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APOE ε4 AND ALZHEIMER'S DISEASE

Positive association in a Colombian clinical series and review of the Latin-American studies

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ABSTRACT - *Objective:* As the strength of the association between the APOE ε4 allele and Alzheimer's disease (AD) varies across ethnic groups, we studied if there was such an association in Colombian patients. *Method:* We performed apolipoprotein E (APOE) genotyping in a clinical sample of 83 unrelated AD patients, predominantly late-onset (>65 yrs) including familial (n =30) and sporadic AD cases (n= 53) diagnosed according to NINCDS-ADRDA criteria and assessed by a multi-disciplinary team. Control subjects (n = 44) had no significant cognitive impairment by medical interview and neuro-psychological testing. *Results:* We found a high association (OR= 5.1 95%CI 1.9 -13.6) between APOE ε4 and AD, in this series with predominantly late-onset cases with familial aggregation in 24 cases (28.9%). A significant negative association was found between ε2 and AD (OR= 0.2 95% CI 0.05-0.75). *Conclusion:* Further population-based surveys in Colombia are warranted to precise a possible dose effect of APOE ε4.

KEY WORDS: Alzheimer's disease, APOE, dementia, risk factors, ethnic groups, Colombia.

Asociación positiva entre apoe ε4 y demencia de Alzheimer en una serie clinica en Bogotá (Colombia) y revision de los estudios latinoamericanos

RESUMEN - *Objetivo:* Como la fortaleza de la asociación entre el alelo ε4 del gen APOE y la enfermedad de Alzheimer (EA) difiere entre grupos étnicos, quisimos evaluar si esta asociación existe en pacientes colombianos. *Métodos:* Realizamos una genotipificación para el gen de la apolipoproteina E (APOE) en una muestra clínica de 83 pacientes con EA no relacionados, de inicio predominantemente tardío (> 65 años), incluyendo casos familiares (n=30) y esporádicos (n=53) diagnosticados según los criterios del NINCDS-ADRDA y evaluados por un equipo multi-disciplinario. Los sujetos control (n= 44) no presentaban deterioro cognoscitivo de acuerdo con la entrevista médica y la evaluación neuropsicológica. *Resultados:* Hallamos una alta asociación (OR= 5.1; IC95% 1.9- 13.6) entre APOE ε4 y EA en la serie de casos de inicio tardío y con agregación familiar en 24 sujetos (28.9%). Una asociación negativa, estadísticamente significativa, fue encontrada entre ε2 y EA (OR= 0.2; IC95% 0.05-0.75). *Conclusión:* En Colombia son necesarios futuros estudios con base poblacional para poder precisar la existencia o no de un efecto de dosis de APOE ε4.

PALABRAS-CLAVES: enfermedad de Alzheimer, APOE, demencia, factores de riesgo, grupos étnicos, Colombia.

An association between the ε4 allele of the APOE gene and Alzheimer's disease (AD) has been confirmed in many studies worldwide, especially in late - onset disease[1]. However the strength of this association varies, being stronger in the Caucasian and Japanese population. Farrer et al[2] confirmed by meta-analysis a weak association between the ε4 allele and AD in «Hispanics". The ε4ε4 genotype carried significantly higher risk of AD than the ε3ε4 genotype in Caucasian population, but not in the

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so-called "hispanics". Few studies have been so far conducted in Latin America whose population is heterogeneous. Thus results obtained in Argentina[3] and more recently in Chile[4] are not readily applicable to the Colombian population. Furthermore, some authors have suggested that the APOE genotype modulates disease's age of onset, with a gene dose effect[1].

In the present study we assessed the association between APOE ε4 and AD in Colombia and its effect on the age at onset both in familial and sporadic late-onset cases.

METHODS

Patients and clinical asessment

Subjects with 3 to 4 Colombian grand-parents (only 2 subjects had 1 foreign grand-parent) were evaluated between 1995 and 1998 at the Dementia Clinic of the Instituto Neurologico de Colombia and University Hospital (Javeriana University) in Bogotá (Colombia), a national reference center. A total of 83 unrelated patients were diagnosed as probable and possible AD according to NINCDS-ADRDA criteria[5]: 39 patients with probable and 44 with possible AD. There were 36 men and 47 women predominantly (75%) with late-onset AD (onset >65 years). Patients were all assessed using a standard protocol by an experienced multi-disciplinary team including neurology, neuro-psychology, psychiatry, genetics and geriatrics specialists. Written informed consent to participate in the study was obtained from the patient or a first degree family member. Age of onset was defined as the age of the patient when cognitive changes were consistently detected in the family environment. Familial aggregation (familial AD) was considered when at least one first degree relative had a clear history and/or a diagnosis of dementia. Since 1997, genealogies were established systematically even in apparently sporadic cases. 44 cases were sporadic (age at onset: 69.1 ± 10.3 yrs), 24 cases with clear familial aggregation (age at onset: 67.6 ± 10.4 yrs) and 6 uncertain. No data on family history were available in 9 cases. These cases with dubious or undefined family history were older than cases with positive family history. To avoid reducing sample size, these cases with a dubious family history were classified as familial and 9 cases with unknown family history as sporadic. When there was a positive family history only the proband was included in the analyses.

The 44 controls were functionnally independent persons, 15 men and 29 women, attending a Day Care Center for the Healthy Elderly. All subjects underwent a medical interview, a battery of screening tests for dementia (MMSE, Blessed scale, IADL scale and Geriatrics Depression Scale), followed by a neuropsychological assessment to rule out very mild cognitive impairment. Mean age of controls at study entry and age at disease onset of cases were similar (65.8±7.1 versus 68.1 yrs). Controls had slightly better educational and main lifetime activity level than cases (data not shown).

Blood samples were obtained by venous puncture, DNA extraction was carried out either by the salting out method[6] or by phenolic extraction. APOE genotyping was made by PCR-RFLP's analysis according to standard protocols[7]. Restriction fragments of polymorphic length were run on a 6% polyacrylamide gel with silver staining. Quality control on 54 samples was performed by one of us (OT) at the Laboratory of Molecular Genetics, Neurogenetics Group of the University of Antwerp (UIA), obtaining full concordance. Genotyping was performed blindly in regard to clinical information.

Statistical analysis

Means of some demographic variables and clinical characteristics were compared by the t-test. Allele frequencies were obtained by allele counting and proportions were compared. Both cross tabulation analysis for association assessment (by chi2 or Fisher test when appropriate) and logistic regression models for association defining 2 categories "at least one ε4 allele" (genotypes ε3ε4, ε4ε4, ε2ε4) and "without ε4" (ε2ε3, ε3ε3, ε2ε2),controlling for sex, age, education,family history and using the apoE ε3ε3 genotype as reference cells.

RESULTS

The characteristics of the case-control study population are summarized in Table 1. This series included more females than males both among cases and controls and a similar number of probable versus possible AD cases. Controls were slightly younger (4 yrs) than cases, taking into account age at study entry but there was no difference between age at onset of cases and controls' age. The MMSE score range was wide among cases, as patients with mild through severe dementia were included, while controls scored around 29 with a narrow standard deviation.

Allele frequencies, genotypes frequencies and corresponding odd's ratios (OR) in the total group and stratified by diagnosis and family history, are shown in Table 2. The ε3ε3 genotype was most frequently found in all diagnostic categories. Allele frequencies for ε2/ε3/ε4 in cases versus controls were respectively 1.9%/74.7%/23.4% and 9.1%/82.9%/8.0%.The ε2 allele was significantly more frequent among controls than cases, at expenses of the ε2ε3 genotype. No subject bore the rare ε2ε2 genotype and only 8 subjects (7 cases and 1control) had the ε4ε4 genotype. There was a significant association between bearing at least 1 ε4 and having a diagnosis of AD (possible or probable) (p=0.001) (OR = 5.1; 95%CI 1.9 -13.6). There was a significant difference between relative risks in probable versus possible AD and the odd's ratio was slightly higher for familial AD than for sporadic disease, with a tendency for

Table 1. Sample characteristics.

	AD Total	Familial AD	Dubious FH	Undefined FH	Sporadic	Controls
Number of subjects	83	24	6	9	44	44
Probable AD	39	17	3	2	17	-
Possible AD	44	7	3	7	27	-
Males/females	36/47	13/11	3/3	3/6	17/27	15/29
Age at study entry	73.3	69.5	79	81	69.8	65.8
(yrs ± SD)	±9.5	±12.4	± 6	± 7.9	± 9.6	± 7.2
Age at onset of disease	68.5	66.1*	74.6	-	65.8	-
	±10.0	±11.5	± 9.1		±7.1	
MMSE	18.7	19.4	-	-	17.3	29.3
	±8.1	±8.3			±8.3	±0.8

*taking off 1 subject with age at onset 35 (and a known PSEN-1 mutation), the age at onset in FAD cases becomes 68.1 ± 8.5 yrs; AD, alzheimer's disease; FH, family history.

Table 2. APOE allele, genotype frequencies and OR in the global sample and stratified by diagnosis and family history.

	Cases (n=83)					Controls (n=44)
Genotypes	AD (n=83)	Probable AD (n= 39)	Possible AD (n=44)	Familial AD (n=30)	Sporadic (n=53)	
"Without ε4"						
ε3ε3	48 (57.8)	20 (51.3)	28 (63.6)	15 (62.5)	33(62.3)	33 (75)
ε2ε3	33.6)	0	3 (6.8)	2 (6.7)	1 (1.9)	6 (13.6)
ε2ε2	0	0	0	0	0	0
"With ε4"						
ε2ε4	0	0	0	0	0	1(2.3)
ε3ε4	25 (30.1)	14 (35.9)	11 (25)	9 (30.0)	16 (30.2)	3 (6.8)
ε4ε4	7 (8.5)	5 (12.8)	2 (4.6)	4 (13.3)	3 (5.6)	1 (2.3)
Allele frequencies:	(2n=166)	(2n=78)	(2n=88)	(2n=60)	(2n=106)	(2n=88)
ε2	3 (1.9)	0	3 (3.4)	2(3.3)	1 (0.9)	7 (8.0)
ε3	124 (74.7)	54 (69.2)	70 (79.5)	41 (68.3)	83 (78.3)	75 (85.2)
ε4	39 (23.4)	24 (30.8)	15 (17.0)	17 (28.4)	22 (20.8)	6 (6.8)
OR (95% CI)						
"With at least 1ε4"	5.1 (1.9 -13.6)	7.4 (2.5 -22.0)	3.3 (1.1-9.8)	6.0 (1.9 -18.7)	4.6 (1.6 -13.1)	-
"With ε2"	0.2 (0.1- 0.8)					

Statistics: Cases versus controls (proportion comparison) : p= 0.0016. Probable versus possible AD: p= 0.016. OR, Odd's ratio; AD, Alzheimer's disease.

the ε4ε4 genotype to be more frequent among familial AD cases. The ε4 allele frequency was slightly higher in the cases with positive familial AD history (37.5%) than in the familial AD group including dubious and undefinite family history cases (30%) but the difference did not reach statistical significance (p=0.7), thus allowing the re-classification used here.

There was no difference between onset age among patients with or without the ε4 allele (68.2 yrs ±10.7 versus 68.3 yrs ±10) nor among familial cases with or without ε4. Stratifying the sample by sex, we found no association between "at least one ε4 allele" and AD in men (p= 0.1); in contrast a very significant association between ε4 and AD diagnosis

was found in women (p= 0.004): women controls without ε4 tended to be more frequent (48% versus 33%) (n.s) and the ε4 allele frequency among female cases was non significantly higher than in males. However these differences between sexes were not confirmed by logistic regression analysis controlling for sex and age . Family history was not included in the regression model, not leading to a significant effect when we controlled for other variables (OR: .925 95% CI .79-1.08). There was a high association with age (in the category "75 yrs and over") (OR= 9.3; 95%CI 3.0 - 28)) and bearing at least an ε4 allele (OR= 4.4 95%CI 1.8 -10.9).

DISCUSSION

This is the first study on APOE genotyping in unrelated patients in Colombia. The cases were obtained from the wide catchment area of the University Hospital in Bogotá, where the study was conducted and includes patients originating from other regions of Colombia. The control group shares a similar profile and the majority of participants belong to middle class families, having had between 3 and 7 yrs of formal education. As found in many studies, demented aubjects in this study tended to be less educated.

Allele frequencies in our controls were very similar to those reported in previous studies[8-13] among "Hispanics" in the USA and in Farrer's meta-analysis[2]. Also, the observed association of ε4 with AD occurs at expenses of the ε3ε4 subgroup rather than the ε4ε4, the latter being very infrequently found in this series, both in cases and in controls; these results are also concordant with previous report[2]. The so called "hispanic group" is ethnically heterogeneous as highlighted by Mendez[14] and populations from Central America and the Andean region have a percentage of Indian ancestry while populations from Cuba and Puerto Rico share a known African admixture with no Indian ancestry. Although there was an important Spanish immigration to Colombia in former centuries, the Colombian population today is relatively stable. APOE ε4 allele frecuencies are lower in European mediterranean countries (Italy, Spain and Greece) than in northern European studies[15,16].

Bogotá, especially in the last decades, has become home to people from the whole country but the main population from the plateau of Bogota and Cundinamarca, was a mixture of European and Native Americans (Chibcha). We did not perform any anthropological studies nor screened for biological markers. The Chilean study refers to a "socio-ethnogenetic stratification" in their country (Valenzuela cited by Quiroga)[4]. This concept is not readily attributable to Colombia where ethnic flows have been steadily dynamic since the Colonial period. No subject among cases and controls belonged to an ethnic minority.

These data confirm a clear association between ε4 and AD; we found that the relative risk was higher than previously reported in available Latin American studies (Table 3). High associations have been reported in studies including familial cases[17-21] and late-onset disease[1,22]. Most pedigrees identified as late-onset AD families show high frequencies of the ε4 allele for affected and non affected members and the ocurrence of multiple cases of AD likely involves the sharing of high-risk APOE alleles and of other risk factors[23,24]. Our sample included 36% of familial cases, predominantly late-onset and a high proportion of late-onset sporadic cases, but although ε4 allele frequency tended to be higher among familial cases, this difference was not significant and age at onset was homogeneous between familial and sporadic cases. Also, some authors have emphasized that research samples are biased and usually enriched in ε4 bearers, but when controlling for earlier age at onset in a research sample, results were similar to those obtained in the community sample[25]. The positive predictive value (PPV) of "at least an ε4 allele" was high (86.5%) and thus might be useful in adjunction to clinical diagnosis, although our team does not use APOE genotyping as a diagnostic tool. The observed negative predictive value (NPV) was low (43.3%), as the majority of AD cases did not bear the at-risk allele, thus APOE genotyping cannot be used to discard a diagnosis of AD. Although these results are based on a clinical diagnosis of AD, they are concordant with those obtained in a series of 67 necropsy-confirmed sporadic AD patients (PPV: 100%; NPV: 42%)[26].

Although various population genetic surveys based on polymorphisms of different apolipoproteins (including APOE) and their effect on lipid profile are available in Native American populations in Mexico and Ecuador, few studies on APOE status in AD have been conducted in Latin America. A study in the large genealogies from Antioquia (Colombia), which probably correspond to a founder effect, confirmed the absence of modulation of the age of onset by APOE genotype in presenilin 1 (PSEN -1) mutation bearers[27]. Also, in 2 Cuban families, there was some relationship between the APOE genotype and age at onset

Table 3. Studies on APOE and AD in Latin American population.

Country	Population	Familial AD/ Sporadic	Cases/ Controls	Age (yrs) Cases	Allele frequencies in Cases/Controls (%)			OR (95%CI)	References
					ε2	ε3	ε4		
Argentina	Clinical	Sporadic	45 /45	74.7±5.5	-	-	-	3.3 (1.2-9)	[3]
Brazil	Clinical	-	55/56	68.3 (65.9-71)	6.4 6.3	72.7 84.8	20.9 8.9		[29]
Brazil	Clinical	-	57/74	70(55-92)	-	-	29.8 11.5	-	[30]
Brazil	Clinical and population	Sporadic	23/100	72.3±3.3 72.4 (61-84)	7.0 7.5	54.0 81.0	39.0 11.5	-	[31]
Chile	Population	Sporadic?	95/187	80.7 (79.2-82.2)	8.4 7.2	51.6 73.5	40.0 19.3	ε3ε4:2.5(CI:-) ε4ε4:12.8 (3.9-47.6)	[4]
"Hispanics" USA	Population	-	61/90	76±9.4	6 3	70 84	12 24	ε3ε4 2.6 (1.6-6.4)	[9]
"Hispanics" USA	Clinical	Familial and sporadic	46	72±8	3	69	28	-	[22]
Hispanics (Cuban)	Clinical	-	180/64	762± 8	-	-	0.26 0.12	ε3ε4: 3.9 (2.0-8.3)	[12]
"Hispanics"	Mixed: meta-analysis	Sporadic	261/267	-	6.3 6.7	74.5 82.3	19.2 11	ε3ε4 2.2(1.3-3.4) ε4ε4 2.2(0.7-6.7)	[2]
"Hispanics" (Caribbean)	Population longitudinal	-	145/516	75.3±5.8	8.3 8.8	76.9 77.1	14.8 14.1	1.1 (0.7-1.6)=n.s.	[13]
"Hispanics" (Cuban)	Clinical	-	188/84	76.0±8.1	0.02 0.04	0.72 0.82	0.26 0.14	3.5 (2.3-5.5)	[32]
Colombia	Clinical	Familial AD + Sporadic	83/44	68.1±8.5	1.9 9.1	74.7 82.9	23.4 8.0	5.1 (1.9 –13.6)	Current study

of disease[28]. In Argentina, in a study based on a small AD sample (45 patients), predominantly late onset AD with only 2 FAD cases, a low OR was reported[3]. In Brazil, a positive association between ε4 and AD was confirmed in a sample including 55 patients with probable or possible AD, without differences in the strength of this association between early-onset and late-onset cases, nor between men and women[29]. This sample included a high percentage of relatively early-onset dementias. Another brazilian study, including 57 AD cases obtained similar results[30].

Also a recent Brazilian survey[31] carried - out on 3 independant samples including 100 young Caucasian subjects assessed for paternity testing, 23 sporadic AD cases detected at a neurology clinic and a group of young Black individuals, showed an over-representation of the APOE ε4 allele among the AD subjects (39%) versus Caucasian controls (11.5%) and higher plasma triglyceride levels in ε4 carriers, especially in women.

Recently in Chile in a population - based sample part of the WHO Age-Associated Dementia Project, were studied 95 patients with AD and 187 controls randomly selected from the Chilean urban sample of 2,449 individuals[4]. APOE phenotyping was carried out by isoelectric focussing. The frequency of the ε4 allele was quite high both in cases and controls (0.40 and 0.19 respectively); in this study a dose effect of ε4 on the RR was shown: OR of 2.5 for the carriers of one ε4 allele and OR = 12.8 in the ε4ε4 carriers compared to ε3ε3, with a highly significant 95%CI (3.9 - 47.6). The mean age at onset was quite late in this study (80.7 yrs); also the family history was not controlled for and might account for the high asso-

ciation reported, similar to European and North American studies.

The study by Harwood et al.[32] included a clinical series of white non Hispanic subjects (392 AD patients, 202 control subjects) and white Hispanic (188 AD patients and 84 control subjects) mainly of Cuban origin and assessed the risk of AD associated with the APOE genotype, along with various environmental factors. This study confirmed a strong association with ε4 in both ethnic groups and a negative association with ε2 only in the white Hispanic group. Among environmental factors, a strong influence of education and hypertension was observed, but neither smoking nor head trauma with loss of consciousness were shown to be risk factors in this study. Family history of dementia was not taken into account.

The lower ε4 allele frequency in the possible AD versus the probable AD group in our sample, as previously shown[22], probably reflects the heterogeneity of this category including both typical AD cases presenting with an additional risk factor and another subgroup of patients with a more dubious profile, who might not have AD. Pathological confirmation should further characterize this sub-group.

A protective effect of ε2 is still controversial and some authors report a significant decrease in ε2 frequency in EOAD[33] and in LOAD[21,22] while other studies do not[32,34]. The ε2ε4 genotype is now known to behave as an "at risk" genotype[2] and was thus computed with the group "at least one ε4 allele". Because of our limited sample size no subject wore the rare ε2ε2 genotype. The ε2 allele frequency of 8.0% in controls reflects significant admixture in our population, e2 frequency is very low in Native Americans as shown in rural areas of Mexico[35], in the Cayapa indians in Ecuador[36] and also in Colombia[37].

Although there were slightly more women than men in our sample, both among cases and controls, this difference cannot account for the very significant association between AD and ε4 found in women and not in men in a cross-table analysis. However, the logistic regression analyses controlling for sex, age and family history, did not show such a sex effect and suggests it might be due to lack of homogeneity between diagnostic groups in our sample.The mentioned Brazilian study[29] did not report differences by sex. Earlier studies suggested that ε4 might be more frequent in women (after controlling for increased longevity), thus it could possibly be a component of the increased risk for women, but ε4 frequency was not different between men and women[2]. Thus women might be more susceptible to develop AD in the presence of ε4 and this susceptibility might be even greater for women in the FAD group[11] although conclusions by Combarros et al. were divergent[38]. Hormonal or other genetic factors might be related to this increased susceptibility.

Age at onset - We found no effect of APOE status on age at onset among familial or sporadic cases. Modulation of AD onset by APOE genotype is controversial and may occur in specific families, such as those bearing an APP717 mutation. In this series we found no APP mutations and the few detected PSEN mutations (unpublished data) are not influenced by APOE status. In our series, APOE ε4 was present in approximately 30% of sporadic AD cases and close to 40% of all familial AD cases and might contribute to familial aggregation in these families without mutations in known causative genes, although APOE ε4 may represent a stronger influence on familial aggregation of AD among whites than Hispanics[24]. These results confirm APOE as a susceptibility gene in this sample of Colombian out-patient population.

Dose effect - Because of our limited sample size we used a global category "at least one ε4 allele" for logistic regression analysis. The OR was higher for ε4ε4 subjects, with broad non significant confidence interval.

The ε4 association was positive in both the sporadic and familial groups but we did not observe an association between age at onset and APOE genotype. We could not definitely assess the existence of a dose effect on the relative risk. So, prevalence and incidence studies on a population basis are needed in Colombia, to further assess an increased susceptibility to APOE related risk in women, the negative association with ε2 found in this study and and the intervention of other genetic and non genetic susceptibility or protective factors.

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