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ScienceDirect
Neuroscience Letters 411 (2007) 185–188
Neuroscience Letters
www.elsevier.com/locate/neulet

Association of APOE with Parkinson disease age-at-onset in women

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Received 7 February 2006; received in revised form 15 May 2006; accepted 3 July 2006

Abstract

APOE polymorphism has received extensive attention as a risk factor for Parkinson's disease (PD), but findings have been equivocal. Analysis of *APOE* variants in an Australian PD case–control sample revealed a robust association between genotype and age-at-onset (AAO) of PD in women ($P = 0.0008$). These data not only further implicate *APOE* in PD, but also provide a stark example of the effects that gender may play in complex disorders.

Keywords: Parkinson's disease; APOE; Age-at-onset; Genetic association studies

Genetic association of *APOE* with AD is one of the few widely recognized examples of common polymorphism contributing to a common illness [7]. The possibility of a genetic mechanism underlying neurodegeneration in AD has led to the investigation of *APOE* in numerous diseases, among which Parkinson's disease has been one of the most studied. In contrast to AD however, data on *APOE* in PD have been controversial suggesting that a genetic effect, if present, is much weaker than for AD. Previous studies have suggested that α-synuclein accumulation can be significantly enhanced by the presence of amyloid precursor protein and Aβ [10]. Thus factors, including *APOE* genotype, that influence the deposition of these molecules in the brain may also alter risk for PD pathology [1]. Recently, sib-pair analysis concluded that the E4 allele was probably responsible for the previously reported chromosome 19 linkage peak for PD [9]. However, a meta-analysis which included 22 eligible case–control studies investigating APOE and PD revealed no evidence for an association between the E4 allele and PD [5]. Interestingly however, a modest positive association between the E2 allele and PD was revealed [5]. In the largest single study performed to date, Li et al. [6], using family based methodologies, reported that the E4 allele increased the risk of PD but intriguingly was also associated with a decreased AAO of PD. Inherent problems in case–control association studies such as population stratification, inappropriate control subjects and underpowered studies may underlie many of the findings for APOE and PD and contribute to the contradictory reports regarding the E4 allele as a PD risk factor. With these limitations in mind we studied the common APOE gene variant in a relatively large sample of Australian PD patients and controls of European descent. We have considered both PD risk and AAO in genetic models, and have also performed stratified analyses of men and women.

The details of our Australian case–control cohort have been published previously [2,11,12]. PD cases ($n = 422$, 239 males, 183 females, average age = 66.95 ± 9.80 (S.D.) years, average onset age = 59.98 ± 10.44 (S.D.) years) were recruited from Movement Disorders clinics. The diagnosis of probable or definite PD was made by a movement disorders neurologist using standard criteria. Cases were excluded if they: had a diagnosis of dementia or exhibited symptoms suggestive of an alternative form of parkinsonism. Controls ($n = 387$, 114 males, 273 females, average age = 64.16 ± 10.83 (S.D.) years) consisted of 156 patient spouses and 231 volunteers from patient neighbourhoods and community organizations. All subjects were examined by one of the authors (PAS). Patients with known *LRRK2* or *parkin* gene mutations were not included in the analysis. Data pertaining to ethnic background, family history of PD (first-degree relatives with PD) and AAO of PD symptoms were obtained from study subjects via face-to-face interviews using a structured questionnaire. AAO was defined as self-reported age at which the patient initially noticed the appearance of a

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0304-3940/$ – see front matter © 2006 Published by Elsevier Ireland Ltd.
doi:10.1016/j.neulet.2006.07.080

Table 1
Gender, age, genotype, and E4 carrier status frequencies for PD patients and controls

	Controls	PD	
Males	114	239	
Females	273	183	
Total	387	422	χ^2, $P<0.0001$
Average age (years)	64.16	66.95	ANOVA, $P=0.0001$
S.D. (years)	10.83	9.80	
Male average age (years)	64.71	67.03	ANOVA, $P=0.045$
S.D. (years)	11.35	9.53	
Female average age (years)	63.93	66.85	ANOVA, $P=0.0037$
S.D. (years)	10.61	10.16	
APOE Genotypes			OR (95%CI)
E3/E3	232	258	1
E2/E2	1	1	N.D.
E4/E4	11	13	0.77 (0.32–1.87)
E2/E3	37	51	1.27 (0.78–2.07)
E2/E4	11	9	0.99 (0.37–2.65)
E3/E4	95	90	0.86 (0.60–1.25)
Total	387	422	
APOE E4 carrier status			
Non-E4 carriers	270	310	1
E4 carriers	117	112	0.83 (0.61–1.13)
Total	387	422	

Odds ratios (OR) with 95% confidence intervals were calculated using logistic regression modeling adjusting for age, gender, smoking exposure and positive family history of PD.

salient parkinsonian symptom. All subjects were of European Caucasian ancestry.

APOE genotyping was performed using a previously described method including primer sequences [9]. All samples were genotyped in duplicate.

Genotype and allele frequency comparisons were made using 2×2 contingency tables, Fishers exact and Pearson's chi-squared tests, logistic regression analyses, Kaplan-Meier survival plots, ANOVA, Fishers PLSD and Hardy-Weinberg equilibrium tests. All probability tests were 2 sided with a level of significance of 0.05.

With regards to our case–control sample, there was a significant over-representation of males in the PD group and controls subjects were younger than cases (Table 1).

Potential effects of *APOE* variants on PD risk were explored using two models. In model 1 we tested possible differences in genotype distributions between cases and controls for E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, and E4/E4 classes (Table 1). In model 2 we compressed genotype groups to two classes consisting of E4 carriers and non-carriers (Table 1). Neither analysis resulted in significant findings. We also performed a stratified analysis of E4-carriers and non-carriers in men and women separately. There was no evidence of association in either group ($\chi^2=1.6$, $P=0.21$ for men and $\chi^2=0.65$, $P=0.42$ for women, respectively).

The distribution of AAO in the PD sample was normal. Average AAO were statistically equivalent for all genotypes in the entire PD set (Table 2). However, the E4 carrier/non-carrier analysis (model 2), identified marginal evidence that E4 carriers had a lower mean AAO (Table 2). Chi-square analysis of all cases stratified on median AAO (60 years) revealed that E4 carriers were marginally over-represented in the early onset group (OR = 1.50, 95% CI = 0.97–2.33); this was more marked in females (OR = 2.40, 95% CI = 1.19–4.82).

Table 2
Age-at-onset (years (S.D.)) for genotype, and E4 carrier status for PD patients

AAO	PD	Males	Females
Genotype			
E2/E2	N.D.	N.D.	N.D.
E2/E3	59.06 (12.00)	57.12 (12.89)	60.92 (11.02)
E2/E4	59.67 (9.64)	66.50 (8.70)	54.20 (6.72)
E3/E3	60.68 (10.13)	60.14 (10.07)	61.37 (10.22)
E3/E4	58.18 (10.68)	60.06 (10.34	55.29 (10.70)
E4/E4	62.00 (8.26)	62.25 (9.48)	61.60 (6.84)
Total average AAO	59.98 (10.44)	60.01 (10.41)	59.95 (10.50)
E4 carrier status			
Non-E4 carriers	60.43 (10.45)	59.74 (10.53)	61.28 (10.33)
E4 carriers	58.75 (10.34)[a]	60.71 (10.15)	55.87 (10.05)[b]

[a] $F_{1,416}=3.2$; $P=0.076$.
[b] $F_{1,180}=9.4$; $P=0.0025$.

We used a second order factorial ANOVA to address a possible interaction with sex, focusing on the E4 carrier model (model 2). The sex × genotype interaction term was significant in this analysis ($F_{1,414}=5.4$, $P=0.02$). In separate gender analyses, the effect of E4 carrier status was found to be significant in the female group ($P=0.0025$), but not in males ($P=0.91$). We also noted a significant effect of genotype on AAO in females ($F_{4,177}=2.8$; $P=0.027$) (Table 2). Post hoc tests (Fishers PLSD) were significant between E2/E3 and E3/E4 classes ($P=0.036$) and between E3/E3 and E3/E4 classes ($P=0.0027$). We observed significant main effects upon AAO of the rs7412 variant (i.e. the Arg176Cys) in females ($F_{2,178}=5.6$; $P=0.0043$), but not in males ($F_{2,232}=0.18$; $P=0.83$).

We used Kaplan-Meier analysis to estimate survival functions, and performed tests with both censored control samples (censored for age-at-exam) and excluding controls. In the female sample, results for models 1 and 2 were highly significant ($\chi^2=13.7$, d.f. = 4; $P=0.0083$) and ($\chi^2=11.3$, d.f. = 1; $P=0.0008$), respectively (Fig. 1). Similar AAO trends were observed for female cases with and without a family history of PD. Exclusion of E2/E4 genotypes from the analysis did not substantially alter the overall result; here female carriers continued to have and earlier AAO ($P=0.006$). E2 carriers did not differ to non-carriers in AAO overall or in gender-stratified analyses.

One of the possible caveats associated with an analysis of *APOE* upon AAO for any disease is that genotypes have been shown to change with age (thus suggesting longevity effects). We used logistic regression to test for the possible dependence of *APOE* genotype upon age in the entire control sample. This was not significant ($P=0.14$), but did indicate that population frequencies of the E4 allele may be diminishing with age. We note that this model is the equivalent inverse of an ANOVA