cellular ferritin, but in an insufficient number of cells to increase serum ferritin concentrations appreciably.

The above hypotheses are testable. In either case, however, a role for iron-chelation therapy in FRDA would appear to be problematic, because total storage cellular ferritin, but in an insufficient number of cells in these patients is already normal. Of particular concern is whether the cytosolic iron concentration in affected cells is low, as suggested by the finding of Rotig and collaborators\textsuperscript{14} that cytosolic aconitase activity is reduced, in which case affected cells may compete more avidly for limited tissue iron supplies than unaffected cells. It is possible that iron-chelation therapy will benefit FRDA patients by producing a more favorable iron concentration gradient between the inside and outside of mitochondria in affected cells. Even if such therapy limits iron availability to unaffected cells, the net effect may still be beneficial. Our results suggest, however, that a great deal of caution would need to be exercised. It is hoped that the clarification of the exact function of frataxin will lead to more directed therapeutic approaches.

References
10. Bidichandani SI, Ashizawa T, Patel PI. Atypical Friedreich baropathy, primary progressive aphasia (or hereditary dysphasic dementia), and the disinhibition–dementia–parkinsonism–amyotrophy complex, comprises 10 to 16% of all primary degenerative dementias.\textsuperscript{3} Antemortem diagnosis is made based on clinical features and supportive neuroimaging findings, but diagnostic accuracy outside of specialized centers is often low.\textsuperscript{4} At least two genetic loci have been identified, and, similar to AD, FTD has genetically diverse etiologies.\textsuperscript{1,2,5}

The apolipoprotein E (apoE) genotype comprises a

The Apolipoprotein E ε4 Allele Is Not a Significant Risk Factor for Frontotemporal Dementia
Dan Geschwind, MD, PhD,† Juliana Karrim, BS,† Stanley F. Nelson, MD,‡ and Bruce Miller, MD$
significant portion of the genetic risk for early- and late-onset AD. Whether the ApoE ε4 allele imparts specific risk for AD or is a more general risk factor for neurodegenerative conditions remains unresolved. An increase in ApoE ε4 frequency has been variably observed in diffuse Lewy body disease but does not appear to be an important risk factor in several other neurodegenerative conditions, namely, progressive supranuclear palsy, amyotrophic lateral sclerosis, and Parkinson’s disease with or without dementia. Studies of ApoE ε4 allele frequency in patients with FTD have produced conflicting results. In many cases, either the number of patients studied was small or there was no pathological confirmation of disease status. Here, we study a relatively large population of rigorously diagnosed familial and non-familial FTD patients and compare the prevalence of ApoE alleles with early- and late-onset AD patients and with elderly non-demented controls.

**Patients and Methods**

**Patient Characteristics**

FTD patients were recruited through the UCLA Alzheimer Disease Center, and the protocols were reviewed and approved by the Human Subjects Protection Committee. After informed consent was obtained, suspected FTD patients received in-depth neurobehavioral, neuropsychological, and imaging studies as previously described. All FTD patients met the research criteria for FTD established by the Lund Manchester Group and modified by our own group. By using these guidelines, we obtain high diagnostic accuracy for FTD, with diagnosis confirmed by autopsy in 14 of 15 cases studied to date. Three additional patients in the Irvine AD center had autopsy-confirmed FTD.

Thirty-three FTD patients were genotyped. Eleven patients had either direct pathological confirmation of disease status (9 patients) or a first-degree relative with FTD pathology (2 patients). In all instances, the pathology was consistent with FTD, with frontotemporal neuronal loss, gliosis, and macrovacuolization of tissue. Four additional FTD patients developed motor neuron disease in association with FTD, which is similar to previous reports.

Single-photon emission computed tomographic (SPECT) imaging was performed with both $^{133}$Xe and $^{99m}$Tc hexamethylpropyleneamineoxime (HMPAO). Visual rating of SPECT super $^{133}$ xenon and HMPAO helped to classify FTD patients. In every subject there was frontal or anterior temporal hypoperfusion, which was greater than parietal hypoperfusion with SPECT (or positron emission tomography in 1 case).

Thirty early-onset (EOAD; age, <65 years) and 30 late-onset (LOAD; age, >65 years) pathologically proven AD cases were randomly selected from the UC Irvine AD center resource. Thirty elderly subjects with pathologically proven normal brains were used as controls. This elderly non-demented group was chosen because they are conservative controls (the ApoE ε4 allele is biased toward the lower end of the population frequency). In addition, there were not enough FTD spouses available to genotype as controls. ApoE genotypes were determined as part of the UC Irvine AD center protocol, using standard methods.

**Molecular Methods**

Genomic DNA was isolated from blood by using the Puregene kit (Gentra Systems, Research Triangle Park, NC) and diluted to a concentration of 20 ng/μl. The ApoE gene was amplified from genomic DNA by using primers, (F) 5' TAAGCTTAGCCACGGCTGTCCAAAGA-3' and (R) 5' ACAGAATTCTGCCCGCCGCCGTGTACACTGCCA-3', in a 50-μl polymerase chain reaction (PCR) reaction containing 1 μM concentration of each primer, 200 μM dNTPs, 1.5 U AmpliTaq DNA polymerase (Perkin-Elmer, Foster City, CA), 10% dimethyl sulfoxide, 1× PCR buffer with 1.5 mM magnesium (Perkin-Elmer), and 40 ng of genomic DNA. After an initial denaturation for 5 minutes at 95°C, 33 cycles of 30 seconds at 94°C, 1 minute at 60°C, and 1.5 minute at 72°C were performed followed by final extension for 7 minutes at 72°C. Five units of HhaI (New England Biolabs, NEB, Beverly, MA) was added to 40 μl of the purified PCR reaction (Qiapquick; Qiogen, Germany) in the NEB reaction buffer in a total volume of 50 μl and incubated at 37°C for 3 hours. Ten microliters of each reaction was loaded onto a 6% nondenaturing polyacrylamide gel and electrophoresed at 30 mA for 3 to 4 hours. Separated DNA strands were visualized by using ethidium bromide (0.2 mg/L). Ten- and 100-base pair ladders (GibcoBRL, Gaithersburg, MD) were run in adjacent lanes for sizing. In addition, approximately 100 pg of three control plasmids, each containing the ApoE ε2, ε3, and ε4 alleles (gift of Dr Robert Malley, UCSF) were PCR-amplified, digested with HhaI, and run adjacent to the experimental lanes to assist in allele-sizing analysis (Fig 1).

**Results**

Thirty-three patients meeting the strict diagnostic criteria for FTD were genotyped at the ApoE locus and compared with the two groups of AD patients and

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**Fig 1. Apolipoprotein E genotyping in frontotemporal dementia (FTD) patients. Restriction-digested samples from 7 FTD patients (first 7 lanes) and control plasmids (last 3 lanes: ε2, ε3, and ε2) visualized with ethidium bromide on a 6% nondenaturing polyacrylamide gel. Band sizes (bp) are marked to the left of the gel. Genotypes are marked across the top of the gel.**

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Table. Demographics and ApoE Genotypes for Patients with AD, with FTD, and for Nondemented Controls

<table>
<thead>
<tr>
<th></th>
<th>FTD</th>
<th>EOAD</th>
<th>LOAD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age of onset, yr (±SD)</td>
<td>55 ± 11</td>
<td>58 ± 5</td>
<td>76 ± 6</td>
<td>73 ± 14*</td>
</tr>
<tr>
<td>Range</td>
<td>32-69</td>
<td>47-63</td>
<td>66-87</td>
<td>41-89</td>
</tr>
<tr>
<td>Sex distribution</td>
<td>M = 16</td>
<td>M = 13</td>
<td>M = 13</td>
<td>M = 13</td>
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<tr>
<td></td>
<td>F = 17</td>
<td>F = 17</td>
<td>F = 17</td>
<td>F = 17</td>
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<tr>
<td>Pathological confirmation or MND</td>
<td>15</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>ApoE allele distribution (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2 E2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>E2 E3</td>
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<td>4</td>
</tr>
<tr>
<td>E2 E4</td>
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<td>2</td>
<td>0</td>
</tr>
<tr>
<td>E3 E2</td>
<td>18</td>
<td>12</td>
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</tr>
<tr>
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</tr>
<tr>
<td>E3 E4</td>
<td>1</td>
<td>6</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

*Age of death.

†Autopsy confirmation (11 patients) or motor neuron disease (MND; 4 patients); see Patients and Methods for FTD cases.

ApoE = apolipoprotein E; AD = Alzheimer’s disease; FTD = frontotemporal dementia; EOAD = early-onset AD; LOAD = late-onset AD.

non-demented, elderly controls. Clinical characteristics including average ages and sex of patients with FTD are summarized in the Table. The mean age of FTD patients was 55 years, close to that of EOAD but significantly less than LOAD patients. Eighty-six percent of FTD cases had onset before age 65. The sex distributions were not significantly different between the groups (see Table). Thirty-two of 33 FTD patients were Caucasian.

The frequency of ApoE ε4 alleles in EOAD and LOAD patients and in normal controls was 38% (23 of 60), 40% (24 of 60), and 13% (7 of 60), which is similar to previous studies in larger populations (Fig 2). As expected from previous studies, the frequency of ApoE ε4 in AD patients was significantly different from controls ($p < 0.0008$). The frequency of ApoE ε4 was 21% (14 of 66) in patients with FTD, which is significantly less than the ApoE ε4 frequency in both populations of AD patients ($p < 0.036$, EOAD; $p < 0.024$, LOAD; $p < 0.013$ for both EOAD and LOAD combined; Fisher’s exact test) but not significantly different from nondemented controls ($p < 0.110$; Fisher’s exact test). There also was no significant difference in ApoE ε4 distribution between the familial (n = 14) and nonfamilial cases (n = 16) of FTD (family history was not obtainable in 3 patients; data not shown).

Only 1 patient with FTD was homozygous for the ApoE ε4 allele (3.3%), which is similar to the non-demented, elderly control group (3%). In contrast, 20% of EOAD and 13% of LOAD patients were ApoE ε4 homozygotes. This difference in ApoE ε4 homozygosity between controls and FTD patients and AD patients only reached marginal significance due to the small number of homozygotes in the FTD and control populations ($p = 0.06$; Fisher’s exact test). It is noteworthy that the one FTD ApoE ε4 homozygote did have the earliest age of onset among FTD patients. The ApoE ε2 allele, which has been shown to be protective for AD, was rare in all three populations, occurring at a frequency of 2% in EOAD, 7% in LOAD, and 4% in FTD, which is similar to its frequency in the general population. 19

Discussion

In this study, the frequency of ApoE ε4 in FTD patients was not significantly different from elderly nondemented controls, but was approximately one-half that of patients with EOAD and LOAD. It is noteworthy that the frequencies of the ApoE ε4 allele in nondemented controls and in patients with AD were similar to the frequencies reported in previous studies of
Large populations of AD patients and controls.\textsuperscript{6,20} Furthermore, ApoE\textsuperscript{ɛ4} homozygosity was not elevated in FTD patients, compared with control subjects, but was elevated in AD patients, consistent with the dose–response relationship observed in AD. In fact, ApoE\textsuperscript{ɛ4} homozygosity in FTD and control subjects was about one-fifth that seen in AD patients.

These results support those of a recent study in 26 patients with pathologically proven lobar atrophy in which the frequency of the ApoE\textsuperscript{ɛ4} allele (17\%) was not elevated relative to controls (14%).\textsuperscript{6} Similar to the present study, these patients with lobar atrophy comprised a heterogeneous population of patients along the FTD spectrum. The present study and that by Pickering-Brown and collaborators\textsuperscript{8} are the two largest studies of ApoE\textsuperscript{ɛ4} frequency in which there was any pathological confirmation of FTD diagnosis. Another recent small study of well-characterized patients with the FTD variant primary progressive aphasia showed a similarly low frequency of the \(ɛ4\) allele in those affected.\textsuperscript{20} The ApoE\textsuperscript{ɛ4} frequency in these two studies and ours taken together ranges between 13\% and 21\%, about one-half the frequency observed in AD populations.

A recently published study that relied entirely on clinical diagnosis found an increased prevalence of ApoE\textsuperscript{ɛ4} alleles in 34 Dutch FTD patients (25\%), mostly due to an increase in FTD ApoE\textsuperscript{ɛ4} homozygotes. The most likely explanation for the slight increase in ApoE\textsuperscript{ɛ4} prevalence in the Dutch study is admixture from misdiagnosed AD patients. Because no clinicopathological data were presented, the accuracy of clinical diagnosis in these FTD patients cannot be estimated. Even with an optimistic 90\% clinical diagnostic accuracy,\textsuperscript{4} 3 or 4 patients with AD could have been mistakenly included in this cohort, enough to have elevated the frequency of ApoE\textsuperscript{ɛ4} alleles to a level of significance.

In the present study, one-half of our patients or family members had their diagnosis pathologically confirmed or had motor neuron disease and dementia, a hallmark of FTD that is rarely observed in AD. In addition, the diagnostic accuracy in the present cohort is at least 93\%, as inferred from autopsy data (see Patients and Methods). Misdiagnosis of AD patients as having FTD in our study would raise the ApoE frequency and bias results toward a significantly higher ApoE\textsuperscript{ɛ4} frequency, which was not observed.

Thus, this study, along with others cited, provides additional evidence that the ApoE\textsuperscript{ɛ4} allele does not comprise a significant portion of the genetic risk for familial or sporadic FTD. However, due to small numbers of ApoE homozygotes, we cannot eliminate the possibility that the ApoE\textsuperscript{ɛ4} allele may somehow influence the age of onset in those with sporadic or familial FTD without being a significant genetic risk factor for its eventual occurrence.\textsuperscript{12,14}

References

Incidence of Exacerbations in the First 90 Days of Treatment with Recombinant Human Interferon β-1b in Patients with Relapsing-Remitting Multiple Sclerosis

Omar A. Khan, MD,*‡ and J. Richard Hebel, PhD†

Interferon β-1b (IFNβ-1b) is effective in reducing the frequency of exacerbations in patients with relapsing-remitting multiple sclerosis (RRMS). Recently, a study suggested that treatment with IFNβ-1b may place MS patients at risk of exacerbations by increasing interferon-γ (IFNγ)-secreting cells in the blood early after onset of treatment. We conducted a retrospective study in 192 RRMS patients treated with IFNβ-1b. We did not observe an increase in the frequency of exacerbations early after the onset of treatment and suggest that the IFNγ-secreting cell surge linked to the onset of treatment with IFNβ-1b may not be clinically significant.


Interferon β-1b (IFNβ-1b) reduces the frequency of relapses in patients with relapsing-remitting multiple sclerosis (RRMS).1 Although unclear, several mechanisms of efficacy of IFNβ-1b in MS have been proposed, including decreased production of interferon-γ (IFNγ) by T cells.2 The inhibitory effect on the production of IFNγ and IFNγ-mediated activity is especially important because IFNγ was shown to increase disease activity and exacerbation frequency when given to RRMS patients.3 However, it has been shown that IFNβ-1b causes an increase in the number of IFNγ-secreting cells in the blood early after onset of IFNβ-1b treatment in MS patients.4 The IFNγ-secreting cell surge was noted to fall back into the normal range 90 days after the onset of treatment, suggesting that MS patients may be at an increased risk of having exacerbations at the start of treatment with IFNβ-1b because of the IFNγ-secreting cell surge. The authors also suggested that the use of prednisone at the onset of treatment with IFNβ-1b, tapered over a period of 4 weeks, was successful in preventing the IFNγ-secreting cell surge and minimizing the associated risk of exacerbations.4

We carried out a study to determine the incidence of exacerbations in the first 90 days of onset of treatment with IFNβ-1b in 192 patients with RRMS.

Methods

We conducted a retrospective study by interviewing and examining the medical records of 192 consecutive RRMS patients. The study was divided into two “time periods.” The “treatment period” was defined as the first 90 days of treatment with IFNβ-1b, and the “control period” was defined as the the 90 days immediately before the onset of treatment with IFNβ-1b (Betaseron, Berlex, Richmond, CA). Each patient was studied before and after the onset of treatment with IFNβ-1b and served as his or her own control. Patient diagnosis and clinical course was confirmed from a patient registry established to enroll MS patients for treatment with IFNβ-1b. Details regarding symptoms, exacerbations, treatment with corticosteroids, and change in medication were carefully reviewed. Patients were given detailed explanation of symptoms that could be related to IFNβ-1b injection therapy. Symptoms believed to be related to local or systemic IFNβ-1b side effects were carefully evaluated if the symptoms persisted for more than 24 hours. An exacerbation was defined as the appearance of a new symptom or worsening of an old symptom, attributable to MS, accompanied by a documented new neurological abnormality lasting at least 48 hours and preceded by stability or improvement for at least 30 days.

Patient age ranged from 23 to 64 years (mean, 38.4 years). One hundred and twenty-six of 192 patients were female (66.1%). No patient stopped treatment with IFNβ-1b during the first 90 days of treatment with IFNβ-1b (treatment period).

Results

Of the 192 patients studied, 117 did not have exacerbations during either control or treatment periods, and thus did not receive treatment with corticosteroids. Twenty patients had exacerbations in the treatment period but not the control period. Of these 20 patients, 16 received corticosteroids to treat exacerbations.

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