Published in final edited form as:

Neurobiol Dis. 2012 May ; 46(2): 389–392. doi:10.1016/j.nbd.2012.02.002.

A large study reveals no Association between *APOE* and Parkinson's disease

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Abstract

Background—Research focusing on the role of *APOE* in Parkinson's disease (PD) has been largely inconclusive, creating a broad discrepancy in association studies.

Objective—To elucidate the role of *APOE* alleles in PD risk by studying a large sample size and controlling for population substructure.

Patients and Methods—In total, 3465 case and control samples were genotyped, obtained from the NINDS Neurogenetics repository.

Results—No significant differences in $\epsilon 4$ dosages exist between PD cases and controls. The frequency of $\epsilon 4$ carriers differed slightly between cases and controls at 24% (580/2412) and 26% (270/1053), respectively. Likewise, mean dosages of *APOE* $\epsilon 2$ were not significantly different between cases and controls. *APOE* $\epsilon 2$ carriers were observed at a frequency of 13.6% (329/2412) among cases and 15% (158/1053) among controls. Logistic regression models evaluating PD as possibly associated with $\epsilon 4$ or $\epsilon 2$ carrier status and allele dosages yielded no significant results. The mean MMSE score among all PD cases was 28.35 (SD = 2.58) and memory loss was reported in only 11.9% (105/879) of cases. Linear regression models comparing MMSE scores as predicted by $\epsilon 4$ or $\epsilon 2$ carrier status and allele dosages were not significant.

Conclusions—There is no association between *APOE* epsilon alleles and Parkinson's disease.

Keywords

Parkinson's disease; genetics; *APOE*

INTRODUCTION

Parkinson's disease (PD), with a prevalence of approximately 2% of the adult population over 65 years of age, is second to Alzheimer's Disease (AD) as the most common neurodegenerative disorder (Nussbaum and Ellis, 2003). The hallmark clinical features are classically characterized as resting tremor, bradykinesia, rigidity and akinesia; it has been suggested that cognitive deficit is a common feature of PD (Aarsland et al., 2003).

Neuropathologically, PD patients exhibit neuronal cell death in the substantia nigra and accumulate the insoluble protein, α -synuclein, which aggregates to form Lewy Bodies. In an

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analogous fashion, those afflicted with AD manifest extensive neuronal cell death while accumulating amyloid β plaques predominately within the cortex and hippocampus (Nussbaum and Ellis, 2003). One of the predominant genetic risk factors for the development of AD, Apolipoprotein E gene (*APOE*), has become a significant target for investigation in neurodegenerative diseases.

Functioning in lipid and lipoprotein transport in plasma and neurons, ApoE is believed to play a role in amyloid clearance and/or metabolism (Bales et al., 2002). *APOE*, located on chromosome 19q13.2, can encode three alleles ($\epsilon 2$, $\epsilon 3$, $\epsilon 4$), with $\epsilon 3$ being the most common universally (Strittmatter and Roses, 1996). In AD, the $\epsilon 4$ allele has been demonstrated to be a key risk factor for acquiring the disease, while possession of the $\epsilon 2$ allele serves as a protective factor towards AD development (Strittmatter and Roses, 1996).

In contrast, research focusing on the role of *APOE* and PD association has been largely inconclusive. Previous work has suggested $\epsilon 4$ as a risk factor for the age of onset, decline in cognitive impairment, and/or development of dementia in PD, $\epsilon 2$ has also been identified as a potential risk factor in PD, however this is at best a weak effect, and inconsistent across studies (Buchanan et al., 2007; Ezquerra et al., 2008; Gallegos-Arreola et al., 2009; Huang et al., 2006; Huang et al., 2004; Kurz et al., 2009; Lopez et al., 2007; Martinez et al., 2005; Pankratz et al., 2006; Ryu and Kwon, 2010; Vefring et al., 2010).

The discrepancy in association studies may be largely attributed to several factors: sample size limitations, methodological bias, and discordance in sample ethnicities. Thus, by utilizing a large sample size ($n = 3465$) and controlling for population substructure using previous immunochip replication data, the purpose of this study was to address inconsistencies previously reported. Excluding a suggestive sex-stratified association of females between age at onset with mean $\epsilon 2$ dosage and $\epsilon 2$ carrier status, there were no *APOE* – disease associations revealed in our analyses.

METHODS

Patients and Controls

In this study, all samples were obtained from the NINDS Neurogenetics repository hosted by the Coriell Institute for Medical research (New Jersey, USA). To be eligible to participate in this study, all patients were required to give written informed consent. A total of 2412 cases and 1053 controls were successfully genotyped.

The medical history of all controls participating in this study was thoroughly investigated through interview sessions. There was no history of AD, amyotrophic lateral sclerosis, ataxia, autism, bipolar disorder, brain aneurysm, dementia, dystonia, or PD in all control participants. Further, the Folstein mini-mental state examination (MMSE) was administered to the entire control population and yielded scores spanning from 26-30. In addition to personal medical history, a comprehensive family history was obtained, revealing the absence of any first-degree relatives with a known primary neurological disorder including: amyotrophic lateral sclerosis, ataxia, autism, brain aneurysm, dystonia, PD, and schizophrenia. Blood samples for control participants were derived from neurologically normal, unrelated, individuals of self-reported European ancestry at several USA sites. The average age of participants during sample collection was 68 years, ranging from 55–88.

Blood was drawn from unique and unrelated European ancestry individuals diagnosed with sporadic/idiopathic PD between 55-84 years of age. The age at which symptoms were initially recognized and recorded determined the age of PD onset among the case population. Symptom characterization required one or more of the following: resting tremor,

rigidity, bradykinesia, gait disorder, and postural instability. A family history regarding parkinsonism, dementia, tremor, gait disorders, and other neurological dysfunction was obtained for all PD subjects. All subjects with three or more relatives afflicted with parkinsonism or with likely Mendelian inheritance of PD were excluded. The case population thus included those without a family history of PD or those with two or fewer relatives manifesting parkinsonism.

Molecular Analysis

To distinguish between APOE $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$ alleles, we performed genotyping of two non-synonymous single nucleotide polymorphisms (SNPs), rs429358 (APOE-C112R) and rs7412 (APOE-R158C). Taqman Assays (Applied Biosystems Assay-On-Demand part numbers C__3084793_20 and C__904973_10) were utilized to genotype these SNPs on a 7900HT Sequence Detection System (Applied Biosystems). Allelic and genotypic analyses of isoforms were conducted using the Computer Software SDS V2.4 2010. Absolute quantification was performed on MBS 384ThermoHybaidPCR cyclers using the following conditions across four stages. Stage 1: consisted of 1 cycle at 50°C for a total of 2 minutes. Stage 2: consisted of 1 cycle at 95°C for a total of 10 minutes. Stage 3: consisted of 40 cycles at 95°C, each for a period of 15 seconds. Stage 4: consisted of 1 cycle at 60°C for a total of 1 minute. Upon completion, each 384 PCR plate was held at 4°C upon returning to the 7900HT Sequence Detection System to perform an allelic discrimination.

Statistical Analyses

The presence of PD and memory loss at time of diagnosis at the time of diagnosis were analyzed using logistic regression for PD status and memory loss outcomes and linear regression for the continuous MMSE (mini-mental state examination) measure models testing the following associations with case/control status: $\epsilon 4$ dosage, $\epsilon 4$ carrier status, $\epsilon 2$ dosage, and $\epsilon 2$ carrier status as independent variables. Linear regression models were utilized to test any association between the age at onset (in years) of PD and MMSE score with identical independent variables in a case-only analysis. To discern sex effect, stratified analyses for gender were performed as well. By rerunning analyses using component vectors 1 and 2 from multidimensional scaling, we were able to account for variation in population substructure within the U.S. cohort of samples. By applying the Bonferroni correction, which was adjusted to 8 tests per phenotype ($0.05/8 = 0.00625$), a slightly conservative p-value threshold was established.

RESULTS

Table 1 depicts the average ages and APOE alleles among subjects. Table 2 depicts the estimates of association levels by logistic regression models 1 and 2 described above. The primary set of regression analyses measured the degree of association between the presence of PD with the following as independent variables: $\epsilon 4$ dosage, $\epsilon 4$ carrier status, $\epsilon 2$ dosage and $\epsilon 2$ carrier status. Within the entire case-control cohort, none of the PD-allelic association estimates were statistically significant ($p < .05$) under both models. Looking at the sex-stratified cohorts of males and females, there were no significant associations between PD presence and any of the allelic independent variables in either model; the closest was females with PD manifesting an association with $\epsilon 4$ carrier status in model 1 ($p = 0.066$). Given our slightly conservative p-value threshold, reverting to a more generous value (i.e. $p = 0.05$) still yields no significant results.

In sex stratified regression analyses, both sexes demonstrated an absence of any association between age at onset and MMSE score with any of the four allelic independent variables for models 1 and 2. The presence of memory loss exhibited a strong association ($p < 0.01$) with

all four allelic dosage and carrier status' in model 1; however, all potentially suggestive associations were eliminated once applying population substructure constraints in model 2.

The linear regression models in the sex-stratified analyses were similar; male cases lacked any significant association between age at onset with each independent variable in models 1 and 2. Analogous to the former suggestive association (between memory loss and all independent variables between both sexes), age at onset with female cases harboring $\epsilon 2$ dosage and $\epsilon 2$ carrier status ($p = 0.031$, $p = 0.0101$), respectively, revealed a similar pattern in model 1. Likewise, upon application and analysis of model 2, these previously reported associations became insignificant ($p = 0.257$, $p = 0.0976$), respectively [3,11]. Hence, all logistic and linear regression models utilized yielded no association and subsequently lacked statistical significance.

DISCUSSION

As one of the three central risk factors for the development of AD pathology (in addition to aging and high plasma cholesterol at mid-life), possession of the $\epsilon 4$ allele (as a carrier or homozygote) has sparked interest regarding PD risk association. As $\epsilon 4$ allelic possession is also linked to an earlier age at onset of AD (Meyer et al., 1998), and $\epsilon 2$ allelic possession exhibits a protective effect in terms of risk for AD, multivariable analyses were utilized to elucidate a potential relationship between APOE genotypic isoforms and PD.

Our data revealed insignificant differences in $\epsilon 4$ mean dosage and carrier status frequencies among cases and controls. We found no evidence for an association between $\epsilon 4$ and risk for PD. Moreover, case-control comparisons of $\epsilon 2$ carrier and mean dosage status were similar, contrasting with the results of a former meta-analysis that suggested $\epsilon 2$ as a PD risk factor (Huang et al., 2004).

Given the likelihood of methodological bias, sample size limitations and ethnic variability, we approached this study with a focus on largely diminishing, if not eliminating these variables. The power of this study is significantly enhanced through its size; by analyzing APOE alleles in 3465 subjects, to our knowledge the largest study of APOE in PD to date, the negative association detected among all variable analyses is noteworthy. Furthermore, since more men than women are diagnosed with PD, sex-stratification analyses allowed us to detect potential gender bias in our study. We acknowledge that the control subjects' average age was significantly lower than that of the case groups (47.97, 65.93, respectively). However, given the incidence of PD in the global population of 0.5 to 1% among persons 65 to 69 years of age and 3% for those above 80 years of age, the likelihood of a significant proportion of controls developing PD pathology later in life is negligible. Thus, we believe this limitation had a minimal effect on the power to detect any association that we evaluated.

As there may be substantial differences between and within ethnicities in genetic risk for developing PD, the incorporation of genome-wide genotyping data obtained from a previous GWAS was essential to allow us to precisely account for genetic risk variation for PD attributable to intra-European population substructure and incorporate these factors into statistical models (International Parkinson Disease Genomics et al., 2011).

Despite previous suggestions of associations, our data reveals a lack of an association between APOE and PD. Notably the online database PDgene also reports a lack of association at this locus in a meta analysis of available Caucasian studies (<http://www.pdgene.org/geneoverview.asp?geneid=21>; data viewed January 12, 2012). Given our approach, and these online data; we believe our results are valid. With an abundance of genetic risk loci affirmatively identified, resource investment should be

directed towards detecting new risk factors as well as furthering an increased understanding of underlying mechanisms and potential therapeutic targets for this devastating disease.

Acknowledgments

This work was supported by the Intramural Research Program of the National Institute on Aging, National Institutes of Health, Department of Health and Human Services; project number Z01 AG000950-09. This study used samples from the NINDS Human Genetics Resource Center DNA and Cell Line Repository (<http://ccr.coriell.org/ninds>), as well as clinical data.

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Highlights

- > in this study we examine APOE genotypes in 2412 Parkinson's disease patients and 1053 neurologically normal controls
- > We failed to find an association between APOE genotype and risk for disease, age at onset, or memory loss
- > These data suggest there is no genetic association between common variants in APOE and Parkinson's disease

Table 1

Allele frequency distribution of APOE and Baseline Characteristics of all subjects Cases with PD Controls

	Cases with PD (n=2412)	Controls (n=1053)	All Males (Cases and Controls) (n=1912)	All Females (Cases and Controls) (n=1553)	Male Cases (n=1527)	Female Cases (n=885)	Male Controls (n=385)	Female Controls (n=668)
Age, mean $\pm$ SD, (years)	65.93 $\pm$ 10.57	47.97 $\pm$ 17.0	63.32 $\pm$ 13.1	56.97 $\pm$ 16.98	66.2 $\pm$ 10.46	65.49 $\pm$ 10.75	51.91 $\pm$ 16.0	45.69 $\pm$ 17.14
APOE isoform: E4 dose (freq)	627 (0.13)	293 (0.14)	528 (0.14)	392 (0.13)	418 (0.14)	209 (0.12)	110 (0.14)	183 (0.14)
APOE isoform: E4 carrier	580 (0.24)	270 (0.26)	490 (0.26)	360 (0.23)	390 (0.26)	190 (0.21)	100 (0.26)	170 (0.25)
APOE isoform: E2 dose	340 (0.07)	164 (0.08)	267 (0.07)	237 (0.08)	208 (0.07)	132 (0.07)	59 (0.08)	105 (0.08)
APOE isoform: E2 carrier	329 (0.14)	158 (0.15)	258 (0.13)	229 (0.15)	202 (0.13)	127 (0.14)	56 (0.15)	102 (0.15)
Age at onset $\pm$ SD (years)	58.93 $\pm$ 11.92	N/A	N/A	N/A	59.33 $\pm$ 11.76	58.24 $\pm$ 12.17	N/A	N/A
Memory loss at time of diagnosis: N (N with measure)	105 (n=879)	N/A	N/A	N/A	79 (n=571)	26 (n=308)	N/A	N/A
MMSE score: N $\pm$ SD (N with measure)	28.35 $\pm$ 2.58 (n=879)	N/A	N/A	N/A	28.21 $\pm$ 2.48 (n=571)	28.70 $\pm$ 2.59 (n=308)	N/A	N/A

Table 2

Logistic regression models 1 and 2 show estimates of the association between $\epsilon 4$ dosage, $\epsilon 4$ carrier status, $\epsilon 2$ dosage and $\epsilon 2$ carrier status with Parkinson's disease, age at onset of PD, presence of memory impairment and average MMSE, respectively. Model 1 is representative of all subjects successfully genotyped within the study. Model 2 utilizes a smaller sample size by eliminating population substructure variation obtained through previous genome-wide immunochip data [10]. Models were calculated based on data from both sexes as well as sex-stratified cohorts.

Samples in model	Outcome	Independent variable	Model 1: self-reported European ancestry with no covariates					Model 2: genotype confirmed European ancestry with population structure adjustment				
			Beta coefficient	Standard error	Z from logistic regression or T from linear regression	P-value	N samples in model	Beta coefficient	Standard error	Z from logistic regression or T from linear regression	P-value	N samples in model
Both genders	Parkinson's disease	$\epsilon 4$ dosage	-0.085	0.07531	-1.021	0.307	3465	-0.021	0.08382	-0.251	0.802	2441
		$\epsilon 4$ carrier status	-0.108	0.08515	-1.003	0.316	3465	-0.043	0.09517	-0.452	0.652	2441
		$\epsilon 2$ dosage	-0.111	0.09919	-1.092	0.275	3465	-0.043	0.10843	-0.393	0.694	2441
		$\epsilon 2$ carrier status	-0.072	0.10472	-1.063	0.288	3465	-0.049	0.11528	-0.426	0.670	2441
	Age at onset (years)	$\epsilon 4$ dosage	-0.021	0.50621	-0.142	0.887	3465	-0.307	0.6255	-0.491	0.623	2441
		$\epsilon 4$ carrier status	-0.964	0.5702	-0.037	0.970	3465	-0.362	0.7178	-0.505	0.614	2441
		$\epsilon 2$ dosage	-1.239	0.6734	-1.432	0.152	3465	-0.973	0.816	-1.192	0.233	2441
		$\epsilon 2$ carrier status	-0.486	0.708	-1.749	0.080	3465	-1.389	0.8689	-1.599	0.110	2441
	Memory loss at time of diagnosis	$\epsilon 4$ dosage	-0.481	0.1446	-3.361	0.001	2422	-0.205	0.20117	-1.021	0.307	1498
		$\epsilon 4$ carrier status	-0.648	0.16078	-2.995	0.003	2422	-0.162	0.22412	-0.721	0.471	1498
		$\epsilon 2$ dosage	-0.710	0.24843	-2.61	0.009	2422	-0.268	0.29256	-0.917	0.359	1498
		$\epsilon 2$ carrier status	-0.159	0.25964	-2.735	0.006	2422	-0.333	0.31407	-1.06	0.289	1498
	MMSE score at time of diagnosis	$\epsilon 4$ dosage	-0.139	0.2012	-0.791	0.429	681	-0.011	0.20691	-0.056	0.956	604
		$\epsilon 4$ carrier status	0.153	0.2295	-0.603	0.546	681	-0.077	0.23502	-0.327	0.744	604
		$\epsilon 2$ dosage	0.145	0.2663	0.574	0.566	681	0.166	0.2652	0.624	0.533	604
		$\epsilon 2$ carrier status	-0.049	0.2796	0.517	0.606	681	0.158	0.2794	0.566	0.572	604
Males only	Parkinson's disease	$\epsilon 4$ dosage	-0.023	0.11527	-0.429	0.668	1912	-0.019	0.12104	-0.155	0.877	1315
		$\epsilon 4$ carrier status	-0.127	0.1302	-0.174	0.862	1912	-0.019	0.14003	-0.133	0.894	1315
		$\epsilon 2$ dosage	-0.110	0.1536	-0.829	0.407	1912	-1.033	-0.167	0.16188	1315	0.302
		$\epsilon 2$ carrier status	-0.027	0.1631	-0.676	0.499	1912	-0.170	0.1735	-0.978	0.328	1315

Samples in model	Outcome	Independent variable	Model 1: self-reported European ancestry with no covariates					Model 2: genotype confirmed European ancestry with population structure adjustment				
			Beta coefficient	Standard error	Z from logistic regression or t from linear regression	P-value	N samples in model	Beta coefficient	Standard error	Z from logistic regression or t from linear regression	P-value	N samples in model
	Age at onset (years)	E4 dosage	0.207	0.62258	-0.043	0.965	1912	-0.034	0.74371	-0.046	0.964	1315
		E4 carrier status	-0.020	0.6934	0.299	0.765	1912	0.011	0.8628	0.013	0.990	1315
		E2 dosage	-0.102	0.85158	-0.023	0.981	1912	-0.642	1.0422	-0.616	0.538	1315
		E2 carrier status	-0.163	0.8907	-0.115	0.908	1912	-0.769	1.1063	-0.695	0.487	1315
Females only	Parkinson's disease	E4 dosage	-0.222	0.10631	-1.536	0.125	1553	-0.113	0.12739	-0.888	0.374	1126
		E4 carrier status	-0.057	0.1208	-1.838	0.066	1553	-0.147	0.14124	-1.041	0.298	1126
		E2 dosage	-0.073	0.13676	-0.419	0.675	1553	0.140	0.15286	0.915	0.360	1126
		E2 carrier status	-0.269	0.1441	-0.506	0.613	1553	0.136	0.16191	0.838	0.402	1126
	Age at onset (years)	E4 dosage	-0.629	0.8674	-0.31	0.757	1553	-1.064	1.1496	-0.926	0.355	1126
		E4 carrier status	-2.373	0.9988	-0.63	0.529	1553	-1.308	1.2898	-1.014	0.311	1126
		E2 dosage	-2.998	1.0981	-2.161	0.031	1553	-1.505	1.327	-1.134	0.257	1126
		E2 carrier status	-0.085	1.1631	-2.577	0.010	1553	-2.354	1.4184	-1.659	0.098	1126