Frequency of the apolipoprotein E ε4 allele in a case-control study of early onset Parkinson’s disease

Alexander S Whitehead, Solange Bertrandy, Finola Finnan, Aileen Butler, George Davey Smith, Yoav Ben-Shlomo

Abstract

Objectives—It has been suggested that Parkinson’s disease and Alzheimer’s disease may share a common or at least overlapping aetiology. The prevalence of dementia among cases of Parkinson’s disease is known to be greater than expected in the general population. The frequency of the apolipoprotein ε4 allele in a large case-control study of early onset Parkinson’s disease has been examined.

Methods—215 patients and 212 population based controls were recruited from the Republic of Ireland between 1992 and 1994. Cases had to have disease onset at 55 years or younger and be born after 1925.

Results—The frequency of the ε4 allele was almost identical between cases of Parkinson’s disease (14.6%) and healthy controls (13.3%). There was no relation between ε4 status and disease onset, disease duration, Hoehn and Yahr score, and disease progression. The frequency of the ε4 allele was not increased among 10 patients with Parkinson’s disease with dementia (10.0%) compared with the other patients without dementia (14.8%). There was no association between ε4 allele status and either a history of smoking, family history of dementia, or Parkinson’s disease, or being born in a rural area. The odds ratio for the ApoE ε4 allele associated with Parkinson’s disease was 1.01 (95% confidence interval 0.68–1.79), adjusting for age group, sex, and residential status. The pooled odds ratio from a meta-analysis of six studies of ApoE ε4 status and Parkinson’s disease was 0.94 (95% CI 0.69–1.27).

Conclusions—The results from our study as well as the pooled meta-analysis exclude any important role for ApoE ε4 status in the development of Parkinson’s disease. Our results similarly do not support its role either in dementia associated with Parkinson’s disease or disease progression.

Keywords: Parkinson’s disease; apolipoprotein ε4; case-control study; meta-analysis

Parkinson’s disease is a progressive neurodegenerative disorder that affects a significant number of the elderly population. Although clinically and pathologically well defined, the underlying aetiology remains poorly understood. It is now generally accepted, however, that the pathogenesis of Parkinson’s disease may well involve both genetic and environmental factors. Apolipoprotein E (ApoE) is a polymorphic protein with three major isoforms, ε2, ε3, and ε4 that is present in high concentrations in brain where it is synthesised by astrocytes and can be internalised by nerve cells utilising a receptor mediated pathway. It may therefore play a part in the aetiology of neurodegenerative diseases.

Several studies have established a significant association between the ε4 allele and both familial late onset Alzheimer’s disease and sporadic late onset senile dementia of the Alzheimer type, and subsequent studies have noted similar associations with senile dementia of the Lewy body type. There are several reasons for examining whether the ε4 allele is also a risk factor in the development of Parkinson’s disease: firstly, some investigators have postulated that Parkinson’s disease and Alzheimer’s disease may have a common, or at least overlapping, aetiology. Some studies have noted familial aggregation of dementia, amyotrophic lateral sclerosis, and Parkinson’s disease; secondly, the Lewy body is the pathognomonic pathological lesion in Parkinson’s disease; thirdly, dementia is more prevalent than expected in patients with Parkinson’s disease; fourthly, one report has noted an increased risk of coronary heart disease among a cohort of patients with Parkinson’s disease compared with a control population. As the ε4 allele is also associated with an increased risk of coronary heart disease, it could provide a common link between these two diseases.

We report the results of a large genetic and epidemiological case-control study of early onset Parkinson’s disease using population based controls. The aims of which were (a) to establish more precise estimates of the risk of Parkinson’s disease associated with the ApoE ε4 allele, (b) to examine the interactions of ε4 with features of clinical disease severity, and (c) to test for interactions between the ε4 allele and other known risk factors for Parkinson’s disease such as smoking habit, family history of disease, and being born and residing in an urban or rural environment.
Subjects and methods

SAMPLE SELECTION

Between 1992 and 1994 a large population based case-control study of early onset Parkinson’s disease was conducted in the Republic of Ireland to examine both environmental and genetic risk factors. Cases were ascertained from all neurologists in the Republic of Ireland, national inpatient data, Parkinson’s disease self help groups, and drug administration records. Only patients fulfilling the Parkinson’s Disease Brain Bank criteria were included—that is, patients were enrolled if they had bradykinesia and at least one of the following criteria: (a) resting tremor (4-6 Hz); (b) muscular rigidity; (c) postural instability unrelated to primary visual, cerebellar, vestibular, or proprioceptive dysfunction. All patients had a physicians’ diagnosis of Parkinson’s disease and were personally examined by one of the study investigators (YB-S) for the above criteria. Additional inclusion criteria for cases were that the initial symptoms of disease had started at the age of 55 or younger and that date of birth was after 1925. All cases were administered modified mini mental state examination excluding the first five preliminary questions. A cut off of 17 or less out of 25 was taken to indicate possible dementia, instead of the normal cut off of 20 or less out of 30.

Population based controls were ascertained from the electoral register, which is computerised and covers 98% of the total population. Controls were randomly selected from district electoral divisions stratified by an urban-rural indicator from the proportion of the population living in towns or cities. One control was found for every case and was frequency matched by sex and age in five year age groups.

Those who were unable to provide a history because of dementia, aphasia, or serious psychiatric illness were excluded from the study both as cases and controls.

EPIDEMIOLOGICAL DATA

Subjects were all interviewed and details concerning clinical history and disability were obtained. The disability was classified according to Hoehn and Yahr criteria. Age at onset of disease and diagnosis were determined and disease progression was dichotomized into good and normal groups. The good group comprised patients who were in the top tertile of disease duration for their Hoehn and Yahr score—that is, a long duration of illness for that degree of severity of disease. Self report data about each residence were collected and these were classified into “urban” (city/town) or “rural” (village/country). A complete cigarette, cigar, and pipe smoking history was obtained and used to classify subjects into never smokers, ex-smokers, and current smokers. A positive family history for dementia or Parkinson’s disease was recorded if subjects stated that a parent, sibling, uncle, aunt, or cousin had had the disease. The positive identification or re-collection of tremor only was not accepted as evidence of Parkinson’s disease.

DNA PREPARATION

Blood samples were drawn into EDTA tubes. All samples were blind coded before transfer for the laboratory analysis. Blood was processed to provide crude cell lysates suitable for amplification by polymerase chain reaction (PCR). Briefly, white blood cell pellets were lysed in 5 ml lysis buffer (50 mM KCl, 2.5 mM MgCl2, 20 mM Tris (pH 8.3), 0.45% nonidet P-40, 0.45% Tween-20) with 0.2 mg/ml protease K (Boehringer Mannheim). After incubation for at least 30 minutes to dissolve the cell pellet completely, lysates were boiled for 10 minutes to inactivate the enzyme.

APOLIPOPROTEIN GENOTYPE ANALYSIS

DNA samples were genotyped for the common ApoE polymorphisms ε2, ε3, and ε4 essentially by the method of Hixson and Vernier. In brief, the oligonucleotide primers 5’-ACAGAATTTCGCCGCCGCTGTACAC-3’ and 5’-TAAGCTTGCCACGGCTGTCCAGA-3’ were synthesized using an Applied Biosystems PCR-Mate model; PCR amplification conditions were 95°C, five minutes, one cycle; 60°C, one minute, 70°C, two minutes, 95°C, one minute, 30 cycles. Products were digested to completion with Hha I and resolved by electrophoresis through 6% polyacrylamide gels. The ApoE alleles represented in each of the coded samples were determined by two investigators independently, without knowledge of case-control status, and before assigning the resulting ApoE genotypes to the corresponding cases and controls.

STATISTICAL ANALYSIS

The frequency of the ApoE alleles were compared by χ² test and Fisher’s exact test if the expected cell count was less than five. Differences in proportions were calculated using the Z statistic. For continuous variables, the Kruskal-Wallis one way analysis of variance was used as the variables were not normally distributed. Logistic regression analysis was used to calculate odds ratios and 95% confidence intervals (95% CIs) and adjusted for potential confounders. As controls were matched by sex, five year age bands, and stratified by an urban-rural indicator, these variables were included in the multivariate model as indicator terms.

All papers that have reported the frequency of the ε4 allele in Parkinson’s disease were identified by using both a Medline search and BIDS, including a citation search. The calculation of the weighted odds ratio in the meta-analysis method was developed by Yusuf and colleagues.

Results

215 cases and 212 controls were recruited into the study and blood samples were obtained from 195 (91%) and 166 (78%) respectively. Five cases that did not fulfil the inclusion criteria were not included in the analysis. Five DNA samples (from one case and four con-
controls) could not be amplified by PCR leaving 351 subjects (189 cases and 162 controls) for whom ApoE genotypes could be determined.

Table 1 shows the basic demographic details of the subjects. The only significant difference between the cases and controls was in a positive family history of Parkinson’s disease ($\chi^2 = 4.2$ on 1 df, $P = 0.04$). The odds ratio of reporting a family history of Parkinson’s disease was 1.88 (95% CI 1.01–3.50) having adjusted for sex, age groups and residential status. Table 2 shows the frequencies of the common ApoE alleles; no significant differences in ApoE allele frequencies were apparent between cases and controls.

Table 3 shows the relation between the ApoE alleles and clinical disease. The mean age of onset for the total sample was 45.6 (SD 7.6) years. Cases with the ε2 allele were more likely to have an older age of onset ($P = 0.046$) but did not differ significantly in any other respect. The prognosis of disease was not associated with the ε4 allele. There were 10 cases with a diagnosis of dementia based on the mini mental state score. There was little difference in the frequency of the ε4 allele among cases of Parkinson’s disease either with or without dementia (10% vs 14.8%).

No significant differences or interactions were noted in the frequency of the ε4 allele with a positive history of smoking (difference in ε4 status for cases compared with controls 2.3% vs 0.8%), a family history of either Parkinson’s disease (6.1% vs 6.9%) or dementia (5.4% vs 3.6%), or with being born in a rural rather than urban environment (1.5% vs 6.7%). The unadjusted odds ratio for the ApoE ε4 allele associated with Parkinson’s disease was 1.12 (95% CI 0.70–1.81). The multivariate odds ratio, adjusting for age group, sex, and residential status, was 1.10 (95% CI 0.68–1.79). There were no significant interaction terms. A repeat analysis of cases and controls that were aged 50 years or younger, with a mean age of onset of 35.5 (range 19–45) years showed the ε4 allele to be less common in cases with Parkinson’s disease but this was not statistically significant (odds ratio 0.35, 95% CI 0.12–1.08).

From the seven published reports only four papers published the genotype data to enable the calculation of an odds ratio associated with ε4 allele status. We were able to obtain additional data from the author of one of the other three reports. Only non-demented cases of Parkinson’s disease were included from the report by Arai et al. The figure shows the results of the meta-analysis. Four of the studies showed a reduced risk associated

### Table 1 Description of sample characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Parkinson’s disease</th>
<th>Control subjects</th>
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<tbody>
<tr>
<td></td>
<td>All</td>
<td>Women</td>
</tr>
<tr>
<td>No (%)</td>
<td>189 (100)</td>
<td>66 (34.9)</td>
</tr>
<tr>
<td>Mean age at entry in study (SD) (y) ε2</td>
<td>56.9 (6.6)</td>
<td>56.8 (7.2)</td>
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<tr>
<td>Mean age at disease onset (SD) (y) ε2</td>
<td>45.6 (7.6)</td>
<td>44.0 (8.7)</td>
</tr>
<tr>
<td>Family history of dementia ε2</td>
<td>18 (9.5)</td>
<td>6 (9.1)</td>
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</tbody>
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### Table 2 ApoE genotypes and allele frequencies

<table>
<thead>
<tr>
<th>Genotype (%)</th>
<th>Parkinson’s disease</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>Women</td>
</tr>
<tr>
<td>ε2/ε2</td>
<td>(n = 189)</td>
<td>(n = 66)</td>
</tr>
<tr>
<td>ε2/ε3</td>
<td>1.6</td>
<td>0.0</td>
</tr>
<tr>
<td>ε3/ε3</td>
<td>58.7</td>
<td>66.7</td>
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<td>ε2/ε4</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>ε3/ε4</td>
<td>23.3</td>
<td>24.2</td>
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<td>ε4/ε4</td>
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<td>9.1</td>
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<tr>
<td>ε2/ε2</td>
<td>2.7</td>
<td>0.0</td>
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### Table 3 Relation between clinical features and allele status

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>ε2 allele (31)</th>
<th>ε3 allele (292)</th>
<th>ε4 allele (55)</th>
</tr>
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<tbody>
<tr>
<td>Age at onset of disease (y)*</td>
<td>48.3</td>
<td>45.2</td>
<td>45.8</td>
</tr>
<tr>
<td>Disease duration (y)</td>
<td>9.0</td>
<td>11.6</td>
<td>11.4</td>
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<tr>
<td>Mean Hoehn and Yahr score</td>
<td>2.2</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Proportion with good prognosis</td>
<td>29.0</td>
<td>33.7</td>
<td>29.1</td>
</tr>
<tr>
<td>Dementia (based on MMSE)</td>
<td>5.0</td>
<td>8.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

*Kruskal-Wallis $\chi^2 = 6.2$ on 2 df, $P = 0.046$. MMSE = mini mental state examination.
with the e4 allele status whereas the other two showed an increased risk. However, all the 95% CIs included unity, reflecting random variation. The pooled odds ratio was 0.94 (95% CI 0.69–1.27). This confirms the lack of any significant association between ApoE e4 status and Parkinson’s disease, and excludes any clinically important raised risk as the 95% CIs for the pooled estimate are reasonably narrow. The homogeneity test showed no significant difference between studies (P = 0.67).

Discussion
RISK OF PARKINSON’S DISEASE
Several studies to date have attempted to assess the possible involvement of the e4 allele in Parkinson’s disease.13 16 41–45 All have determined the ApoE genotypes of small samples; although no significant increase in e4 allele frequency was found, none of these studies have had sufficient statistical power to exclude an odds ratio less than three. These studies did not always have representative samples of patients and may have been biased towards more atypical cases. No study has specifically examined possible interactions between the e4 allele and various environmental factors and its association with early onset patients, in whom the risk conferred by a given genetic component might be more pronounced.

Our results provide the most precise estimates of the risk associated between ApoE e4 status and Parkinson’s disease. We confirm previous reports that there is no association between ApoE polymorphism and the risk of Parkinson’s disease regardless of age at onset. When our results are pooled with other published reports in the meta-analysis, we can confidently conclude that if there is any “true” risk associated with e4 status, it is small and of very limited clinical relevance.

PARKINSON’S DISEASE PROGNOSIS
ApoE and the low density lipoprotein receptor have been postulated to play a part in repairing and maintaining the nervous system.8 This function, when compromised, may have a role in the progression of nerve cell degeneration and thus be related to clinical disease progression. Furthermore, there is compelling evidence that ApoE is massively expressed after experimentally induced nerve damage.8 It is possible that although the e4 allele is not associated with risk of developing Parkinson’s disease, it could be related to accelerated disease progression and hence worse prognosis. Our results, however, do not show any relation between e4 status or clinical features, progression, and disability.

PARKINSON’S DISEASE AND DEMENTIA
We failed to find an association between e4 status and patients with Parkinson’s disease with dementia, a finding similar to that of two other studies44–45 but unlike another.47 A recent report of 50 patients with Alzheimer’s disease with concomitant pathological damage associated with Parkinson’s disease, did show an association with e4 status. These patients were selected on the basis of Alzheimer’s disease and the parkinsonian pathology “might represent merely the coincidental occurrence of another common disorder of the elderly.”46 The number of cases of dementia in our sample was small and the diagnosis was based on the mini mental state examination. It is possible that we failed to show an association because of misclassification and an insufficient number of cases. Alternatively, dementia in Parkinson’s disease may be of mixed aetiology; some patients may have concurrent Alzheimer’s pathology and hence an association with e4 status whereas other patients do not.47 For example, one study which compared five non-demented patients with Parkinson’s disease with seven cognitively impaired patients failed to detect any difference in the accumulation of paired helical filaments in the cingulate and occipital cortices48 suggesting a non-Alzheimer’s cause of dementia.

APOE AND OTHER ENVIRONMENTAL FACTORS
Riggs49 has postulated that the inverse relation found between smoking and both Parkinson’s disease and Alzheimer’s disease may be linked to a genetic predisposition to accelerated aging (and therefore premature death) that interacts with smoking. His hypothesis suggests that studies of aged (older average age of onset) patients may be depleted of those carrying the e4 allele, because of premature death from other causes, thereby, at least partly, masking any inherent risk of Parkinson’s disease in carriers of the e4 allele due to selective loss of such people. It is therefore important to examine the genetic and environmental interactions between smoking, ApoE genotype, and Parkinson’s disease in a cohort of young patients in whom premature mortality from e4 associated disease(s) will be less problematic. The results of van Duijn and colleagues failed to show any relation between e4 status, smoking, and Alzheimer’s disease.50 We too failed to show any difference in e4 status between smokers and never smokers either in cases or controls. These results do not provide any support for Riggs’ hypothesis, but cannot refute it as it is still possible that this hypothesis may be valid for another “genetic candidate”.

MISDIAGNOSIS OF PARKINSON’S DISEASE
Our study was based on a clinical diagnosis of Parkinson’s disease. We know that even among patients diagnosed by clinicians with a special interest in movement disorders, around 25% of patients are misdiagnosed.51 This is reduced to 18% if the Parkinson’s Disease Society Brain Bank criteria are applied,1 as has been done in this study. If it is assumed that the “other” conditions wrongly labelled as Parkinson’s disease have no relation with ApoE status, then our study will have underestimated the potential true relation. This is unlikely to be a serious problem as our negative results are very similar to smaller studies which in some cases have been validated at postmortem18 and the small degree of misclassification would be insufficient to totally remove a twofold to threefold association.
Conclusions

The results of this and other research clearly indicate no relation between ApoE status and either the risk of developing Parkinson’s disease or its natural history. Furthermore, the lack of an association in this young sample reduces the possibility that selective mortality might have depleted the ApoE e4 gene pool and thus produced a spurious negative result. Similarly, no associations were seen with various environmental risk factors. The differentiation between Parkinson’s disease, and both Alzheimer’s disease and the “Lewy body variant of Alzheimer’s disease” suggests that ApoE cannot provide a unifying explanation for these conditions.

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