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Familial Alzheimer Disease in Latinos: Interaction Between APOE, Stroke and Estrogen Replacement

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Abstract

Background—Factors that modify risk related to APOE variants have been examined primarily in unrelated patients and controls, but seldom in family-based studies. Stroke, vascular risk factors, estrogen replacement therapy (ERT), head injury (HI) and smoking have been reported to influence risk of sporadic but not familial AD.

Objectives—To examine the potential relationship between these risk factors and APOE, we used a family study design in a population in which the APOE-ε4 variant is strongly associated with risk of AD.

Methods—Latino families primarily from the Caribbean Islands in which two or more living relatives had dementia were identified in the New York City metropolitan area, the Dominican Republic and Puerto Rico. 1,498 participants from 350 families underwent a clinical interview, medical and neurological examinations, neuropsychological testing and APOE genotyping. Diagnosis was made by consensus using research criteria for AD.

Results—APOE-ε4 was associated with a nearly two-fold increased risk of AD. A history of stroke was also associated with a four-fold increased risk. A statistical interaction between APOE-ε4 and stroke was observed. Women with an APOE-ε4 who took ERT did not have an increased risk of AD, but in women with a history of stroke ERT was a deleterious effect modifier.

Conclusions—APOE-ε4 and stroke independently increase risk of familial AD among Latinos, and may interact to further increase AD risk. Among women, the risk of AD associated with APOE-ε4 may be attenuated by a history of ERT.

Keywords

Alzheimer disease; estrogen; stroke; APOE

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Introduction

Apolipoprotein E (APOE) represents the most robust genetic risk factor for late-onset Alzheimer disease (AD) (1–3). Stroke, vascular risk factors, estrogen replacement therapy (ERT), head injury (HI) and smoking are risk factors suspected of influencing the risk of AD (4–14). Little is known about the potential interaction between these risk factors and APOE in AD. To investigate the relationship between APOE and these risk factors we studied Latino families from the Caribbean, previous known to have strong APOE-ε4 association (15).

Family-based studies facilitate the investigation of the effects of putative modifiers on genetic variants and disease risk because they are less susceptible to population stratification than case-control studies. They have been relatively under-utilized in the neurology literature, especially with regard to the study of non-genetic risk factors because of the difficulty in identifying large groups of families with a specific disease and ascertaining the appropriate information(16–18). For this report, we used a family-based study to simultaneously examine the modifying effects of several risk factors on the APOE associated risk of AD.

Methods

Setting and subjects

Details of the source population and recruitment, ascertainment of probands and their family members, and diagnostic methods have been reported in detail previously (15). 1,498 participants from 350 families were recruited from The Alzheimer Disease Research Center/ Memory Disorders Center, physicians' private offices, general medical services at Columbia University Medical Center, an ongoing population-based community study of dementia in the Washington Heights-Inwood community of northern Manhattan, New York City, and from clinics in the Dominican Republic and Puerto Rico.

Data collection

Patients with AD and both affected and unaffected family members underwent standardized clinical interviews and medical, neurological and neuropsychological examinations. Information regarding risk factors was obtained directly from the individual if their cognitive function was intact (as determined by the interviewer) using a structured questionnaire (19); for patients with more severe cognitive dysfunction, the information was obtained from an informant, usually a relative or caregiver. The risk factor questionnaire has been previously shown to be highly reliable (19). History of stroke, hypertension (HTN), diabetes mellitus (DM), and myocardial infarction (MI) were defined as ever being diagnosed or treated for these conditions. ERT was defined as ever having taken female hormone pills after menopause, head injury (HI) was defined as ever having had a injury that resulted in loss of consciousness or amnesia, and smoking was defined as having ever smoked at least one cigarette per day for at least one year. We corroborated information obtained on clinical interview with available family members or the participant's medical record.

Diagnostic evaluations

Patients diagnosed with AD were required to meet National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINDCS-ADRDA) research criteria for probable or possible AD (20). Brain imaging was available and reviewed 201 affected individuals. We also offered imaging as medically necessary to support the NINDCS-ADRDA criteria. Most patients had rountine laboratory studies. Patients in the Dominican Republic and Puerto Rico were offered the same diagnostic examinations. Neuropsychological examinations were given in Spanish (21–23) to all participating family members and probands regardless of affection status. All available

clinical data were reviewed at a consensus conference of neurologists and neuropsychologists, the methods and development of which have been previously outlined in detail (21,22).

Blood samples were collected from all patients with AD, their living siblings and other family members. A modification (24) of the methods described by Hixson and Vernier (25) was used to determine APOE genotype.

Data analysis

Statistical analyses for categorical variables consisted of chi-square and Fisher's exact tests for comparisons between affected and unaffected persons. Student's t-test was used for comparisons involving continuous variables. A Bonferroni correction was applied where appropriate to correct for multiple testing. Logistic regression was used for preliminary multivariate analyses using statistical software (SPSS version 11.0, SPSS Inc., Chicago, IL). Multivariate analyses were also completed using generalized estimating equations (GEE) in which each family was treated as a cluster, and odds ratios were derived from these analyses. Models were completed using the GENMOD procedure in SAS (SAS Institute, Cary, NC) (26). To investigate the influence of environmental risk factors on AD GEE logistic regression analyses for each risk factor (stroke, HTN, DM, MI, ERT, HI and smoking) were performed with AD as the outcome variable. When we conducted analyses for stroke, HTN, DM, MI, HI and smoking we included APOE-ε4 status, age, gender and years of education as covariates. Analyses for ERT were limited to women, and we adjusted for ε4 status, age and years of education. Each model was inspected to determine the effects of the non-genetic factors adjusting for APOE-ε4. We also calculated the percent change in beta estimates for APOE-ε4 in each model to determine whether or not the non-genetic factor modified the point estimate for APOE-ε4. *A priori* we considered a 10% change in beta estimate from the estimate for APOE-ε4 in the reduced model as the criterion for effect modification. For stroke and ERT, interaction terms were formed by multiplying the individual variables (stroke and APOE-ε4 in one analysis, ERT and APOE-ε4 in a second). In GEE logistic regression models we included the covariates age, education, sex and APOE-ε4. In a second set of analyses dummy variables were created according to ERT and APOE-ε4 and included in the GEE logistic regression model with age, education and ERT use as covariates. Patients lacking both a history of ERT and APOE-ε4 were used as the reference group. A similar analysis was used to investigate the potential interaction between stroke and ε4 status. Finally, a GEE logistic regression analysis was performed that included stroke, ERT and APOE-ε4 with age and education as covariates.

Results

Demographics and clinical data

There were 385 families with an average of four participating family members per family. 39 families (7.5%) had four or more affected individuals. 778 of the 1,498 participants (51.9%) were diagnosed with AD (table 1). Of 85 patients who had a history of stroke, 67 (78.8%) were diagnosed with AD. Among 96 women who used ERT, 19 (19.8%) had AD. Family characteristics and demographic, risk factor and APOE genotype data are presented in Table 1. Patients with AD were older, had fewer years of education, were more likely to have a history of stroke and were less likely to have a history of ERT than unaffected family members. History of HTN, DM, MI, HI and smoking did not significantly differ between groups with and without AD. Patients with AD were more likely to have one or more APOE-ε4 alleles than their unaffected family members.

Risk of AD associated with APOE-ε4

Presence of one or more ε4 alleles was associated with increased risk of AD in unadjusted analysis (OR 1.5, 95% CI 1.2–1.9). Adjusting for age, gender and education increased the point

estimate for the association (overall OR=1.8, 1.4–2.4; homozygosity OR=2.3, 1.5–3.5; heterozygosity OR=1.7, 1.3–2.3).

Individual risk factors, APOE and AD risk

The effects of non-genetic risk factors and APOE on risk of AD are summarized in Table 2. Stroke was associated with a three-fold increase in risk of AD (crude: OR= 3.9; 2.3–6.9, adjusted: OR=3.3; 1.8–6.1), while ERT was associated with a decreased risk of AD (crude: OR=0.2; 0.1–0.3, adjusted: OR=0.3; 0.2–0.6). Among cardiovascular risk factors, DM, MI, HI, HTN and smoking were not associated with AD. Although a history of HTN was not associated with AD in crude analysis, in adjusted analysis an apparent decreased risk was observed (crude: OR=1.2; 1.0–1.5, adjusted: OR=0.7; 0.6–0.9). As those with HTN were older ($p<.001$) and less educated ($p<.001$) than those without HTN among the non-diseased, and both older age and lower education were associated with AD (shown above), this effect appeared to be the result of joint (positive) confounding by age and education on an underlying negative HTN – AD association. In regression analyses, adjustment for HTN, DM, MI, HI and smoking did not modify the ε4-associated risk of AD.

Stroke, APOE and AD risk

Of forty-four patients with both a history of stroke and at least one APOE-ε4 allele, 39 (88.6%) were diagnosed with AD, compared to 28 of 41 patients with stroke and no APOE-ε4 allele (68.3%). Although regression analysis failed to show multiplicative interaction the effects did appear to be strongly additive. As shown in Table 3, compared to those with no APOE-ε4 allele and no history of stroke, those with one or more APOE-ε4 alleles and a history of stroke had an eleven-fold increased risk of AD.

Estrogen use, APOE and AD risk

Adjusting for ERT use resulted in greater ε4-associated AD risk (change in beta estimate = 19.4%; OR 2.1, 95% CI 1.4–3.0), suggesting that ERT may act as a protective effect modifier. ERT was not associated with ε4 status among the unaffected family members ($p=.555$), and thus did not meet criteria for a potential confounder of the ε4–AD association. Compared to those with no APOE-ε4 allele and no history of ERT, women without APOE-ε4 who took ERT had an 80% AD risk reduction (OR= 0.2; 0.1–0.5). Remarkably, women with APOE-ε4 but no history of ERT had a two-fold increased risk of AD (OR=1.9; 1.3–2.9), while those with both APOE-ε4 and a history of ERT had no increased risk (OR=0.8; 0.3–1.8, Table 4).

Stroke, estrogen use, APOE and AD risk

Among women, inclusion of ERT in a regression model including stroke, APOE-ε4, age, and education revealed evidence of mild positive effect modification of ERT on the stroke-associated AD risk (change in beta estimate = −13.7%). Controlling for ERT, the stroke-associated risk of AD remained elevated (OR=2.8; 1.4–5.7), less than in the model without ERT above. ERT remained protective in this analysis (OR=0.3; 0.2–0.6), and was not modified by the presence of stroke in the model (change in beta estimate < 1%). Overall, APOE-ε4 (OR=2.0; 1.3–3.0), stroke, and ERT appeared to be independent risk factors among women for AD, with partial positive effect modification of ERT on stroke-associated AD risk.

Discussion

APOE is strongly associated with late-onset familial AD in Latinos from the Caribbean (15). The focus of this study was to investigate the modifying effects of several risk factors on APOE-ε4 associated on risk of AD. We found that the risk of AD associated with APOE-ε4 and stroke was strongly additive even after restricting the analysis to patients with Probable AD. A history

of ERT was associated with a protective effect in the absence of an APOE-ε4 allele, and appeared to attenuate the APOE-ε4 -associated risk among women.

Systemic vascular disease has been reported to be associated with an increased rate of cognitive decline (27,28) and increased risk of AD (29) in the setting of APOE-ε4. One longitudinal study has shown an increased rate of cognitive decline associated with DM among persons with APOE-ε4 (27). While no association was observed between DM, MI and APOE-ε4 here, stroke was associated with a three-fold increase in AD risk. The relationships between vascular risk factors, stroke, APOE and AD are at present unclear. In one longitudinal study (13), a history of stroke was associated with an increased risk of AD, and the stroke-associated risk was strengthened by the presence of HTN, DM and heart disease. Although APOE genotype was not considered, data from the Cache County Study (30) support the findings of increased stroke-associated risk of AD in the presence of vascular risk factors. The presence of APOE-ε4 has been shown to be a risk factor for dementia with stroke in two population-based case-control studies (31,32).

Our results suggest that a history of stroke in the presence of APOE-ε4 increases risk of AD substantially. One potential explanation is that stroke in persons with APOE-ε4 may promote the pathologic process of AD. Alternatively, the presence of APOE-ε4 may modify the brain's response to vascular injury (33,34), increasing the degree of cognitive dysfunction resultant from a given stroke in a patient with incipient AD. Although little is known about the possible role of APOE in the molecular events following stroke in the setting of AD, the relationship between vascular injury, APOE and AD on a molecular level is deserving of further study.

ERT may have a protective effect on the risk of AD associated with the APOE-ε4. There are conflicting results in the literature with regard to ERT effects on AD risk. Estrogen use in postmenopausal women has been associated with AD risk reductions of about 50% in several case-control (35–38) and cohort (5–7) studies. However, in the Women's Health Initiative Memory Study (39–42) both unopposed conjugated equine estrogens alone or in with progesterone were not protective, and were accompanied by an increased risk of dementia and declining cognitive test performance. Additionally, randomized controlled trials of conjugated equine estrogens in women already diagnosed with AD (43–45) have failed to show benefit in terms of cognitive performance, global assessment of change, activities of daily living, mood or regional brain perfusion. Notably, the majority of these studies (6,35–43,45) have not controlled for APOE genotype. In the present study, of the 65 women who were APOE-ε4 homozygous, only 7 had taken ERT. Although we cannot address the effects of ERT on AD risk associated with ε4 homozygosity, our results do suggest that a history of ERT may attenuate the ε4-associated risk of late-onset familial AD among women in this population.

One possible alternative explanation for an apparent protective effect of ERT on the development of AD is that hormone users tend to be healthier and lead more healthy lifestyles (46), a limitation of several previous observational studies (47). Although subjects in our sample who took ERT were younger ($p<.001$) and better educated ($p<.001$), there was no difference from those who did not take ERT with regard to history of several systemic diseases, stroke, seizure, alcohol or tobacco use, or APOE status (Table 5). We adjusted for age and education in our multivariate analyses involving ERT.

We did not demonstrate an association between HI and APOE-ε4 in this study, consistent with other investigations (48–50). Smoking has been reported to increase risk of AD in patients lacking an ε4 allele in two population-based cohort studies (11,12), but this association was not observed in the current study. Although the sample size in our study was large enough, a limitation of family studies is "over matching" due to similar environmental exposures between affected and unaffected relatives (16,17).

Methodological limitations of many of the previously published observational studies of dementia include use of proxy respondents to ascertain history of risk factor exposure and lack of rigor in classification of patients based on dementia status. Although our study is susceptible to information bias, our data was obtained in a structured interview with each individual participant, using another informant as the primary source only if the participant was unable to provide accurate information. We attempted to corroborate information obtained on clinical interview with available family members and medical record data, and used standardized risk factor and family history questionnaires previously shown to be reliable and valid in this setting (19,23). Each participant underwent a standardized neurological and neuropsychological evaluation leading to a consensus diagnosis based on research diagnostic criteria.

The concept of gene-environment interaction is controversial primarily because it is unclear whether the term implies a true biological interaction or a statistical one (51). The results of the current study imply that a history of stroke substantially increases the risk of AD among individuals with an APOE-ε4 allele, while ERT use could lessen the APOE-ε4 associated risk of AD. We have not established biological explanations for either interaction or modification, but instead have observed effects on the strength of the associations based on statistical analysis. Clearly if these analyses are replicated by others, biological studies should follow.

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Table 1

Demographics, risk factors and APOE genotype of study participants

Individual characteristics	**No dementia (n=720)**	**AD (n=778)**	**Significance***
Age (mean) **	62.4 yr	73.6yr	**p <.001**
Gender (%women)	64.9	69.3	p =.069
Years education (mean)	9.4 yr	6.0 yr	**p <.001**
Stroke	2.7%	9.8%	**p <.001**
Hypertension	45.5%	49.7%	p = .115
Diabetes mellitus	14.5%	16.4%	p = .315
Myocardial infarction	3.3%	5.1%	p = .098
Head injury	10.2%	10.4%	p =.880
Smoking	37.9%	36.8%	p = .673
APOE-ε4***	43.9%	54.0%	**p <.001**

AD = probable and possible Alzheimer disease; percentages reflect those positive for each risk factor;

* = Corrected for multiple comparisons using Bonferroni method (for α = .05, corrected significance level = .05/11 = .005). P-values in bold denote significant differences at the p<.005 significance level;

** = age at follow-up;

*** = homozygous or heterozygous for ε4 allele

Table 2

Summary of the effect of non-genetic factors and APOE on risk of AD

Risk factor		OR	(95% CI)	Significance
Stroke*	C	3.9	2.3–6.9	**p < .0001**
	A	3.3	1.8–6.1	**p < .001**
Hypertension*	C	1.2	0.9–1.5	p = .110
	A	0.7	0.6–0.9	**p = .015**
Diabetes mellitus*	C	1.2	0.9–1.6	p = .323
	A	1.0	0.7–1.4	p = .852
Myocardial infarction*	C	1.7	0.9–3.1	p =.060
	A	1.1	0.6–2.2	p = .770
Estrogen use**	C	0.2	0.1–0.3	**p < .0001**
	A	0.3	0.2–0.6	**p < .001**
Head injury*	C	1.0	0.7–1.5	p = .870
	A	1.0	0.7–1.6	p = .936
Smoking*	C	0.9	0.7–1.2	p = .740
	A	1.0	0.7–1.3	p = .776
APOE-ε4 (one or two)	C	1.5	1.2–1.9	**p < .001**
APOE-ε4 (one or two)***	A	1.8	1.4–2.4	**p < .001**
APOE-ε4 homozygous***	A	2.3	1.5–3.5	**p < .001**
APOE-ε4 heterozygous***	A	1.7	1.3–2.3	**p < .001**

OR = odds ratio; CI = confidence interval; C= crude, A=adjusted;

* = adjusted for ε4 status, age, gender, and education;

** = adjusted for ε4 status, age, and education (all subjects female);

*** = adjusted for age, gender and education.

Table 3

Stroke, APOE genotype and risk of AD among study participants

ε4	Stroke	Number at Risk	AD	OR*	95%CI	Significance
−	−	643	285 (44.3%)	1.0	--	Reference
−	+	41	28 (68.3%)	2.0	1.0–4.1	p = .066
+	−	624	334 (53.5%)	1.7	1.3–2.2	**p < .001**
+	+	44	39 (88.6%)	11.3	3.6–35.2	**p < .001**

* = adjusted for age, gender, and education

Table 4

Table 4a. Estrogen use among women study participants

	ERT USED	No ERT
Age	63.97 (9.98) years	74.4 (13.4) years
Education	11.6 (4.6) years	7.1 (8.4) years
AD	19 (5%)	361 (95%)
Unaffected	77 (21%)	289 (79%)

Table 4b. Estrogen use, APOE genotype and risk of AD among women study participants

ε4	ERT	Number at Risk	AD	OR*	95%CI	Significance
−	−	348	178 (51.1%)	1.0	--	Reference
−	+	53	5 (9.4%)	0.2	0.1–0.5	**p = 0.002**
+	−	305	185 (60.7%)	1.9	1.3–2.9	**p < 0.001**
+	+	43	14 (32.6%)	0.8	0.3–1.8	p = 0.533

Differences are statistically significant, p<0.001

* = adjusted for age and education

Table 5

Comparison of participants with and without history of ERT

	No ERT (n=653)	ERT (n=96)	Significance*
Head injury	7.2%	6.3%	p=.760
Smoking	29.1%	26.0%	p=.531
Alcohol use	1.5%	3.1%	p=.229
Hypertension	51.2%	47.4%	p=.489
Diabetes	17.1%	10.4%	p=.096
Myocardial infarction	4.7%	2.1%	p=.414
Pulmonary disease	6.1%	7.4%	p=.624
Thyroid disease	5.6%	9.6%	p=.135
Liver disease	5.9%	8.4%	p=.345
Renal insufficiency	1.1%	0%	p=.604
Stroke	7.1%	2.2%	p=.076
Seizure	1.9%	1.1%	p=1.000
Cancer	3.4%	3.9%	p=1.000
Arthritis	31.0%	32.6%	p=.753
APOE ε4**	46.7%	44.8%	p=.725

ERT = postmenopausal estrogen replacement therapy; percentages reflect those positive for each risk factor;

* = Corrected for multiple comparisons using Bonferroni method (for α = .05, corrected significance level = .05/17 = .003). P-values in bold denote significant differences at the p<.003 significance level;

** = homozygous or heterozygous for ε4 allele