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Does apolipoprotein E genotype modify the clinical expression of ALS?

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Background: The presence of the apolipoprotein E (ApoE) 4 genotype is associated with an earlier age of onset for Alzheimer's disease (AD) and several other neurodegenerative disorders. The objective of this study was to investigate the effect of ApoE genotypes on the clinical course of amyotrophic lateral sclerosis (ALS).
Methods: Eight hundred and fifty-two consecutive patients with sporadic ALS evaluated at a tertiary care center were investigated for the effect of ApoE genotype on age of onset, rate of motor disease progression, cognitive functioning, and survival in ALS.
Results: The frequencies of individual ApoE genotypes did not differ between patients with ALS and ALS-free Caucasian populations. Patients with different ApoE genotypes did not differ in the age of onset for ALS (years) (ApoE2 = 57.8 ± 13.7, ApoE3 = 57.3 ± 13.7, ApoE4 = 57.7 ± 13.2; $P = 0.97$), the rate of disease progression (Appel ALS score/month) (ApoE2 = 2.91 ± 2.66, ApoE3 = 2.67 ± 2.66, ApoE4 = 2.61 ± 2.47; $P = 0.89$), cognitive status (% cognitively impaired) (ApoE2 = 31.7, ApoE3 = 26.8, ApoE4 = 34.3, $P = 0.28$), or survival in years (ApoE2 = 3.79 ± 3.70, ApoE3 = 3.17 ± 2.27, ApoE4 = 3.05 ± 1.75; $P = 0.85$).
Conclusions: Our results suggest that ApoE genotype does not modify clinical course of sporadic ALS, in stark contrast to the influence of ApoE genotype on the disease course of AD and other neurodegenerative disorders.

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that manifests as motor weakness, and in many instances, it is accompanied by cognitive changes of varying severity [1]. To gain insight into the pathophysiology underlying ALS, factors that may influence the course of the disease have been sought. Thus far, evidence suggests that the duration of ALS is longer when the age of onset is younger (< 45), in men, and in limb-onset disease [2,3]. Certain metabolic perturbations have also recently been associated with an altered course for ALS, including dyslipidemia and diabetes mellitus [4,5].

Apolipoprotein E (ApoE) genotype has been implicated as a risk factor for several neurological disorders, because of its role in lipid transport, neuronal repair, calcium homeostasis, and antioxidant activity [6–9]. There are three major isoforms of ApoE: ApoE2, ApoE3, and ApoE4, some of which have been identified as risk factors or are known to influence the disease course of neurological diseases. ApoE4, for example, is a major risk factor for the earlier onset sporadic AD. It has a dose-dependent effect on age of onset [10] and rate of cognitive decline [11]. Additionally, the ApoE4 genotype may influence the risk, onset, or progression of a number of other neurological conditions, including Wilson's disease [12], multiple sclerosis [13], Creutzfeldt-Jakob disease [14], traumatic brain injury [15], anuerysmal subarachnoid hemorrhage [16], cerebral amyloid angiopathy [17], Parkinson's disease [18], and dementia with Lewy bodies [19].

Apolipoprotein E has also been suggested to have a role in the clinical expression of frontotemporal dementia (FTD), which has a high degree of clinical, genetic, and pathological overlap with ALS. ApoE2 and E4 have been variably shown to increase the risk of FTD [20,21], whereas ApoE4 has been suggested to have a disease-modifying effect in FTD [22].

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Apolipoprotein E genotype has been proposed as a risk factor and disease-modifier in ALS; however, there has not been a consensus regarding the relationship between ApoE and the clinical characteristics of ALS. Apo 2/3 genotype has been suggested to have a protective effect in ALS [23,24]. Other studies suggest an earlier onset of ALS symptoms in patients with the E4 allele [25,26]. However, one study reported that ALS patients with the E4 isoform have a comparable age of onset to other Apo isoforms [27]. No studies reported a difference in allelic frequencies between normal populations and patients with ALS.

Given the unclear relationship between ApoE and ALS and its role in other neurodegenerative disorders especially FTD, it is important to further evaluate its influence on the course of ALS. This knowledge may help to ascertain the prognostic significance of ApoE genotype in patients with ALS and may offer more clues about the pathophysiology underlying the motor and cognitive impairment observed in ALS. We prospectively investigated the influence of ApoE genotypes on age of onset, rate of disease progression, cognitive functioning, and survival rate in a large cohort of patients diagnosed with ALS.

Methods

Study design and participants

This was a prospective study of ALS patients with varying ApoE genotypes. The sample was recruited from the Baylor College of Medicine (BCM) Vicki M. Appel MDA/ALS clinic from January 2000 through October 2007. Inclusion criteria were that patients had to have a diagnosis of definite/probable ALS based on El Escorial criteria [28]. Patients were excluded if their diagnosis of motor neuron disease was uncertain; there was a diagnosis of a motor neuron disease other than ALS.

The study was approved by Institutional Review Board of BCM. The funding source did not have a role in study design, patient recruitment, clinical assessment, blood sampling, ApoE testing, data storage, data analysis, and manuscript writing.

Clinical assessment

All patients received a baseline motor assessment by two board-certified neurologists blinded to the study design, on their first clinic visit. The severity of motor impairment was gauged using the Appel amyotrophic lateral sclerosis score (AALSS). AALSS is a validated measure of severity of motor impairment in ALS, which consists of five subscores: bulbar, respiratory, muscle strength, lower extremity function, and upper extremity function. The total AALSS ranges from 30 for healthy subjects to 164 for those with maximal impairment [29]. Patients were also queried about the duration of their motor symptoms at baseline.

Definitions

The age of onset was defined as the chronological age of the patient at time of ALS symptom onset. This information was collected at the time of the first clinical visit by two board-certified neurologists blinded to the study design.

The rate of disease progression was calculated using serial AALSSs, including baseline and each follow-up visit. The severity of motor symptoms was gauged by the AALSS. The difference between the first and the last available AALSS scores for each individual patient divided by the number of months between the two evaluations determined the rate of disease progression. This variable was calculated at the completion of the study period by a research technician unblinded to the study design. It was expressed in AALSS/month.

Two hundred and sixty-five patients from the cohort also received a comprehensive neuropsychological evaluation at baseline by two clinical neuropsychologists blinded to the study design. The patient cohort was classified according to whether cognition was intact or impaired (ALSci) according to the consensus criteria defined by Strong *et al.* [3]. The neuropsychological battery of tests used to determine this classification included the Stroop Color and Word test [30], lexical fluency (COWA-FAS) [31], semantic fluency (Animals) [31], and Trail Making Test parts A and B [32]. Only test subscores for which motor performance was controlled in the measure (Stroop interference, Trails B-A) were considered to be a valid reflection of cognitive abilities [3].

Survival was defined as the number of years a patient with ALS survived after the onset. This information was collected at the completion of the study period by two board-certified neurologists blinded to the study design. For the patients who were alive at the completion of the study (October 2007), the information was sought again after 18 months (March 2009). This was done to ensure that the survival data were available for a maximum number of patients.

ApoE genotyping

DNA was extracted from venous blood specimens using a standard procedure. ApoE genotyping was determined as described by Hixson and Vernier [33]: The extracted DNA was amplified using polymerase chain

reaction, digested by restriction enzyme *HhaI*, and DNA fragments were separated using 8% polyacrylamide gel electrophoresis, then visualized under UV light.

The rs-numbers for individual apolipoprotein genotypes are: ApoE2/2: rs76353203; ApoE2/3: rs429358; ApoE2/4: rs7412; ApoE3/3: rs1800206; ApoE3/4: rs28391574; ApoE4/4: rs63750110. These were extracted by performing genotype name search on dbSNP database available at http://www.ncbi.nlm.nih.gov/snp.

Participants who had genotype ApoE2/2 and ApoE2/3 were classified as ApoE2 carriers, those with genotype ApoE3/4 and ApoE4/4 were classified as ApoE4 carriers, and those with ApoE3/3 were classified as ApoE3 carriers. ApoE2/4s were excluded because of their small number ($n = 10$) and ambiguity of classification.

Statistical analysis

The data were validated through dual entry and was examined using SPSS version 16 (SPSS Inc, Chicago, IL, USA). Associations between demographics and clinical characteristics (gender, ethnicity, site of onset) and different ApoE genotypes were analyzed through chi-squared tests. The effect of ApoE polymorphism on ALS age of onset, rate of disease progression, and survival was tested using non-parametric analyses of variance (Kruskal–Wallis ANOVA). Associations were also examined by comparing the disease variables between patients with and without one particular ApoE allele using Mann–Whitney *U*-tests. The association between different ApoE genotypes and cognitive status of the patients was tested through chi-squared test.

Results

Demographics

A total of 971 patients qualified for inclusion. Of these, 862 patients consented for ApoE genotyping. Ten patients were excluded because of incomplete data. Hence, a total of 852 patients were included in the final analyses. The distribution of individual ApoE genotypes in our sample was E2/2 0.5% ($n = 4$), E2/3 10.4% ($n = 89$), E3/3 63.0% ($n = 537$), E2/4 1.2% ($n = 10$), E3/4 23.1% ($n = 197$), and E4/4 1.8% ($n = 15$). These ApoE gene frequencies were consistent with those reported in disease-free Caucasian population studies (Fig. 1) [31,32].

The range of AALSS scores for the cohort was 35–95 with a mean score of 55.8 ± 15.9. The mean duration of symptoms before the diagnosis of ALS was 10.5 ± 5.1 months. There were discrepancies between the patients and the caregiver's reports regarding the date of motor symptom onset, which reduces the reliability of this subset of data. The clinical and demographic characteristics of the population are presented in Table 1. The distribution of ApoE genotypes did not differ between groups based on gender, ethnicity, or the site of symptom onset (Table 1).

ApoE genotype and ALS motor disease variables

There were no differences in the age of onset, rate of disease progression, and survival in patients with ApoE genotypes 2/2 and 2/3 vs. 3/3 vs. 3/4 and 4/4 (Table 2). There were also no associations between age of onset, rate of progression, and survival when we compared particular ApoE alleles versus the remaining sample; for example, patients with an ApoE4 allele versus those with no E4 alleles (Table 3).

ApoE genotype and Neuropsychological performance of patients with ALS

We compared the cognitive status between patients with different ApoE genotypes (Table 4). The percentage of patients with impaired cognition (ALSci) did not differ significantly between the genotypes.

Discussion

The frequency of ApoE genotypes did not differ between our cohort and two large disease-free groups.

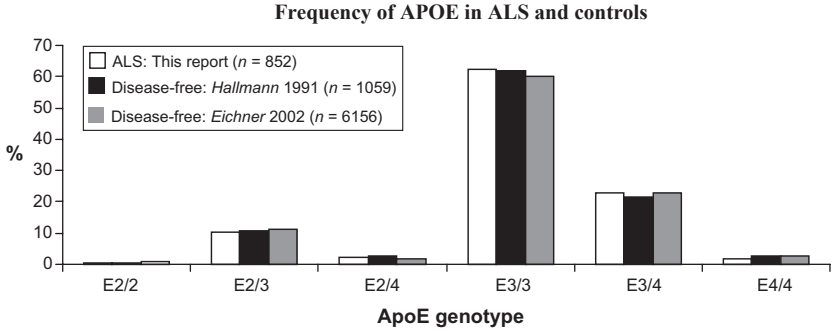

Figure 1 Apolipoprotein E genotype distribution in amyotrophic lateral sclerosis patients and disease-free Caucasians.

Table 1 There were no differences in apolipoprotein E (ApoE) genotype distribution according to gender, ethnicity, and site of onset of amyotrophic lateral sclerosis using the Mann–Whitney *U*-test[a]

	ApoE genotypes			
	E2 (2/2, 2/3) (n = 93)	E3 (3/3) (n = 537)	E4 (3/4, 4/4) (n = 212)	All patients
Gender				
Male/female (%)	60.9/39.1	64.9/35.1	61.4/38.6	63.5/36.5
Ethnicity				
Caucasian/other (%)	90.9/9.10	87.5/12.5	87.7/12.3	87.9/12.1
Site of onset				
Bulbar/limb/unknown (%)	28.2/71.8/0.00	29.1/70.3/0.60	29.5/69.0/1.50	29.1/70.1/0.80

[a]Patients with ApoE 2/4 were excluded from this analysis.

Table 2 There was no association between apolipoprotein E (ApoE) genotypes and age of onset, rate of disease progression, and survival in amyotrophic lateral sclerosis using the Kruskal–Wallis test[a]

	ApoE genotype			
	E2 (2/2, 2/3) (n = 93)	E3 (3/3) (n = 537)	E4 (3/4, 4/4) (n = 212)	P-value
Age of onset (years)				
Mean ± SD	57.5 ± 13.5	57.3 ± 13.7	57.7 ± 13.2	0.97
Rate of disease progression				
Mean ± SD (AALSS/month)	2.91 ± 2.66	2.67 ± 2.66	2.61 ± 2.47	0.89
Survival (years)				
Mean ± SD	3.79 ± 3.70	3.16 ± 2.38	3.05 ± 1.75	0.85

[a]Patients with ApoE 2/4 were excluded from this analysis. AALSS, Appel amyotrophic lateral sclerosis score.

Table 3 There were no significant associations between age of onset, rate of disease progression, and survival and apolipoprotein E (ApoE) genotypes when comparing each genotype to all patients without that genotype using the Mann–Whitney *U*-test[a]

	ApoE genotype					
	E2 (n = 103)	No E2 (n = 749)	E3 (n = 823)	No E3 (n = 29)	E4 (n = 222)	No E4 (n = 630)
Age of onset (years)						
Mean ± SD	57.8 ± 13.7	57.4 ± 13.5	57.4 ± 13.6	59.7 ± 11.5	57.9 ± 13.2	57.3 ± 13.7
Rate of disease progression (AALSS/month)						
Mean ± SD	2.91 ± 2.66	2.65 ± 2.60	2.68 ± 2.61	2.53 ± 2.63	2.62 ± 2.46	2.70 ± 2.66
Survival (years)						
Mean ± SD	3.79 ± 3.70	3.13 ± 2.22	3.17 ± 2.27	3.34 ± 3.84	3.05 ± 1.75	3.26 ± 2.63

[a]Patients with ApoE 2/4 were included in this analysis. AALSS, Appel amyotrophic lateral sclerosis score.

There were also no differences in the distribution of ApoE genotypes in patients with ALS when parsed by gender, ethnicity, or site of onset. Similarly, there was no association between age of onset, rate of disease progression of motor symptoms, or survival in ALS and ApoE genotypes (2/2 and 2/3 vs. 3/3 vs. 3/4 and 4/4). This was also true when each individual genotype was compared to all patients without that genotype; for example, E2 versus no E2. Moreover, the distribution of ALS patients with normal cognition versus impaired cognition did not differ significantly amongst ApoE genotypes.

Previous investigations

The effect of ApoE gene polymorphisms on disease course of ALS has been a focus of extensive research in the last few years, but the results have been variable and inconclusive. Moulard *et al*. [23] were the first to report longer survival in ALS patients with ApoE2/3 genotype

Table 4 The percentage of patients with normal versus impaired cognition did not differ between apolipoprotein E (ApoE) genotypes

Cognitive classification (%)	ApoE			
	E2 (2/2, 2/3) (n = 35)	E3 (3/3) (n = 170)	E4 (3/4, 4/4) (n = 60)	P-value
Intact cognition	68.3	73.2	65.7	
ALSci	31.7	26.8	34.3	0.28

in a case–control study of 130 patients with ALS. To the best of our knowledge, this finding has not yet been replicated. A protective role for ApoE2 in ALS was also suggested by Li *et al.* [24] who noted that ApoE2 genotype is associated with a later onset of motor symptoms in ALS (3 years) based on a familial association analysis.

On the other hand, ApoE4 genotype has been suggested to have a deleterious effect on the disease course of ALS. Drory *et al.* [25] observed a 50% reduction in survival in ALS patients carrying the ApoE4 genotype. Similarly, Zetterberg *et al.* [26] showed an earlier age of onset for ALS in patients carrying ApoE4 genotype. However, one study (n = 170) did not show an association between ApoE4 genotype and age of onset in ALS [27]. One study showed a correlation between plasma ApoE levels and survival in ALS; however, no significant effect of specific ApoE genotypes on ALS disease course was observed [34].

The variability in the results of these studies could be attributable to a number of factors. First, there is a variety of ways in which the effect of apolipoprotein on ALS disease parameters has been analyzed previously. This includes a comparison amongst genotype groups, amongst individual alleles or family-based association analysis. Second, the specific ApoE genotype frequencies have varied in these studies, which may have altered the inferential statistics and hence the results.

To the best of our knowledge, we have carried out the largest prospective study of the effects of ApoE polymorphisms on the motor findings of ALS and the first study to examine the association between ApoE polymorphisms and cognitive functioning in ALS. This was carried out to try to clarify the role of ApoE2 and 4 in the clinical characteristics of ALS, considering previous reports. Our cohort was large enough to have the same distribution of ApoE genotypes as disease-free Caucasian populations in the region [35,36]. And, we examined potential associations between ApoE genotype and multiple clinical features, both motoric and cognitive. However, we did not find an association between any ApoE alleles and any clinical parameters.

This appears to be a very important negative result. It suggests that it may not be useful to devote more resources to the question. Based on the previous studies and the evidence presented here, it may be inferred that specific ApoE genotypes do not have a prognostic significance in ALS. However, a correlation between plasma ApoE levels and survival in ALS has previously been suggested, which warrants further investigation [34].

Cognition in ALS

As noted, we found no relationship between cognitive function and ApoE genotypes. However, there are several potential complications in studying cognitive function in ALS that need to be considered. First, the motor impairment of the disease can confound the neuropsychological test results. We addressed this confounder in our analyses by including only those neuropsychological tests that excluded motor abilities or had a motor control incorporated in the measure. Second, numerous co-morbid medical and psychiatric diseases can confound the neuropsychological test results.

Third, pre-morbid intellectual functioning can confound the results of a cross-sectional group investigation. We used the AMNART [37] as an estimate of premorbid intellectual functioning at the baseline evaluation to ensure intellectual equality within our sample. Fourth, the neuropsychological evaluation was conducted only at one time point at the baseline evaluation. Most of the patients were in the initial stages of the disease, as suggested by the mean AALS score of 55, which limits the generalizability of the findings. It is difficult to ascertain the influence of ApoE gene polymorphisms on cognition along the full course of the disease because of the lack of longitudinal neuropsychological data. Nonetheless, it appears that at least at baseline, cognitive function is not influenced by the ApoE genotype.

Pathophysiology

ApoE4 genotype is known to confer vulnerability to a wide variety of neurodegenerative processes. It is hypothesized that this effect may result from an impaired neuronal remyelination and axonal regeneration in ApoE4 carriers [38]. It is unclear why this effect would be more prominent in certain neurodegenerative processes, like AD, than in others, like ALS. One possibility is that certain neurons may be selectively vulnerable. For example, neurons may respond differently when subjected to a similar metabolic stressor [39]. Thus, whilst ApoE genotype may confer vulnerability to a particular stressful condition in neurons, this may be more deleterious for one type of neuron than others, which are spared.

Conclusion

This study suggests that ApoE genotype does not have a disease-modifying effect on patients with ALS. This is in striking contrast to AD where there is a significant association between ApoE4 genotype and lower cognitive performance. This study supports the data, suggesting that different neurodegenerative disorders have different underlying risk factors for the disease, their profile, and their rate of progression.

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