Neurogenetics (2004) 5:209–213
DOI 10.1007/s10048-004-0193-0

ORIGINAL ARTICLE

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Apolipoprotein E is associated with age at onset of amyotrophic lateral sclerosis

Received: 14 June 2004 / Accepted: 16 August 2004 / Published online: 2 October 2004

Abstract Apolipoprotein E (APOE) is a confirmed risk factor for Alzheimer disease. APOE is also involved in several other neurodegenerative disorders, including Parkinson disease and multiple sclerosis. Previous studies of amyotrophic lateral sclerosis (Lou Gehrig disease, ALS) have investigated the effect of APOE on the risk of developing ALS, age at onset, site of onset, and duration of the disease. The results have been inconsistent, possibly due to small sample sizes and complete reliance on case-control data. No family-based association studies were performed. To address these limitations, we investigated the relationship between APOE functional polymorphisms and age at onset of ALS in a large set of 508 families. We treated age at onset as a quantitative trait and performed family-based association analysis using the TDT_{Q5} method. APOE-2 is protective against earlier onset (P=0.001) with an average age at onset of APOE-2 carriers approximately 3 years later than that of non-APOE-2 carriers. Similar to our previous report, we did not find APOE associated with ALS risk. Our findings suggest that APOE may express its strongest effect through age at onset rather than on risk.

Keywords Amyotrophic lateral sclerosis · Apolipoprotein E · Age at onset · Association · Quantitative trait

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Introduction

Amyotrophic lateral sclerosis (Lou Gehrig disease, ALS) is an age-dependent, neurodegenerative disorder of motor neurons with both sporadic and familial forms. It is a rapidly progressive disease that results in progressive paralysis until death occurs, usually within 5 years of onset. The cause of most types of ALS is unknown and the disease is untreatable. ALS arises as a dominantly inherited trait in approximately 10% of ALS cases. Approximately 25% of autosomal dominant ALS is caused by mutations in the Cu/Zn superoxide dismutase (SOD1) gene [1, 2]. A recessive form of juvenile-onset ALS has been associated with ALS in chromosome 2q33 [3, 4]. Additional chromosomal regions have been identified in

chromosomes 9q [5], 15q [6], 16q [7, 8, 9], 18q [10], 20 [8], and X [11] for familial ALS. However, the underlying genetic causes of the remaining forms of ALS are still unknown.

The apolipoprotein E (*APOE*) gene is a confirmed genetic risk factor that influences both risk and age at onset in Alzheimer disease [12, 13] and Parkinson disease [14, 15]. *APOE* functional polymorphisms have been examined for association with different phenotypes of ALS, including the risk of developing ALS, age at onset, site of onset, and duration of the disease [16, 17, 18, 19]. Most studies found no association between *APOE* and the risk of developing ALS [16, 17, 19]. However, for other phenotypes, no consensus has been made [17, 19, 20, 21, 22]. In particular, the studies of *APOE* affecting age at onset and site of onset in ALS have been the most inconsistent. Moulard et al. [19] found that bulbar-onset ALS patients with an *APOE-4* allele showed an average 6 years earlier age at onset than those without *APOE-4*. Conversely, two later studies [17, 20] reported no significant difference in age at onset between patients with and without an *APOE-4* allele in either the overall ALS dataset or the bulbar-onset ALS subset. These previous studies all used unrelated case-control samples to test allelic association between the groups of interest. No family-based association tests have been performed. In addition, most studies examining age at onset of ALS compared the average ag at onset between different *APOE* genotypes. This approach does not extract the full amount of information from the data.

In this study, we used family-based association approaches for quantitative and qualitative traits to assess the relationship of *APOE* with age at onset of ALS and the risk of developing ALS, respectively.

Materials and methods

Family data

The dataset consists of 446 singleton families with only 1 affected in each family (238 parent-child trio and 208 discordant sibpairs) and 62 multiplex families with at least 2 affected relatives (Table 1). All families have at least 1 affected individual with age at onset information available. The family ascertainment was conducted by two clinical sites, Northwestern University Medical School (396 families) and Massachussetts General Hospital (112 families)**.** All patients diagnosed with ALS were examined by a neurologist (T.S, R.B. and colleagues) and met the El Escorial criteria [23] for the diagnosis of ALS. Clinical information, including patient's age at onset (defined as the time at which symptoms first occurred), was obtained at the time of examination. Because ALS is characterized by sudden onset and rapid progression, age at onset is essentially equivalent to age at examination. All participants or their legal representatives gave informed consent prior to joining the study, and data were collected according to protocols approved by each contributing group's institutional review board.

Genotyping

Genomic DNA was extracted from whole blood according to established protocols [24]. We genotyped *APOE* functional polymorphisms in a total of 1,462 samples. *APOE* genotyping was performed as previously described [25]. Substantial quality control procedures were followed [26]. Genotype data were uploaded into the PEDIGENE® database and merged into the LAPIS management system for data analysis [27].

Table 1 Summary of the family data, age at onset, *and* APOE allele frequencies for the overall amyotrophic lateral sclerosis (ALS), early onset ALS (EOALS), and late-onset ALS (LOALS) datasets

	ALS	EOALS[a]	LOALS[b]
No. of families			
Overall data	508	173	335
Multiplex families	62	18	44
Singleton families	446	155	291
Average age at onset±SD (No. of affected with age at onset)			
Overall data	48.1±13.1 (601)	36.7±9.6 (217)	54.6±9.7 (384)
Multiplex families	53.4±12.7 (155)	45.2±12.4 (62)	59.0±9.4 (93)
Singleton families	46.3±12.6 (446)	33.3± 5.3 (155)	53.3±9.4 (291)
***APOE* frequencies**			
APOE-2	8.7%	8.0%	9.3%
APOE-3	75.9%	79.7%	73.4%
APOE-4	15.4%	12.3%	17.3%

[a] EOALS families with at least one affected with age at onset <40 years
[b] LOALS families with all affecteds with age at onset ≥40 years

Statistical analysis

All genotype data were examined for mendelian consistency, using the PedCheck program [28]. *APOE* functional polymorphisms were tested for Hardy-Weinberg equilibrium (HWE) in the affected group (1 affected from each family) and the unaffected group (1 unaffected from each family). An exact test implemented in Genetic Data Analysis (GDA) [29] was used to test HWE, in which 3,200 replicate samples were simulated for estimating the empirical *P* value.

To investigate the effect of *APOE* on age at onset of ALS, we treated age at onset as a quantitative trait. We used the TDT_{Q5} method [30], which utilizes parent-child triad data, implemented in the QTDT program [31] (http://www.sph.umich.edu/csg/abecasis/QTDT/) to test the allelic association between *APOE* and age at onset of ALS. The method proposed by Rabinowitz [32], which uses parents and multiple siblings data, served as a second method to confirm the results. To test whether ascertainment center has an effect on the results, we incorporated center as a covariate in both tests.

The pedigree disequilibrium test (PDT) [33] was employed to test *APOE* association with ALS risk. Two PDT statistics were used: the PDT-sum statistic [34], which examines allelic effects, and the genotype-PDT, which examines genotypic effects [35]. We performed the PDT analysis on the full dataset as well as stratified datasets based on an age at onset cutoff of 40 years. The late-onset subset (LOALS) consists of families with all affecteds having age at onset greater than or equal to 40 years. The early onset subset (EOALS) consists of families with at least 1 affected with age at onset less than 40 years.

Results

For each *APOE* genotype, the number of unaffected and affected individuals of all family members is summarized in Table 2. Since these counts were taken from family

Table 2 Summary of *APOE* genotypes for the affecteds and unaffecteds in the overall ALS, multiplex, and singleton family datasets

	Total genotype counts (affected vs. unaffected)		
***APOE* genotype**	**ALS (1,462)[a]**	**Multiplex families (362)**	**Singleton families (1,100)**
APOE-22	5 (2, 3)	0 (0, 0)	5 (2, 3)
APOE-32	189 (58, 131)	50 (13, 37)	139 (45, 94)
APOE-33	849 (325, 524)	209 (78, 131)	640 (247, 393)
APOE-42	58 (23, 35)	7 (4, 3)	51 (19, 32)
APOE-43	331 (110, 221)	86 (25, 61)	245 (85, 160)
APOE-44	30 (7, 23)	10 (2, 8)	20 (5, 15)

[a]Total number of samples genotyped for *APOE* in each dataset

data, one should take into account familial correlation in interpretation of the data. Overall, *APOE-33* is the most-frequent genotype. The allele frequencies in our overall ALS family dataset were 8.7% for *APOE-2*, 75.9% for *APOE-3*, and 15.4% for *APOE-4*. A similar range of *APOE* allele frequencies was observed in the overall ALS, EOALS, and LOALS subsets (Table 1).

Age at onset ranged from 18 to 81 years, with average age at onset (±SD) being 48.1±13.1 years in the overall ALS dataset. Although the average age at onset tends to be higher in multiplex than singleton families in all three subsets, a large standard deviation of age at onset is found in the multiplex families due to their small sample sizes (Table 1). No deviation from HWE was found in either affected or unaffected samples for *APOE*.

In the TDT $_{Q5}$ analysis, *APOE-2* showed the strongest evidence of positive association with age at onset (P= 0.001) and *APOE-3* showed a marginally significant P value (P=0.058). Similar results were obtained with the Rabinowitz method (P =0.008 for *APOE-2* and P=0.054 for *APOE-3*). Furthermore, the association between *APOE-2* and age at onset was not changed after taking into account the ascertainment effect as a covariate in the tests. The positive association between *APOE-2* and age at onset indicates that age at onset increases as the number of *APOE-2* alleles transmitted from parents increases. This is also seen in the mean age at onset in each *APOE-2* carrier group, in which the average age at onset was 48±13.2 years for non- *APOE-2* carriers (433 individuals), 51±11.3 years for one- *APOE-2* carriers (81 individuals), and 64±3.5 years for two- *APOE-2* carriers (2 individuals). Overall, we found approximately a 3-year difference between *APOE-2* carrier and non- *APOE-2* carrier groups (51 vs. 48 years) for age at onset.

To update our analyses [17] of *APOE* and the risk of ALS, we examined all 508 families for both allelic and genotypic effects. No significant results were observed (global P=0.276 for alleles and global P=0.491 for genotypes). For the association between *APOE* and the risk of ALS in the stratified subsets, we found that *APOE-2* significantly reduced the risk of ALS in the EOALS subset (P=0.012), but not in the LOALS subset.

In order to investigate the relationship of *APOE-2* with the risk and age at onset of ALS, we also examined the pattern of *APOE-2* carriers in each dataset. We randomly selected 1 affected and 1 unaffected with *APOE* genotype available from each family. The percentages of *APOE-2* carriers in affected and unaffected groups, respectively, for each subset are depicted in Fig. 1. The frequencies of *APOE-2* carriers in unaffecteds are similar (15.9%–17.6%) among EOALS, LOALS, and the overall ALS datasets. For the affecteds, the EOALS subset clearly consists of far fewer *APOE-2* carriers than the other two datasets (8.7% vs. 18.6% and 15.5%) as well as all unaffecteds. The differences in the numbers of *APOE-2* carriers between affected and unaffected groups in the EOALS subset may lead to significant association between *APOE-2* and the risk of ALS.

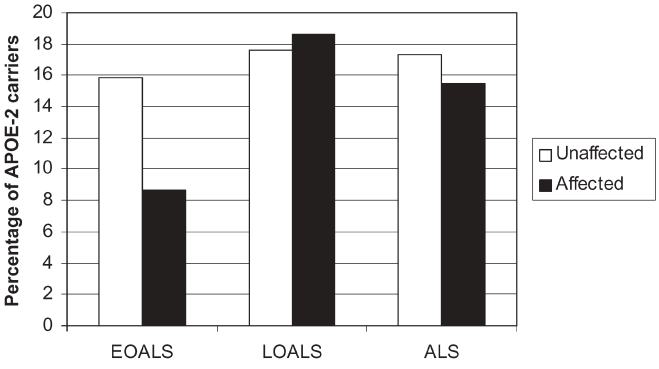

Fig. 1 Percentages of *APOE-2* carriers for affecteds and unaffecteds in each amyotrophic lateral scleosis (ALS) subset. Early onset ALS (*EOALS*) consists of families with at least 1 affected with age at onset <40 years and late-onset ALS (*LOALS*) consists of families with all affected with age at onset ≥40 years. We selected 1 affected and 1 unaffected individual from each family for each subset for the calculation of percentages of *APOE-2* carriers

Discussion

APOE has been implicated in several neurodegenerative diseases [13, 15, 36]. To our knowledge, there are at least eight publications using unrelated samples with inconsistent conclusions on the role of *APOE* in ALS. Some datasets are larger than the others, for instance, no effect of APOE on age at onset of ALS was found using 360 ALS cases versus 351 controls [17] in either the overall data or bulbar-onset subset, while significant results were found for age at onset of bulbar-onset patients using 130 ALS cases versus 161 controls [19]. The current study is different from the previous studies in several respects. In particular, we used the family based association approach to dissect the effect of *APOE* on the age at onset of ALS. This approach protects against spurious association due to

population substructure [37]. In this study, age at onset is treated as a quantitative trait, increasing the amount of information extracted from the data.

Overall, the present study showed a strong association of later onset with increasing number of *APOE-2* alleles. The protective effect of *APOE-2* on age at onset of ALS may have been because the early onset ALS patients tended to receive less *APOE-2* from parents than the late-onset ALS patients. This leads to a plausible scenario that parents transmit *APOE-2* less than expected to ALS affected individuals within the early onset group, as shown in Fig. 1. This is probably the main reason that we detected negative association between *APOE-2* and ALS risk in the early onset group. We, therefore, concluded that the effect of *APOE-2* on age at onset is the main cause of the APOE risk effect in early onset ALS.

Previous studies have focused on the risk effect of *APOE-4* in ALS [17, 20, 21]. Clearly, the strong dose effect of *APOE-4* in Alzheimer disease has impacted the direction of previous research on *APOE* in ALS. Overall, there are few studies of the risk of *APOE-2* in ALS. Moulard et al. [19] showed a difference for *APOE-23* genotype frequency between 130 cases and 540 controls (7.7% vs. 14%, respectively), but they did not stratify the data by age at onset. Our comprehensive analysis examined risk and age at onset effects of each allele in overall as well as stratified datasets, which provides the first in-depth analysis of *APOE-2*.

Unlike the dose effect of *APOE-4* in Alzheimer disease [13], we did not find that *APOE-4* affected age at onset of ALS (P=0.75), which is consistent with our previous report of no significant differences in age at onset between patients with *APOE-X/X* and *APOE-4/4* or *APOE-4/X* genotypes [17]. Furthermore, we did not detect any association between *APOE* and the risk of ALS, which is consistent with our previous report using sporadic ALS data [17] and several others [16]. Rather, we found that *APOE* affects the risk of ALS within age at onset-specific groups. The use of an age at onset cutoff of 40 years to stratify the dataset is rather arbitrary, because there is no clear definition for the early and late-onset ALS. We also investigated the subsets based on other cut-offs (e.g., 45 and 50 years), but similar results were generated. In conclusion, our findings suggest that *APOE* may express its strongest effect on the risk of ALS through age at onset.

Acknowledgements We would like to thank the families and patients with ALS for their participation in this study. Without their help this work would not have been possible. This research was supported by the National Institute of Neurological Disorders and Stroke (NS 37912, NS021442), Les Turner ALS Foundation, Vena E. Schaff ALS Research Fund, Harold Post Research Professorship, Herbert and Florence C. Wenske Foundation, Ralph and Marian Falk Medical Research Trust, Abbott Labs Duane, and Susan Burnham Professorship. R.H.'s laboratory also receives support for this work from the NINDS, the NIA, project ALS, the Angel Fund, the ALS Association, and the Al-Athel ALS Research Foundation. P.S. is supported through the laboratory of H.R. Horvitz, an investigator of the Howard Medical Institute, Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass.

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