The Role of \textit{APOE} in the Occurrence of Frontotemporal Dementia in Amyotrophic Lateral Sclerosis

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\textbf{IMPORTANCE}  Amyotrophic lateral sclerosis (ALS) is a heterogeneous disease with a wide spectrum of involvement of cognitive functions. The mechanisms of this heterogeneity are still largely unknown, but genetic variants may account for this variability.

\textbf{OBJECTIVE}  To assess the influence of the apolipoprotein E (\textit{APOE}) and \textit{C9ORF72} genotypes on cognitive impairment in a population-based series of Italian patients with ALS.

\textbf{DESIGN, SETTING, AND PARTICIPANTS}  All 504 patients with ALS living in Piemonte, Italy, diagnosed between January 1, 2009, and December 31, 2013, and identified through the Piemonte and Valle d'Aosta register for ALS, were eligible to participate in the study. Controls were 223 age- and sex-matched individuals identified through the patients' general practitioners. Data analysis was performed from June 1 to December 31, 2014.

\textbf{MAIN OUTCOMES AND MEASURES}  The presence of \textit{APOE} and \textit{C9ORF72} genotypes was assessed. Patients were cognitively classified as having ALS with normal cognition, ALS with frontotemporal dementia (FTD), ALS with executive or nonexecutive impairment, and ALS with behavioral impairment.

\textbf{RESULTS}  Of the 504 patients with incident ALS, 357 (70.8%) were included in the study; 154 were women, 203 were men, they had a mean (SD) age at onset of 64.8 (10.2) years, and 37 of them carried a \textit{C9ORF72} repeat expansion. Cognitive testing revealed that 184 patients (51.5%) had ALS with normal cognition, 51 (14.3%) had ALS with FTD, 103 (28.9%) had ALS with executive or nonexecutive impairment, and 19 (5.3%) had ALS with behavioral impairment. Distribution of \textit{APOE} haplotypes did not significantly differ between patients and controls or among patients with different levels of cognitive impairment. According to multivariate logistic regression, the presence of \textit{C9ORF72} repeat expansions was the strongest determinant of FTD (odds ratio, 13.08; 95% CI, 4.75-36.02; \( P < .001 \)); however, the presence of an \textit{APOE} ε2 allele significantly increased the risk of FTD (odds ratio, 2.61; 95% CI, 1.14-6.10; \( P = .03 \)). Presence of an \textit{APOE} ε4 allele was ineffectual.

\textbf{CONCLUSIONS AND RELEVANCE}  \textit{C9ORF72} repeat expansions have a primary role in increasing the risk of cognitive impairment in patients with ALS; the \textit{APOE} ε2 allele, to a lesser extent, also increases the risk of FTD. These study findings highlight the importance of considering the genetic background of patients with ALS when analyzing the putative effect of genetic modifiers.

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Amyotrophic lateral sclerosis (ALS) is a multisystem neurodegenerative disorder characterized by the progressive impairment of lower motor neurons at the spinal and bulbar levels and of upper motor neurons, with progressive loss of voluntary muscle functions. In approximately 50% of cases, ALS is also characterized by the involvement of prefrontal cortical neurons, with various degrees of frontotemporal cognitive impairment, up to frontotemporal dementia (FTD).1,2 Of patients with ALS, 10% to 15% have a family history of ALS and/or FTD.3 More than 20 genes related to ALS have been identified,3 the majority of which can cause both ALS and FTD.4 However, FTD or more subtle cognitive impairment is also found in patients with ALS who do not carry known genetic mutations.

The genotype of apolipoprotein E (APOE [OMIM +107741]) is the most extensively studied potential genetic risk in Alzheimer disease (AD).5 Human APOE exists as 1 of 3 isoforms: ε3, ε2, and ε4.6 The presence of 1 or 2 ε4 alleles increases the risk of AD and anticipates age of the patient at onset,5 whereas the presence of 1 ε2 allele delays the onset.5 APOE has a similar effect, albeit less strong, in other disorders characterized by dementia, such as FTD, vascular dementia, and dementia with Lewy bodies.7 In ALS, the APOE genotype is not associated with an increased risk of disease8; however, to date, no studies have assessed the influence of the APOE genotype on cognitive impairment in patients with ALS. The aim of this study was to assess the influence of the APOE genotype on cognitive impairment in a population-based series of Italian patients with ALS.

Methods

Patients and Controls

Patients were 504 individuals living in the provinces of Torino and Cuneo of Piemonte region, Italy. They received their diagnosis of ALS between January 1, 2009, and December 31, 2013, and were identified through the Piemonte and Valle d’Aosta register for ALS.9 If they met El Escorial revised diagnostic criteria10 for definite, probable, and possible laboratory-supported ALS, they were invited to participate in the study.

Controls were 223 age- and sex-matched Italian individuals identified through patients’ general practitioners. The study was approved by the local ethical committee (Comitato Etico Interaziendale Azienda Ospedale Università Città della Scienza di Torino, Azienda Ospedaliera Ordine Mauriziano, Azienda Sanitaria Locale Torino 1). Patients and controls provided written informed consent. Databases were treated with due respect for Italian privacy regulations, including deidentification of patients and controls.

APOE Genotype

A single amplicon of APOE containing both amino acid position 112 and 158 has been amplified via polymerase chain reaction and sequenced using BigDye termination v.1.1 (Life Technologies) according to standard protocol. Sequencing products were separated using 3130 Genetic Analyzer (Life Technologies) and analyzed using SeqScape software, version 2.6 (Applied Biosystems).

Other Genes

All patients with ALS were also tested for SOD1 (OMIM *147450) (all exons), TARDBP (OMIM *605078) (exon 6), FUS (OMIM *137070) (exons 14 and 15), ANG (OMIM *105850), and C9ORF72 (OMIM *614260) using the methods described elsewhere.11 Patients with familial ALS were also tested for OPTN (OMIM *602432) (all exons) and MATR3 (OMIM *164015) (all exons).

Neuropsychological Assessment

The selection of the neuropsychological tests, evaluating executive function, memory, visuospatial function, and language, was based on clinical diagnostic criteria for frontotemporal lobar degeneration12 and consensus criteria for ALS with FTD.13 The neuropsychological battery included the Mini–Mental State Examination,14 Wisconsin Card Sorting Test,15 Trail Making Test A and B,16 Stroop Color and Word Test,17 Letter and Category Fluency Test,18 Wechsler Memory Scale Revised,19 Rey Complex Figure Test,20 Token Test,21 Wechsler Adult Intelligence Scale Revised,22 Raven’s Coloured Progressive Matrices,23 and Frontal Assessment Battery.24 Neurobehavioral dysfunction was assessed through direct observation and the patient’s history and with the Frontal Systems Behavior Scale using the family form completed by a close relative of the patient. Evaluation of anxiety and depression was based on the Hospital Anxiety and Depression Scale.

Patients with a history of neurologic disorders affecting cognition (eg, major stroke, severe head injuries, or mental retardation), alcohol and drug dependence, severe mental illness, and use of high-dose psychoactive medications were tested but not included in data analysis. Patients living in the area but who were not native Italian speakers were assessed only through an unstructured interview and therefore were excluded from the analysis. Patients were tested at the time of the diagnosis or during the first scheduled follow-up visit (approximately 2 months later) and were interviewed at home or at the ALS clinic.2 Further details about the neuropsychological battery and the testing procedure have been reported elsewhere.2
Cognitive Classification
Clinical diagnosis and cognitive classification were established by neurologists and neuropsychologists with expertise in ALS and FTD. Patients were subdivided into 4 groups based on cognition:
1. ALS with normal cognition
2. ALS cases fulfilling the diagnostic criteria for FTD (ALS-FTD)
3. ALS cases with cognitive impairment not meeting the criteria for FTD but presenting with impairment in 2 tests for executive functions or in 2 tests for nonexecutive abilities (ALS-Ci)
4. ALS cases not meeting the criteria for FTD but presenting with a prevalent behavioral impairment and a deficit in none or only 1 test of executive function and no impairment in nonexecutive domains (ALS-Bi)

Statistical Analysis
Data analysis was performed from June 1 to December 31, 2014. Comparisons between categorical variables were made with the χ² test. Logistic multivariate regression was used to test the association between cognitive status and genetic, clinical, and demographic variables. Data were processed using SPSS statistical package, version 22 (IBM Corp).

Results
Of the 504 patients in the study diagnosed with ALS, 105 did not undergo the neuropsychological examination (32 declined to participate, 51 were unable to undergo testing, and 22 died before testing). Thirteen patients were excluded because they were not native Italian speakers, 13 had no DNA available, and 16 underwent the examination but were excluded from the analysis because they did not meet inclusion criteria.

Overall, 357 patients (70.8%) of the original population were included in the present study: 154 women and 203 men, with a mean (SD) age at onset of 64.8 (10.2) years. The site of onset was bulbar in 126 cases (35.3%). A total of 223 age- and sex-matched population controls were also assessed. Sixty patients (16.8%) carried a known genetic mutation related to ALS (C9ORF72, 37 patients; TARDBP, 12; SOD1, 6; FUS, 2; OPTN, 2; and MATR3, 1). Cognitive testing revealed that 184 patients (51.5%) had normal cognition, 51 (14.3%) had ALS-FTD, 103 (28.9%) had ALS-Ci, and 19 (5.3%) had ALS-Bi.

APOE Genetic Status
Most patients were homozygous for ε3/ε3 (276 [77.3%]), 37 (10.4%) had ε2/ε3, 37 (10.4%) had ε3/ε4, 6 (1.7%) had ε2/ε4, 2 were homozygous for ε2/ε2, and 1 was homozygous for ε4/ε4. The frequency of APOE genotypes and alleles in patients was not significantly different compared with that of controls (Table 1). No significant differences of APOE alleles and genotypes were found when comparing patients carrying the C9ORF72 pathogenetic expansion, patients who carried other gene mutations related to ALS, and patients without known genetic mutations (Table 2).

Table 1. Patients With ALS and Age- and Sex-Matched Population Control Individuals

<table>
<thead>
<tr>
<th>Genotype</th>
<th>APOE Patients With ALS (n = 357)</th>
<th>Controls (n = 223)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2/ε2</td>
<td>2 (0.6)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ε2/ε3</td>
<td>36 (10.1)</td>
<td>24 (10.8)</td>
<td>.73</td>
</tr>
<tr>
<td>ε3/ε3</td>
<td>275 (77.0)</td>
<td>169 (75.8)</td>
<td></td>
</tr>
<tr>
<td>ε3/ε4</td>
<td>37 (10.4)</td>
<td>22 (9.9)</td>
<td></td>
</tr>
<tr>
<td>ε2/ε4</td>
<td>6 (1.7)</td>
<td>7 (3.1)</td>
<td></td>
</tr>
<tr>
<td>ε4/ε4</td>
<td>1 (0.3)</td>
<td>1 (0.4)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alleles*</th>
<th>APOE Patients With ALS (n = 357)</th>
<th>Controls (n = 223)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2</td>
<td>46 (6.4)</td>
<td>31 (7.0)</td>
<td>.85</td>
</tr>
<tr>
<td>ε3</td>
<td>623 (87.3)</td>
<td>384 (86.1)</td>
<td></td>
</tr>
<tr>
<td>ε4</td>
<td>45 (6.3)</td>
<td>31 (7.0)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: ALS, amyotrophic lateral sclerosis.

* Data were obtained for 714 patients with ALS and 446 controls.

APOE and Cognitive Status
Genotype and allele distribution of APOE according to different cognitive conditions is shown in Table 3. There was no difference in APOE status among patients with C9ORF72 mutations compared with controls overall or in any of the 4 categories of cognitive impairment. In contrast, patients without C9ORF72 mutations displayed a significant difference in APOE status for those with ALS-Ci. This difference was owing to a higher frequency of ε2 (ALS-Ci: ε2 vs non-ε2, P = .02; ALS-FTD: ε2 vs non-ε2, P = .02).

Multivariate Logistic Regression Analysis
To assess whether APOE genotype influences the onset of FTD independently from the genetic background of cases, a logistic regression analysis was performed (Table 4). Results of this analysis showed that the presence of an APOE ε2 allele significantly modulates the risk of FTD in ALS independent of C9ORF72 mutation status (odds ratio [OR], 2.61; 95% CI, 1.14-6.10; P = .03), whereas ε4 (OR, 0.68; 95% CI, 0.25-1.85; P = .46) was ineffectual. Conversely, none of the APOE genotypes modulated the intermediate forms of cognitive impairment in ALS (ALS-Ci and ALS-Bi). Dominant and/or additive effects of ε2 and ε4 alleles were not calculated because of the small number of homozygous carriers.

The presence of a C9ORF72 repeat expansion was a stronger determinant of FTD than was APOE genotype (OR, 13.08; 95% CI, 4.75-36.02; P < .001). C9ORF72 also was a determinant of ALS-Ci (OR, 2.85; 95% CI, 1.11-7.31; P = .03) but not of ALS-Bi (OR, 1.18; 95% CI, 0.13-9.78; P = .92). The only other factor independently related to cognitive status was increasing age, which was a factor for both ALS-FTD and ALS-Ci but not for ALS-Bi.

Discussion
We analyzed a large population-based series of Italian patients with familial and sporadic ALS to determine if the APOE
and C9ORF72 genotypes affect the risk of developing cognitive impairment. In our series, C9ORF72 expansion was the strongest determinant of both comorbid FTD and, to a lesser degree, one of the intermediate forms of cognitive impairment (ALS-CI) but not ALS-Bi. Conversely, the presence of an APOE ε2 genotype significantly increased the risk of FTD by approximately 2.5-fold but did not increase the risk of both intermediate forms of cognitive impairment. The other APOE genotypes and alleles did not modify the risk of cognitive impairment in patients with ALS.

In AD the role of the APOE ε4 allele is well established. For other dementias, there are indications that the APOE ε4 allele represents a risk factor for vascular dementia, dementia associated with Parkinson disease, and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, but to a lesser extent than in AD. The few studies that assessed the correlation between FTD and APOE genotypes or alleles gave contrasting results. The oldest studies reported that the ε2 but not the ε4 genotype was significantly associated with FTD; more recent articles, including a meta-analysis, have found that the ε4 genotype could represent a moderate risk factor for FTD. In general, all studies performed on the correlation between FTD and APOE are quite small (ranging from 6 to 198 patients with FTD), used different diagnostic criteria for FTD, and are based on clinical series. Moreover, interestingly enough, no studies to date have assessed the possible interaction of genetic mutations with the effect of APOE, even if 30% to 50% of patients with FTD have a familial type and genetic mutations are detected in at least 25% of patients with familial FTD and 10% of those with apparently sporadic FTD. The most commonly detected genes in such cases are progranulin, C9ORF72, and microtubule-associated protein tau.

Possible mechanisms of the APOE ε2 genotype in ALS can be considered. First, APOE ε2 binds less well than ε3 and ε4 to cell surface receptors, thus impairing lipoprotein metabolism, a factor that has been associated with risk and survival of ALS. Second, the mechanism can be explained by molecular positive heterosis, which is a phenomenon occurring when individuals who are heterozygous for a specific genetic polymorphism show a significantly greater phenotypic effect (positive heterosis) for a quantitative or dichotomous trait than homozygotes for either allele.

C9ORF72 expansions are known to be related to an increased risk of FTD or more subtle cognitive impairment
that ALS-Ci and ALS-Bi may represent distinct forms of cognitive impairment (ALS-Bi). These findings support the notion that APOE ε4 and bulbar onset were not independently associated with cognitive impairment. The correlation between bulbar onset and comorbid FTD in ALS is still debated.1,2,44-47 Possible limitations of this study should be considered. First, although the cohort is relatively large, the group of patients with ALS-Bi is relatively low; therefore, it is possible that some effect of C9ORF72 or APOE was not detected. Second, the number of patients with SOD1, FUS, and TARDBP mutations was relatively small, and they could not be assessed separately. To further assess these results, it would be important to replicate our findings in an independent cohort of patients with ALS. Strengths of this study are that it is a population-based study, that it includes more than 70% of patients with incident ALS, and that the distribution of APOE haplotypes and alleles in the control cohort as well as in the patients with ALS without cognitive impairment is in keeping with the data from the general Italian population.48

Conclusions

We have shown that the APOE haplotype influences the risk of developing FTD in patients with ALS. However, in our series C9ORF72 was a stronger determinant of cognitive impairment; in particular, of ALS-FTD and ALS-Ci phenotypes. Our findings highlight the importance of considering the genetic background of patients with ALS when analyzing the putative effect of genetic modifiers.

Table 4. Multivariate Logistic Regression*

<table>
<thead>
<tr>
<th>Patients With ALS-FTD</th>
<th>Odds Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C9ORF72 vs non-C9ORF72</td>
<td>13.08 (4.75-36.02)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>APOE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε2 vs non-ε2</td>
<td>2.61 (1.14-6.10)</td>
<td>.03</td>
</tr>
<tr>
<td>ε4 vs non-ε4</td>
<td>0.68 (0.25-1.85)</td>
<td>.46</td>
</tr>
<tr>
<td>Sex, female vs male</td>
<td>1.25 (0.63-2.48)</td>
<td>.53</td>
</tr>
<tr>
<td>Site of onset, bulbar vs spinal</td>
<td>1.97 (0.98-3.93)</td>
<td>.05</td>
</tr>
<tr>
<td>Age group, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥70 vs &lt;50</td>
<td>7.43 (1.61-34.71)</td>
<td>.01</td>
</tr>
<tr>
<td>60-69 vs &lt;50</td>
<td>3.00 (0.64-14.04)</td>
<td>.16</td>
</tr>
<tr>
<td>50-59 vs &lt;50</td>
<td>1.46 (0.29-7.28)</td>
<td>.64</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patients With ALS-Ci</th>
<th>Odds Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C9ORF72 vs non-C9ORF72</td>
<td>2.85 (1.11-7.31)</td>
<td>.03</td>
</tr>
<tr>
<td>APOE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε2 vs non-ε2</td>
<td>1.01 (0.46-2.20)</td>
<td>.99</td>
</tr>
<tr>
<td>ε4 vs non-ε4</td>
<td>0.68 (0.33-1.42)</td>
<td>.30</td>
</tr>
<tr>
<td>Sex, female vs male</td>
<td>1.27 (0.76-2.13)</td>
<td>.36</td>
</tr>
<tr>
<td>Site of onset, bulbar vs spinal</td>
<td>0.93 (0.54-1.61)</td>
<td>.80</td>
</tr>
<tr>
<td>Age group, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥70 vs &lt;50</td>
<td>2.88 (1.01-8.21)</td>
<td>.048</td>
</tr>
<tr>
<td>60-69 vs &lt;50</td>
<td>2.85 (1.01-7.99)</td>
<td>.047</td>
</tr>
<tr>
<td>50-59 vs &lt;50</td>
<td>1.74 (0.59-5.13)</td>
<td>.31</td>
</tr>
</tbody>
</table>

Abbreviations: ALS, amyotrophic lateral sclerosis; ALS-Ci, ALS with cognitive impairment; ALS-FTD, ALS with comorbid frontotemporal dementia.

*Data for patients with ALS with behavioral impairment are not reported because all values were nonsignificant.

in patients with ALS: in large population-based cohorts of patients with ALS, more than 50% of those carrying the C9ORF72 mutation show some degree of cognitive impairment.36,37 In our cohort, we found that the presence of a C9ORF72 expansion confers a 10-fold increase in the risk of developing FTD and a 2.5-fold increased risk of developing a pattern of milder cognitive impairment (ALS-Ci), but it did not modify the risk of an isolated behavioral impairment (ALS-Bi). These findings support the notion that ALS-Ci and ALS-Bi may represent distinct forms of cognitive impairment in the clinical continuum of ALS and FTD, as also suggested by magnetic resonance imaging and fluorodeoxyglucose F18-labeled positron emission tomography studies.38-40

The only other factor included in multivariate logistic regression that influenced the risk of developing cognitive impairment was increasing age, which was a factor for both ALS-FTD and ALS-Ci but not for ALS-Bi. The effect of aging on increased risk of cognitive impairment is not surprising since it has been reported in several epidemiologic studies.41-43 The other examined factors (sex and bulbar onset) were not independently associated with cognitive impairment. The correlation between bulbar onset and comorbid FTD in ALS is still debated.1,2,44-47

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Possible limitations of this study should be considered. First, although the cohort is relatively large, the group of patients with ALS-Bi is relatively low; therefore, it is possible that some effect of C9ORF72 or APOE was not detected. Second, the number of patients with SOD1, FUS, and TARDBP mutations was relatively small, and they could not be assessed separately. To further assess these results, it would be important to replicate our findings in an independent cohort of patients with ALS. Strengths of this study are that it is a population-based study, that it includes more than 70% of patients with incident ALS, and that the distribution of APOE haplotypes and alleles in the control cohort as well as in the patients with ALS without cognitive impairment is in keeping with the data from the general Italian population.48

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