 14.6%, *P*≤ 0.001, OR: 4.33, CI: 3.25-5.77 or versus 22.2%, *P*≤0.001, OR: 2.60, CI: 1.82-3.71, respectively, **Table 3**).

Discussion

According to our results, the effect exerted by *APOE* alleles is specific for the development of AD, whereas ε2 and ε4 alleles likely do not influence the susceptibility to FTLD, VaD or LBD. The presence of the ε2 allele is a protective factor against the development of AD and also delayed age at onset in comparison to ε3/ε3 carriers; conversely, the ε4 allele is associated to an increased risk for AD, but in this case we did not find a modulation of age at onset among ε3/ε4 or ε4/ε4 carriers in comparison to ε3/ε3 patients. However, the ε4 homozygous status is associated with an almost 10-fold risk to develop AD and this value is even higher (about 15-fold) when considering patients with early-

Table 3. Combination of risk ε4 and protective ε2 alleles in patients and controls.

APOE	AD n=735	FTLD	n=75	LBD	n=40	VaD	n=97
Allele							
ε2	41 (2.8)*	10 (6.7)		4 (5.0)		11 (5.6)	
ε3	1060 (72.1)	123 (82)		65 (81.2)		160 (82.6)	
ε4	369 (25.1)**	23 (11.3)		11 (13.8)		23 (11.8)	
Genotype							
ε2/ε2	1 (0.1)	0 (0)		0 (0)		1 (1.0)	
ε2/ε3	29 (3.9)	10 (13.3)		4 (10.0)		8 (8.3)	
ε2/ε4	10 (1.4)	0 (0)		0 (0)		1 (1.0)	
ε3/ε3	382 (52.0)	48 (64.0)		25 (62.5)		68 (70.1)	
ε3/ε4	267 (36.3)	17 (22.7)		11 (27.5)		16 (16.5)	
ε4/ε4	46 (6.3)	0 (0)		0 (0)		3 (3.1)	

*P≤0.001; OR: 0.34, CI: 0.22-0.54 ε2+/ε4- carriers, AD versus controls; P≤0.001; OR: 0.35, CI: 0.20-0.62, ε2+/ε4- carriers, AD versus other dementias; **P≤0.001; OR: 4.33, CI: 3.25-5.77, ε2-/ε4+ carriers, AD versus controls; P≤ 0.001; OR: 2.60, CI: 1.82-3.71, ε2-/ε4+ carriers, AD versus other dementias

onset AD). In accordance with these data, the lowest OR was found in *APOE* ε2+/ε4- carriers.

Whereas both ε2 and ε4 alleles have been repeatedly demonstrated to play an opposite role in the development of AD, to date controversial results on such role for other types of dementia have been obtained. Regarding the ε2 allele, Bernardi et al. [19] demonstrated a protective role of this allele in an Italian population with FTD. However, this study was conducted in a population from a region of Southern Italy (Calabria), which can be considered genetically homogeneous due to its geographic and historical isolation, possibly explaining the discrepancies with our results. A recent meta-analysis [25] demonstrated a risk effect of the ε2 on the susceptibility to develop FTLD, but this effect was mainly observed in patients with familial forms of the disease. Concerning the ε4 allele in FTLD, our results are in agreement with previous findings demonstrating an absence of a susceptibility effect [22, 25, 37].

In accordance with previous data [38], the frequency of *APOE* ε2 allele has been shown to be unchanged in LBD as compared with normal population. Concerning the ε4 allele, its role in LBD is more controversial. Whereas some studies showed a distribution similar to AD [38], others showed an increased frequency of the ε4 allele in patients with coexisting clinical and pathological features of both LBD and AD [37]. However, as the clinical diagnosis of AD+LBD has been criticized [39], in this study we didn't consider the possibility of this diagnostic variant in our patients. This our data are in agreement with previous findings [38], but should be considered cautiously because the sample analyzed is quite small. Therefore, the role of *APOE* in the susceptibility of LBD remains still debated and further studies on a larger population are certainly needed to clarify this issue.

The role of *APOE* ε4 allele in VaD is doubtful as well, with some evidences of an association [27] and others failing to demonstrate a role in the susceptibility to the disease [31]. Interestingly, Engelborghs et al. [24] demonstrated an effect of the ε4 allele on the risk of mixed dementia. The concomitant occurrence of microvascular brain disease in AD is a matter of great debate and represents a yet unsolved dilemma in the pathogenesis of AD [40, 41].

The specificity of the ε4 allele in influencing the susceptibility to AD but not to other types of dementia could be linked to the amyloid-related pathogenetic mechanism at the basis of AD. In fact, compelling *in-vitro* evidence demonstrates that lipidated APOE E4 binds preferentially to Aβ

than APO E2 or E3 isoforms [42]. In addition, several studies confirmed an increased amyloid deposition in APOE E4 carriers [43, 44], thus suggesting a role in AD but not in other types of dementia, in which other proteins are supposed to be responsible for neuronal death [45, 46]. The observation of an association between an increased frequency of the ε4 allele in patients having coexisting clinical and pathological features of AD+LBD, i.e. amyloid plaques and Lewy bodies but not in patients with a pure neuropathological form of LBD [37] strongly supports an amyloid-related mechanism at the basis of the observed association between *APOE* alleles and the development of AD.

In accordance with previous findings, carriers of the ε4/ε4 genotype have the greatest risk to develop AD. Moreover, this genotype seems correlated with an earlier onset of the disease, as also previously suggested [8]. Conversely, the effect of carrying the ε2/ε2 genotype is hard to evaluate given the very low frequency of this genotype in our as well in other populations.

In conclusion, our findings confirm that the *APOE ε2* allele has a protective role towards the development of AD, where it can also delay the age at onset, but has no effect in other forms of dementia. Nevertheless, its role in VaD and LBD remains questionable, because of the small sample analyzed with respect to the very low frequency of this allelic variant. The *APOE ε4* allele is a strong susceptibility factor for AD, particularly in the homozygous ε4/ε4 form, whereas it does not influence the risk to develop other types of dementia. Again, the borderline significance obtained between AD and LBD does not allow to draw reliable conclusions on a possible role of *APOE ε4* allele in LBD.

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