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ORIGINAL COMMUNICATION

Apolipoprotein E genotype as a risk factor for susceptibility to and dementia in Parkinson's Disease

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Abstract Further to the well-established association between apolipoprotein E (APOE) and Alzheimer's disease, this gene has also been implicated in both susceptibility to, and dementia in, Parkinson's disease (PD). However studies to date have produced contradictory findings. We conducted a case-control study in a population of 528 PD patients and 512 healthy controls and found no significant difference in allele or genotype distribution of APOE between the two groups. An updated meta-analysis showed a modest increase of APOE-ε2 carriers amongst PD patients compared to controls [$P = 0.017$, OR = 1.16 (95 % CI 1.03–1.31)]. 107 of our patients were incident cases participating in a population-based epidemiological study. Longitudinal follow-up of this cohort over a mean of 5.0 ± 0.7 years from diagnosis revealed no significant impact of APOE-ε4 carrier status on risk of dementia or rate of cognitive decline. An updated meta-analysis indicated an over-representation of APOE-ε4 carriers amongst PD dementia compared to non dementia cases [OR 1.74 (1.36–2.23), $P = 1 \times 10^{-4}$], although small sample sizes, heterogeneity of odds ratios and publication bias may have confounded this finding. In conclusion, our study does not support previously reported associations between APOE genotype and susceptibility to, or cognitive decline in, PD. An updated meta-analysis indicates any association with PD susceptibility is at most modest, an observation with important implications for further study of this issue. Large scale longitudinal studies would be best placed to further evaluate any impact of APOE genotype on cognitive decline in PD.

Key words Parkinson's disease · apolipoprotein E · dementia · genetics

Introduction

Although a number of causative genes for Parkinson's disease (PD) have been identified over the past decade, these account for only a proportion of familial cases [19]. In the vast majority of sporadic cases, the aetiology is likely to be more complex, resulting from a combination of multiple environmental and genetic risk factors [10], the majority of which are yet to be elucidated. Apolipoprotein E (APOE) genotype has been proposed as a potential risk factor for both susceptibility to PD and/or with the occurrence of cognitive impairment and dementia in PD in the light of the well-established association between this gene and susceptibility to Alzheimer's disease (AD) [7]. APOE has 3 alleles: APOE-ε2,

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APOE-ε3 and APOE-ε4. The APOE-ε4 allele is associated with both AD risk and lower age at disease onset, whilst the APOE-ε2 allele appears to be protective [7]. There are significant similarities between PD and AD: both are characterized pathologically by neuronal loss and protein aggregation, and their clinical features overlap, with dementia and extrapyramidal symptoms occurring in both disorders. Thus the APOE-ε4 allele is also a plausible candidate for influencing the neurodegenerative process in PD and PD dementia (PDD). Furthermore, recent evidence implicates APOE in the molecular pathway of alpha-synuclein mediated neurodegeneration [12].

Most studies investigating a role for APOE in PD, however, have been relatively small and have reached contradictory conclusions. Previous meta-analyses have suggested association of the APOE-ε2 allele with susceptibility to PD and of the APOE-ε4 allele with PD dementia [16, 17].

We investigated the role of APOE as a susceptibility locus for PD in the largest single study population hitherto. Further analysis of a subset of incident cases prospectively followed up for 5 years from diagnosis allowed us to investigate the longitudinal effect of APOE-ε4 on cognitive decline and the development of dementia in PD. Finally, our data, together with data from relevant studies identified in the literature, were used to extend previous meta-analyses with a more than doubling of the number of cases included in an attempt to clarify whether APOE genotype contributes significantly to the risk of PD and PD dementia.

Methods

The study population consisted of 528 PD patients recruited from the East Anglia region and 512 controls of UK Caucasian origin. Patients were 60 % male, with a mean (SD) age of onset of 62.5 (11.8) years. All fulfilled the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria for PD, although positive family history (in 14 %) was not used as an exclusion criterion, given that individuals with a family history of PD are expected to carry relevant genetic factors at a higher frequency. Two individuals carrying the mutation in the *LRRK2* gene that is known to cause familial forms of PD were excluded [28]. Controls included 144 spouses of patients with PD and 368 blood donors. 48 % of controls were male and none had a diagnosis of PD at the time of DNA collection. Ethical approval was granted by the Local Research Ethics Committee, Cambridge, UK.

APOE ε2, ε3 and ε4 alleles can be differentiated by typing two non-synonymous single nucleotide polymorphisms (SNPs), rs429358 (APOE-C112R) and rs7412 (APOE-R158C). We genotyped these SNPs in our samples using Taqman Assays (Applied Biosystems Assay-On-Demand part numbers C__3084793_20 and C__904973_10) on a 7900HT Sequence Detection System (Applied Biosystems). Allelic and genotypic analyses were performed using the Unphased package version 3.0.4 [6]. The meta-analysis previously presented by Huang et al. [17] was updated with our own data and all available datasets on APOE genotype and susceptibility to PD identified in PubMed for which cases were identified as clinically or pathologically defined PD and genotype frequencies were available for both cases and controls. Meta-analysis for APOE carriage rate and susceptibility to PD over different strata was done with the Mantel-Haenszel test statistic assuming fixed effects and calculated in Statsdirect.

Amongst the patients, 107 were incident PD cases from Cambridgeshire participating in a longitudinal population-based study of the heterogeneity of PD [9, 27]. All incident patients underwent standardized neurological, neuropsychological and functional assessments at baseline (mean 0.3 ± 0.3 years from diagnosis) and follow-up assessments approximately 3 years later (see [27] for full details of the assessment battery). Surviving patients underwent further assessment at approximately 5 years from diagnosis. We performed cross-group comparisons to investigate the impact of APOE-ε4 carrier status on both the development of PD dementia and rate of cognitive decline. Dementia was diagnosed on the basis of both a Mini-mental State Examination (MMSE) score ≤ 24 and fulfilment of the DSM-IV criteria at follow-up. No incident patients met criteria for dementia at baseline. Rate of cognitive decline was measured using mean change in MMSE per year, calculated for each individual by subtracting the last available MMSE score from the baseline score and dividing by the time interval between assessments. We also updated a previously published meta-analysis of studies investigating the association between the APOE-ε4 allele and PD dementia [16] with all available datasets from the literature, identified via PubMed, and our own results, using the Mantel-Haenszel test statistic as above.

Results

Genotyping success rates were 97.6 % and 96.4 % for SNP rs429358 and rs7412, respectively, enabling the APOE genotype to be determined in 94.5 % of samples. Neither SNP showed deviation from Hardy-Weinberg equilibrium (P > 0.40) and no inconsistencies were seen amongst 56 samples typed in duplicate. There was no significant difference in allele or genotype distribution of *APOE* between PD patients and controls (Table 1). Adjusting for gender did not change this observation.

A further nine studies[1–3, 13, 15, 20, 22, 24, 26] were identified to update the previous meta-analysis of APOE genotype and PD susceptibility [17], which, in addition to our own data, approximately doubles the total number of included cases (N = 4,198) and increases the number of controls by 28 % (N = 10,066). No significant heterogeneity of odds ratios was observed between studies (Breslow-Day test $P = 0.91$, Cochran Q test 0.95). The frequencies of APOE-ε4 carriers were virtually identical between cases and controls [$P = 0.99$, OR = 1.00 (95 % CI 0.91–1.10)]. The previously reported over-representation of APOE-ε2 carriers amongst PD patients compared to controls [17] continues to be observed in this updated meta-analysis [$P = 0.017$, OR = 1.16 (95 % CI 1.03–1.31)] (Fig. 1).

Of the 107 patients in our incident PD cohort, all were reassessed at the first (3 year) follow-up visit whilst 9 patients died before the second (5 year) follow-up visit [3 of 31 (10 %) APOE-ε4 carriers versus 6 of 76 (8 %) non-carriers]. The mean (SD) total duration of follow-up across all 107 patients was 5.0 ± 0.7 years from diagnosis (range 2.3 to 5.9 years). Nineteen patients (18 %) developed dementia during the follow-up period. Eight

Table 1 Association between APOE and susceptibility to PD

Haplotype rs429358- rs7412	APOE isoform	PD (N = 505)	Freq	Control (N = 478)	Freq	*P*	Overall *P*	*P* adjusted for gender
T-T	APOE-ε2	84	0.083	79	0.083	0.97	0.25	0.16
T-C	APOE-ε3	792	0.784	725	0.758	0.18		
C-C	APOE-ε4	134	0.133	152	0.159	0.09		
	APOE genotype	PD	Freq	Controls	Freq	*P*	Overall *P*	*P* adjusted for gender
	ε2/ε2	4	0.008	5	0.010	0.67	0.68	0.48
	ε2/ε3	63	0.125	54	0.113	0.57		
	ε2/ε4	13	0.026	15	0.031	0.59		
	ε3/ε3	311	0.616	277	0.580	0.24		
	ε3/ε4	107	0.212	117	0.245	0.22		
	ε4/ε4	7	0.014	10	0.021	0.39		
	ε2+	80	0.158	74	0.155	0.88		
	ε4+	127	0.251	142	0.297	0.11		

of 31 individuals (26 %) carrying the APOE-ε4 allele developed dementia, versus 11 of 76 (14 %) non-carriers ($P = 0.16$, Chi-squared test), corresponding to an odds ratio of 2.06 (0.74 to 5.74). Considering the rate of cognitive decline, no significant difference in 'change in MMSE per year' was observed between APOE-ε4 carriers and non-carriers (Mann-Whitney U test, $P = 0.27$). Furthermore, rate of cognitive decline as a function of age did not differ for APOE-ε4 carriers and non carriers (Fig. 2; F test, $P = 0.20$).

A previous meta-analysis suggested a weak association between the APOE-ε4 allele and dementia in PD [OR 1.6 (1.0–2.5)] [16]. We identified a further 7 datasets from the literature [1, 4, 8, 18, 23, 24, 26] in addition to our own study to update this meta-analysis (Table 2), thus approximately tripling the entire study population to a total of 501 PD dementia (PDD) cases and 1145 PD cases without dementia (PDnD). This analysis still suggests an over-representation of APOE-ε4 carriers amongst PDD compared to PDnD cases [OR 1.74 (1.36–2.23), $P = 1 \times 10^{-4}$]. However, the number of PDD cases in each study is small (average 39, min. 8–max. 98), and there are indications of heterogeneity of odds ratios (Breslow-Day test $P = 0.02$) and of possible bias (Horbold-Egger test for asymmetry of funnel plot $P = 0.02$).

Discussion

In our study population, the largest in a single study on this subject to date, we did not observe any evidence for an association between APOE and PD. Our updated meta-analysis in a total of 4,198 cases and 10,066 controls continues to show the previous trend towards association of APOE-ε2 carrier status with increased susceptibility to PD. However, the modest odds ratio, if true, would require any single study population to comprise > 5,000 cases and controls to have sufficient power to be detected, an observation that has important consequences for the interpretation of the multitude of reports and the design of future studies on this topic. None of the relevant SNPs tagging APOE alleles were included in the two published whole-genome association screens [11, 21], rendering these studies uninformative to this specific research question.

One limitation of our study is that our controls were not age-matched with our patient cohort, and thus a small proportion of them may have developed PD at a later age. Although this may theoretically have reduced our power to detect any association, such an effect would be expected to be minimal given that the low estimated prevalence of PD in the general UK population (approximately 0.1 %, [25]).

Although our updated meta-analysis of APOE-ε4 and PD dementia suggests a highly significant positive association, a number of factors, including small sample sizes, heterogeneity of odds ratios between studies and probable publication bias, may impact on the validity of this finding. In addition there are significant methodological differences between studies. In particular, the criteria used to diagnose PD and PDD have varied widely, and thus misdiagnosis of AD as PDD is a possibility in some studies. Furthermore, comparisons of APOE-ε4 frequency in PDD and PDnD cases drawn from cross-sectional datasets may be overly simplistic and misleading given that this genetic risk factor might be expected to influence time to onset of dementia in PD, analogous to its effect on age of onset in AD [5]. Our study represents the first longitudinal analysis of the impact of APOE genotype on the evolution

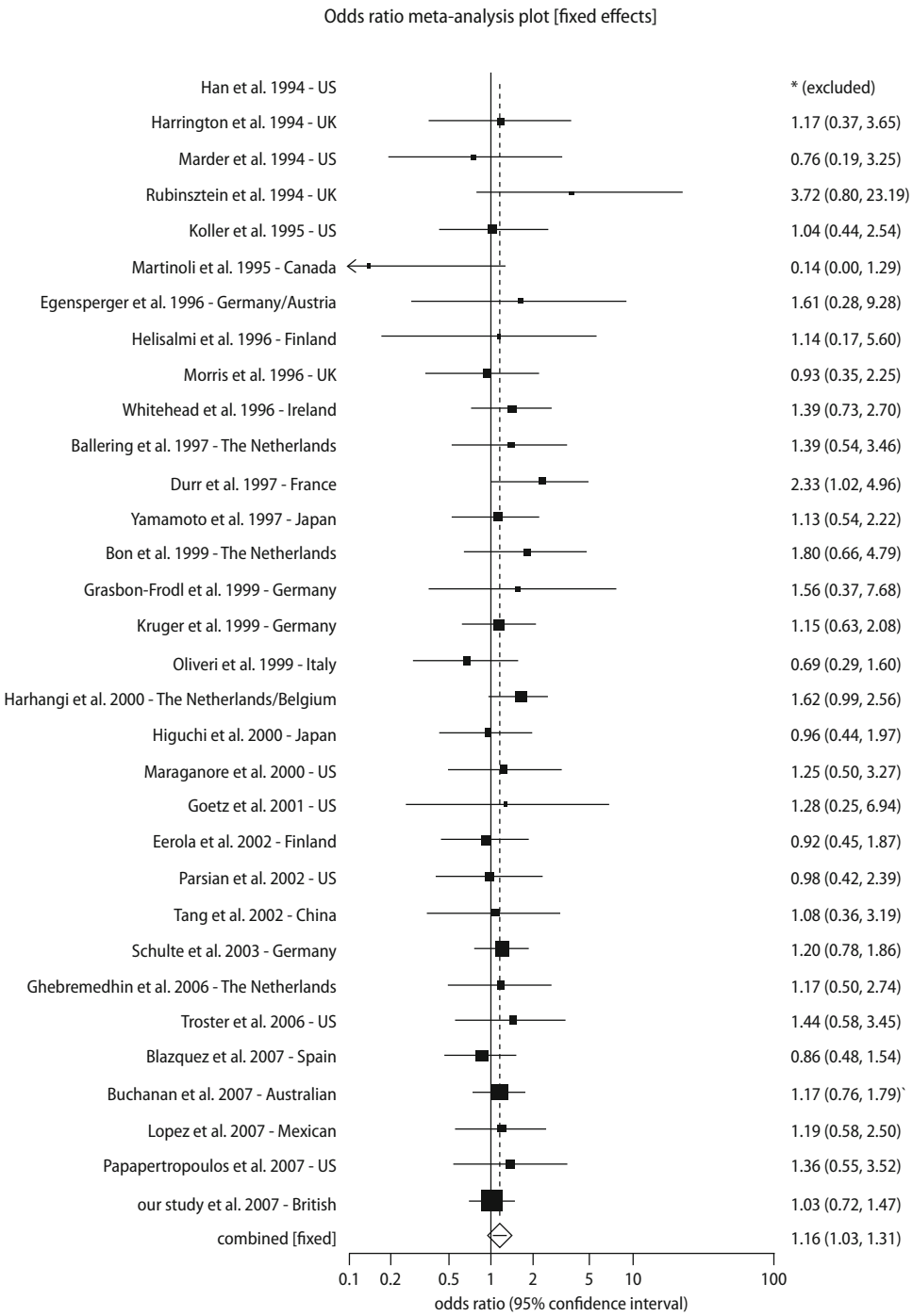

Fig. 1 Meta-analysis of APOE-ε2 carriers and susceptibility to PD, updated from Huang et al. [17]. Black squares represent weights, inversely proportional to the variance of each study. Breslow-Day and Mantel-Haenszel tests are used for testing heterogeneity of odds ratios between studies and for meta-analysis, respectively (* indicates no APOE-ε2 carriers)

of cognitive decline in PD from the time of diagnosis onwards. In our unbiased, population-representative incident PD cohort, we observed no differences between APOE-ε4 carriers and non-carriers in terms of mean rate of cognitive decline or the relationship between cognitive decline and age. We have previously observed two biological factors influencing cognitive decline using a similar longitudinal method in this incident population, namely age and the microtubule-associated protein tau H1/H1 genotype [14]. However, in spite of the strengths of this longitudinal approach, the power of our study to detect small effects is limited by the cohort size.

In conclusion, our study does not support previously reported associations between APOE genotype and susceptibility to PD and the updated meta-analysis suggests

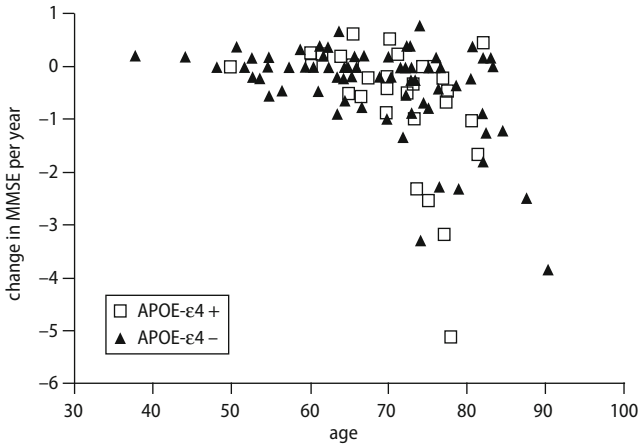

Fig. 2 Rate of cognitive decline (change in MMSE per year) as a function of age at baseline assessment in APOE-ε4 carriers and non-carriers with incident Parkinson's disease

that if there is an association, it is at best modest. This effect size implies that large study populations involving several thousands of patients and controls will be needed to draw reliable conclusions. Our study can only provide equivocal support for the hypothesis of elevated risk of dementia among APOE-ε4 carriers, and meta-analysis fails to convincingly resolve this issue. Longitudinal studies would be best placed to address this question in the future.

Conflict of interest The authors declare no conflict of interest.

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Table 2 Studies included in the meta-analysis of APOE-ε4 carrier status and PD dementia, updated from Huang et al. [16]

Study		N	Mean age, y	ε4 carriers	Non-carriers
Han et al., 1994	PD	5	82	0	5
	PDD	8	75	7	1
Marder et al., 1994	PD	57	71	15	42
	PDD	22	79	3	19
Koller et al., 1995	PD	61	67	17	44
	PDD	52	75	16	36
Martinoli et al., 1995	PD	10	69	2	8
	PDD	18	68	7	11
Egensperger et al., 1996	PD	20	76	4	16
	PDD	15	76	4	11
Helisalmi et al., 1996	PD	15	71	3	12
	PDD	8	73	5	3
Morris et al., 1996	PD	36	74	10	26
	PDD	17	75	6	11
Wakabayashi et al., 1998	PD	10	65	1	9
	PDD	12	75	5	7
Harhangi et al., 2000	PD	81	76	16	65
	PDD	26	82	10	16
Camicioli et al., 2005	PD	19	78	3	16
	PDD	28	78	9	19
Pankratz et al., 2006	PD	274*	NR	62	212
	PDD	50*	NR	19	31
Feldman et al., 2006	PD	49	71	10	39
	PDD			11	27
Troster et al., 2006	PD	42	72	11	31
	PDD	20		9	11
Blazquez et al., 2006	PD	245	71	25	220
	PDD	31		6	25
Papapetropoulos et al., 2007	PD	33	78	3	30
	PDD	39		18	21
Jasinska-Myga et al., 2007	PD	100	62	35	65
	PDD	98	71	30	68
This study	PD	88	68	23	65
	PDD	19	73	8	11
TOTAL	**PD**	**1145**		**240**	**905**
	PDD	**501**		**173**	**328**

* familial cases, 1 randomly selected case from each family included

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