PAPER

The effect of the apolipoprotein E gene polymorphisms and haplotypes on behavioural and psychological symptoms in probable Alzheimer's disease

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Background: Patients with Alzheimer's disease and dementia commonly suffer from behavioural and psychological symptoms of dementia (BPSD). A genetic component to BPSD development in Alzheimer's disease has been demonstrated. Several studies have investigated whether the exon 4 $\epsilon 2/\epsilon 3/\epsilon 4$ haplotype of the apolipoprotein E (APOE) gene is associated with BPSD, with variable results.

Objective: We investigated the exon 4 polymorphisms and extended this study to include promoter polymorphisms and the resultant haplotypes across the gene.

Methods: Our large independent cohort of 388 patients with longitudinal measures of BPSD assessed by the Neuropsychiatric Inventory was used to analyse whether any of these variants were associated with the presence of BPSD.

Results: We revealed several significant relationships before correction for multiple testing. The exon 4 haplotype was associated with hallucinations and anxiety, A-491T with irritability, T-427C with agitation/aggression and appetite disturbances, and T-219C with depression. Haplotype analyses of all variants did not reveal any statistically significant findings.

Conclusions: Our data and a review of previous studies showed a diversity of relationships, suggesting that these findings might be due to chance and so collectively do not support a role for the APOE gene in BPSD.

Many patients with dementia display behavioural and psychological symptoms of dementia (BPSD). Unlike cognitive decline, BPSD do not continuously exist in a patient once they have occurred. Genetic determinants of BPSD in Alzheimer's disease have been proposed from studies on families.^{1–3} It has been hypothesised that the genes that increase the risk for Alzheimer's disease may also determine the presence of BPSD.⁴ The $\epsilon 4$ allele of the apolipoprotein E (APOE) gene is the only risk factor robustly associated with Alzheimer's disease. However, previous investigations on APOE have produced inconsistent findings on BPSD, with some researchers reporting associations with a variety of different symptoms and alleles^{4–16} (summarised in the table provided online at <http://jnnp.bmjjournals.com/supplemental>), whereas others find no relevant relationships.^{17–33} We used a large independent clinical cohort of patients with Alzheimer's disease, with longitudinal data on BPSD to further extend these studies, and additionally investigated promoter polymorphisms of APOE, which have been shown to independently incur risk of Alzheimer's disease in some studies.³⁴

METHODS

Our cohort consists of 388 patients, classified as having mild to moderate dementia (Mini Mental State Examination score 10–25) at baseline, with Diagnostic and Statistical Manual of Mental Disorders-IV criteria for dementia of Alzheimer type. Also, 15% had an additional diagnosis of vascular dementia. The mean (standard deviation (SD)) age of onset of dementia was 74.4 (6.99) years and 56% were women.³⁵ Ethical approval and informed consent were obtained from participants and relatives (for a complete description of the cohort, see Courtney *et al*³⁵). Patients were assessed using the carer rated Neuropsychiatric Inventory up to 12 times over 166 weeks; all patients had at least three assessments, and the mean (SD) number of visits was 7.00 (2.65). Patients were dichotomised

into those having experienced a particular symptom and those that did not, over the study period. Genotype:phenotype correlations were examined using Pearson's χ^2 test, and by logistic regression using genotype, sex and age as covariates. Linkage disequilibrium and haplotypes were assessed using the Haploview program (<http://www.broad.mit.edu/mpg/haploview/>) and power calculations using <http://calculators.stat.ucla.edu/powercalc/>.

RESULTS

For a symptom existing in 50% of patients, our cohort had 80% power to detect associations of a modest effect size of 1.75. Statistical significance is presented before correction for multiple testing. Previous associations have been reported with the $\epsilon 2/\epsilon 3/\epsilon 4$ haplotype for delusions, hallucinations, psychosis, depression, agitation/aggression and anxiety, therefore these symptoms were investigated. A significant increase in the $\epsilon 3/\epsilon 3$ genotype and a decrease in the $\epsilon 3/\epsilon 4$ genotype were observed in patients with hallucinations compared with those without ($\chi^2 = 12.02$, $p = 0.017$; table 1). A significant decrease in the $\epsilon 3/\epsilon 3$ genotype and an increase in the $\epsilon 3/\epsilon 4$ genotype were observed in patients with anxiety compared with those without ($\chi^2 = 15.84$, $p = 0.003$; table 1). No associations were found with delusions, psychosis, depression or agitation/aggression (table 1).

As no previous studies have examined the promoter polymorphisms, all symptoms were considered. Four significant associations were found (table 1). An increase in the AT genotype of the A-491T polymorphism was observed in patients with irritability, compared with those without ($\chi^2 = 6.27$; $p = 0.04$). An increase in the TT genotype of the T-427C polymorphism was observed in patients with agitation/aggression ($\chi^2 = 7.19$;

Abbreviations: APOE, apolipoprotein E; BPSD, behavioural and psychological symptoms of dementia

Table 1 Apolipoprotein E gene $\epsilon 2/\epsilon 3/\epsilon 4$ and promoter polymorphism genotype and allele frequencies in patients with or without specific behavioural and psychological symptoms, measured in our cohort of 388 patients with Alzheimer's disease

	Genotype						Allele						
	%	22/24	23	33	34	44	χ^2	p Value	2	3	4	χ^2	p Value
$\epsilon 2/\epsilon 3/\epsilon 4$													
Delusions absent	38	0.01	0.12	0.28	0.47	0.13	7.98	0.92	0.07	0.57	0.36	4.70	0.095
Delusions present	62	0.03	0.06	0.24	0.50	0.18			0.05	0.52	0.43		
Hallucinations absent	57	0	0.10	0.24	0.51	0.15	12.02	0.017*	0.05	0.52	0.41	0.33	0.85
Hallucinations present	43	0.04	0.06	0.28	0.46	0.16			0.06	0.54	0.40		
Agitation/aggression absent	18	0	0.06	0.29	0.52	0.12	2.95	0.57	0.03	0.58	0.38	2.25	0.32
Agitation/aggression present	82	0.02	0.09	0.25	0.48	0.16			0.06	0.53	0.41		
Depression absent	20	0	0.10	0.26	0.51	0.14	2.12	0.72	0.05	0.56	0.39	0.35	0.84
Depression present	80	0.02	0.08	0.26	0.48	0.16			0.06	0.54	0.41		
Anxiety absent	13	0.04	0.04	0.46	0.30	0.17	15.84	0.003*	0.06	0.63	0.31	3.9	0.14
Anxiety present	87	0.02	0.09	0.23	0.51	0.15			0.05	0.53	0.42		
	Genotype						Alleles						
%							χ^2	p Value	Alleles		χ^2	p Value	
A-491T													
Irritability absent	18	AA		AT	TT		6.27	0.04*	A	T		0.71	
Irritability present	82	0.82 0.80	0.12 0.19	0.06 0.01					0.88 0.89	0.12 0.11	0.14		
T-427C													
Agitation/aggression absent	18	TT		CT	CC		7.19	0.03*	T	C		0.19	
Agitation/aggression present	82	0.77 0.86	0.24 0.12	0 0.02					0.88 0.92	0.12 0.08	1.71		
Appetite disturbances absent	35	0.79	0.18	0.04			6.64	0.04*	0.88	0.12	6.82	0.009*	
Appetite disturbances present	65	0.87	0.12	0.01					0.93	0.07			
T-219G													
Depression absent	20	TT		TG	GG		4.69	0.09	T	G		0.02	
Depression present	80	0.21 0.33	0.40 0.42	0.39 0.26					0.41 0.54	0.59 0.46	5.45		

*Statistically significant before correction for multiple testing.

$p = 0.03$) and with appetite disturbances ($\chi^2 = 6.64$; $p = 0.04$). Finally, an increase in the T allele of the T-219G polymorphism was found in patients with depression ($\chi^2 = 5.45$; $p = 0.02$). Haplotype analysis showed linkage disequilibrium between the variants ($D = 0.86-1$), which only correlated weakly ($r^2 = 0.01-0.03$), and no significant associations with any of the symptoms (data not shown).

Logistic regression showed that small (between 1% and 4%), but statistically significant, contributions were made by age and sex to a model predicting symptoms, but no effect of any of the *APOE* variants was found.

DISCUSSION

Apolipoprotein E has many roles, and one or more of these actions could be involved in the development of BPSD.³⁴ Inconsistent findings have been found in previous studies of the exon 4 *APOE* polymorphisms with BPSD, with statistically significant relationships to anxiety, delusions, hallucinations, psychosis, depression and agitation/aggression being reported.⁴⁻¹⁶ It should be noted that in several reports, corrections for multiple testing were not made. Our findings do not remain significant after correction for multiple testing, and so our data and a large number of previous studies conclude that there is no association with BPSD.¹⁷⁻³³

Not all measures of BPSD reported in the literature include an assessment of anxiety. Two studies using longitudinal assessments with the Neuropsychiatric Inventory found no association with anxiety,^{22, 24} and no association was found when assessed by the Behavioural Pathology in Alzheimer's Disease Rating Scale in a cross-sectional study design.²⁹ Robertson *et al*¹⁶ reported higher levels of anxiety in $\epsilon 4/\epsilon 4$ carriers compared with $\epsilon 3/\epsilon 3$ in both men and women ($p < 0.05$) and higher levels compared with $\epsilon 3/\epsilon 4$ only in men ($p < 0.05$), in 125 patients. We do not support these findings using the same methods of analysis (data not shown). Interestingly, Robertson *et al* reported that *APOE* null mice carrying the human $\epsilon 4$ transgene had higher anxiety ratings than those expressing either wild-type murine *APOE* or the human $\epsilon 3$.¹⁶ We found an association with the $\epsilon 3/\epsilon 4$ genotype, but not with the $\epsilon 4/\epsilon 4$ genotype or the $\epsilon 4$ allele.

Previous associations with hallucinations have been contradictory, with two copies of $\epsilon 4$ being reported as protective,¹⁴ and $\epsilon 4$ being associated with increased risk.¹³ We found association with the $\epsilon 3/\epsilon 4$ genotype, adding further inconsistencies to reported findings. Hallucinations are sometimes incorporated into a measure of psychosis. Previous associations have again been inconsistent, with significant associations being reported for the $\epsilon 4$ allele,⁹ and for $\epsilon 3/\epsilon 4$ versus $\epsilon 4/\epsilon 4$ carriers.⁷ To allow similar comparisons, we investigated a measure of psychosis in our cohort; we observed a non-significant increase in the $\epsilon 4$ allele (data not shown). However, a larger number of studies found no associations with hallucinations or measures incorporating this symptom.^{12, 17, 19-22, 25, 29}

The associations we found between anxiety and hallucinations with the $\epsilon 3/\epsilon 4$ heterozygote but not with the $\epsilon 4/\epsilon 4$ genotype or the $\epsilon 4$ allele are difficult to explain by a simple model. Nevertheless, the possibility that there are complex mechanisms whereby the heterozygote form may modify risk cannot be ruled out. However, it is more likely our findings are due to chance.

Our findings do not support the protective effect of the $\epsilon 2$ allele,¹⁵ or the risk associated with absence of the $\epsilon 4$ allele¹⁰ for depression. Our findings are consistent with those of others.^{7, 9, 13, 14, 21, 23, 26, 27, 29-32} Our findings also do not support the previous association for the $\epsilon 4$ allele being a risk factor for agitation/aggression.¹²

This is the first reported investigation of associations between BPSD and A-491T, T-427C and T-219G *APOE* promoter polymorphisms. Our preliminary hypothesis-generating findings require further exploration in large independent cohorts.

We have reviewed and extended the literature reporting studies of *APOE* in BPSD. Despite some reasonably sized cohorts having been investigated, it is clear that there are no consistent effects between studies, unlike the robust effect of the $\epsilon 4$ allele and the risk of Alzheimer's disease. The inconsistencies observed could be due to spurious associations, particularly where studies are subject to considerable multiple testing. Variability in study design is also a likely contributing factor, particularly where there is focus on certain symptoms, different assessment tools and analytical procedures used, variation between cohort selection criteria, and size and stage of dementia examined. We, along with some others in the field, advocate the use of longitudinal follow-up because BPSD are often transiently expressed. It is also possible that *APOE* plays only a minor part in the development or modulation of certain behavioural symptoms that will only be detectable in appropriately large and well-designed studies. However, collectively, our findings and those reviewed do not support a role for *APOE* in BPSD.

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Supplementary table available at <http://jnnp.bmjournals.com/supplemental>

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