Apolipoprotein E ε4 Allele Frequency and Age at Onset of Alzheimer’s Disease


Introduction

Genetic variations represent major risk factors for Alzheimer’s disease (AD). Familial, autosomal dominant, early onset AD (EOAD) is associated with mutations in the amyloid precursor protein (APP) and presenilin (PSEN1 and PSEN2) genes. However, for late onset AD (LOAD; i.e. those with age of onset, AAO, after 65 years) only the ε4 allelic form of the apolipoprotein E gene (APOE) has so far been firmly established as a genetic risk factor [1–3], though several studies have suggested this might also increase the risk of developing EOAD (i.e. in those patients with AAO before 65 years of age) [3–5].

There have been a vast number of studies in AD emphasising the genetic association between possession of the APOE ε4 allele and disease. Nonetheless, the age profile of the genetic effect has been less studied. In early studies [2] they claimed that possession of APOE ε4 allele decreased AAO of disease in AD in a dose-dependent way [2]. However, such a conclusion was drawn from studies in which mainly LOAD cases had been analysed [2]. A later meta-analysis by Farrer et al. [3], based on data contributed by 40 research teams and encompassing 5,930 patients with AD and 8,607 controls, succinctly demon-
strated the increasing risk of disease with possession of APOE epsilon 4 allele and APOE epsilon 4/epsilon 4 genotype especially between 40 and 60 years, with a declining effect after 65 years. Furthermore, Bickeboller et al. [6], in a study which included 134 patients with AD with AAO less than 60 years, among a total of 417 AD patients, compared to 1,020 controls, reported the following odds ratios according to possession of at least one APOE epsilon 4 allele: 1.9 (0.96–3.7) for AAO under 60 years; 4.1 (2.3–7.5) for AAO between 60 and 69 years; 3.0 (1.7–5.2) for AAO between 70 and 79 years, and 1.7 (0.9–3.4) for AAO over 79 years. Such data challenge the concept of AD as a dichotomous disease, and cast doubt on both the validity and utility of ‘traditional’ approaches of classification into EOAD and LOAD simply on the grounds of AAO of disease. Therefore, in order to further understand the impact of possession of APOE epsilon 4 allele on age at onset of disease, we have investigated this relationship in a cohort of 630 patients with AD in which patients ‘traditionally’ classed as EOAD or LOAD were relatively evenly represented.

### Patients and Methods

The study group consisted of three cohorts of patients with AD, totalling 630 patients (305 males, mean age at onset 63.4 ± 9.5 years, range 35–89 years, and 325 females, mean age at onset 63.8 ± 10.3 years, range 39–90 years). These were mostly ascertained through 2 specialist referral centres (Clinical Old Age Psychiatry Services, South Manchester University Hospital) and through outpatient clinics at the Cerebral Function Unit (CFU) at The Greater Manchester Neurosciences Centre, Hope Hospital, Salford.

The first AD cohort (AD1) from Clinical Old Age Psychiatry Services consisted of 97 individuals (49 males, mean age at onset 70.9 ± 8.2 years, range 45–89 years, and 48 females, mean age at onset 72.5 ± 8.9 years, range 53–90 years). In these, the diagnosis of AD was made according to DSM III-R criteria [7].

The second AD cohort (AD2) from CFU consisted of 365 individuals (173 males, mean age at onset 60.8 ± 8.4 years, range 39–86 years, and 192 females, mean age at onset 60.8 ± 8.9 years, range 41–84 years). Diagnosis of AD was consistent with NINCDS-ADRDA criteria [8].

The third AD cohort (AD3) consisted of 168 individuals dying with autopsy-verified AD, diagnosis being made according to CERAD criteria for pathological diagnosis of AD [9]. All cases were at Braak stages 5 or 6. In this group, there were 83 males (mean age at onset 64.3 ± 10.0 years, range 35–87 years) and 85 females (mean age at onset 65.8 ± 10.7 years, range 39–90 years). One hundred and five of these patients had been ascertained through CFU clinics, 45 through Old Age Psychiatry Services with the remaining 18 cases being represented by tissue donations on the part of relatives of the deceased under the auspices of the Alzheimer’s Society (UK). None of the patient samples within the autopsy group was doubly represented in either of the clinical groups, even though in life 16 of the deceased patients had been investigated in CFU clinic and blood samples had been taken for DNA analysis. All brains had been collected with approval by Local Ethical Research Committee, and full consent by next of kin of the deceased.

The 630 cases were stratified into EOAD (with age at onset under 65 years of age – 357 patients, mean age at onset 56.5 ± 5.6 years), and LOAD (with age at onset at or over 65 years of age – 273 patients, mean age at onset 72.9 ± 5.8 years), groups. Data concerning previous family history were only reliably available for 300 patients. Of these, 109 patients (36%) were reported as having another family member affected, though not necessarily affecting a first degree relative or with multiple family involvement. Patients with previous family history were distributed fairly evenly across the decades, and there was no preferential clustering with respect to APOE genotype (i.e. with or without APOE epsilon 4 allele) within any decade class. Thirty-five patients had AAO within that range associated with a mutation in APP or PSEN1 genes (i.e. under 50 years of age). However, only 5 of these patients also showed a family history of multiply affected first-degree relatives with similar AAO compatible with autosomal dominant transmission of disease, and therefore likely to be bearers of a mutation in either APP or PSEN1 gene.

Control data were derived from a cohort of 756 healthy people over the age of 50 years without cognitive impairment (mean age at ascertainment/venepuncture 63.1 ± 6.3 years) comprising 227 males (mean age at ascertainment/venepuncture 62.8 ± 6.3 years) and 529 females (mean age at ascertainment/venepuncture 63.8 ± 6.2 years) resident within the same Greater Manchester region from which the AD patients were drawn. A full description of how this control cohort was ascertained and investigated psychologically has previously been given [10, 11]. Although there were significantly more females than males within the control group compared to those in the total AD group (χ^2 = 49.2; p < 0.0001), there were no significant differences in mean AAO for the total AD group compared to mean age at ascertainment (venepuncture) for the control group, either for males and females combined or separately.

All subjects, patients and controls, were Caucasian. For all living persons, AD or controls, blood samples (2 ml) were collected in EDTA tubes. DNA was extracted by routine methods from blood samples, or from frozen cerebral cortex or cerebellum in the case of deceased patients where no blood sample had been previously available. The APOE genotype was determined by PCR [12], Genetic analysis for mutations in APP, PSEN1 and PSEN2 genes was not performed. Comparisons of APOE allele and genotype frequency were made using uncorrected χ^2 test, whereas comparisons of mean age at onset between APOE genotypes, and epsilon 2 or epsilon 4 allele bearers and non-bearers, were made using ANOVA or t test, respectively. The threshold for significance was set at p < 0.05.

### Results

APOE allele and genotype frequencies for each AD cohort, separately and combined, or when stratified by gender or into EOAD and LOAD cases, are given in table 1.
Also given in table 1 are APOE allele and genotype frequencies for all controls together, and again stratified by age into those under 65 years of age (younger controls) and those over or equal to 65 years of age (older controls). All allele and genotype frequencies were in Hardy-Weinberg equilibrium.

There were no significant differences in either frequency of APOE genotypes ($\chi^2 = 1.66; p = 0.704$) or APOE $\varepsilon 4$ alleles ($\chi^2 = 0.002; p = 0.995$) between any of the 3 AD cohorts (table 1). Genotype data were therefore pooled. As expected, the pooled APOE $\varepsilon 4$ allele frequency in the combined AD group (493/1,260 alleles, 0.39) was significantly higher ($\chi^2 = 227.0; p < 0.0001$) than that derived from all individuals within the normal control cohort (213/1,512 alleles, 0.14; table 1). In the combined AD group, 46% patients bore one APOE $\varepsilon 4$ allele and 16% were homozygous for APOE $\varepsilon 4$ allele, whereas in the control group 25% patients bore one APOE $\varepsilon 4$ allele but only 1.6% were homozygous for APOE $\varepsilon 4$ allele. Similar data have been reported on numerous occasions over the past decade [for review, see 13].

Similarly, the APOE $\varepsilon 4$ allele frequency in both the EOAD (253/714 alleles, 0.35) and LOAD (240/546 alleles, 0.44) groups was significantly higher than that derived from younger (152/970 alleles, 0.14) and older (61/542 alleles, 0.11) persons ($\chi^2 = 88.1$ and $\chi^2 = 145.2$, respectively; $p < 0.0001$ in both instances), from the mentally normal cohort (table 1). Interestingly, the APOE $\varepsilon 4$ allele frequency in the EOAD group was significantly lower ($\chi^2 = 9.8; p = 0.002$) than that of LOAD group, and the APOE $\varepsilon 4$ allele frequency in the older normal subjects (0.11) was also significantly lower ($\chi^2 = 5.6; p = 0.021$) than that in the younger normal subjects (0.14). There were no significant differences in APOE genotype or APOE $\varepsilon 4$ allele frequencies when AD patients were stratified into males and females (data not shown).

There were no significant differences in APOE $\varepsilon 2$ allele frequency between any of the 3 AD cohorts ($\chi^2 = 0.771; p = 0.68$; table 1). As expected, the pooled APOE $\varepsilon 2$ allele frequency in the combined AD group (42/1,260 alleles, 0.033) was significantly lower ($\chi^2 = 30.8; p < 0.0001$) than that derived from the mentally normal cohort (127/1,512 alleles, 0.080), as was that between EOAD and younger controls ($\chi^2 = 12.2; p < 0.0001$) and LOAD and older controls ($\chi^2 = 20.6; p < 0.0001$; table 1). However, there were no significant differences in APOE $\varepsilon 2$ allele frequency between EOAD and LOAD groups ($\chi^2 = 0.490; p = 0.486$), or between younger and older controls ($\chi^2 = 1.6; p = 0.211$; table 1).

Mean age at onset of disease differed across APOE genotype groups, both overall ($F_{5,624} = 4.0; p = 0.001$), and for EOAD ($F_{4, 351} = 2.57; p = 0.027$) and LOAD ($F_{4, 267} = 5.67; p < 0.001$) cases separately (table 2). However, because of the high proportion (54%) of patients with onset age before 65 years of age in this present AD cohort, we observed that, within all 630 AD cases, the mean age at onset in APOE $\varepsilon 4$ allele bearers (64.6 ± 9.3 years) was significantly later ($p = 0.001$) than that in non-bearers (61.9 ± 10.6 years; table 2), and not earlier as reported previously [2]. However, when we stratified our cases into

### Table 1. APOE genotype and allele frequencies in 3 AD cohorts, separately and combined, and in control subjects

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Patient</th>
<th>APOE genotypes</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\varepsilon 2/\varepsilon 2$</td>
<td>$\varepsilon 2/\varepsilon 3$</td>
<td>$\varepsilon 2/\varepsilon 4$</td>
</tr>
<tr>
<td>AD1</td>
<td>97</td>
<td>0 (0)</td>
<td>3 (3.0)</td>
</tr>
<tr>
<td>AD2</td>
<td>365</td>
<td>1 (0.3)</td>
<td>21 (5.7)</td>
</tr>
<tr>
<td>AD3</td>
<td>168</td>
<td>1 (0.6)</td>
<td>8 (4.8)</td>
</tr>
<tr>
<td>Combined</td>
<td>630</td>
<td>2 (0.3)</td>
<td>32 (5.1)</td>
</tr>
<tr>
<td>EOAD</td>
<td>357</td>
<td>1 (0.3)</td>
<td>22 (6.1)</td>
</tr>
<tr>
<td>LOAD</td>
<td>273</td>
<td>1 (0.4)</td>
<td>10 (4.5)</td>
</tr>
<tr>
<td>AD males</td>
<td>305</td>
<td>0 (0)</td>
<td>19 (6.0)</td>
</tr>
<tr>
<td>AD females</td>
<td>325</td>
<td>2 (0.6)</td>
<td>13 (4.0)</td>
</tr>
<tr>
<td>All controls</td>
<td>756</td>
<td>2 (0.3)</td>
<td>108 (14.3)</td>
</tr>
<tr>
<td>Younger controls</td>
<td>485</td>
<td>2 (0.4)</td>
<td>60 (12.4)</td>
</tr>
<tr>
<td>Older controls</td>
<td>271</td>
<td>0 (0.0)</td>
<td>48 (17.7)</td>
</tr>
</tbody>
</table>

The combined AD group is stratified into EOAD and LOAD cohorts, and into male and female groups, and the control group into younger and older control groups. Percentage values are shown in parentheses.
EOAD and LOAD groups, we found that the mean age at onset in EOAD cases bearing APOE ε4 allele (57.3 ± 5.3 years) was significantly later (p = 0.004) than that in those EOAD cases without ε4 allele (55.5 ± 5.9 years), whereas in LOAD (and consistent with previous studies [see 2]) there was a trend (p = 0.052) towards earlier onset in cases bearing APOE ε4 allele (72.4 ± 5.6 years) than in cases without ε4 allele (73.9 ± 6.1 years). There were no significant age at onset effects involving APOE ε2 allele.

We next analysed the APOE allele and genotype frequencies across each decade. The distribution of APOE ε4 allele bearers and non-bearers across the decades for the whole cohort of 630 patients is shown in figure 1. The data are expressed in terms of actual frequencies (fig. 1a) and percentages (fig. 1b). The relative frequency of at least one APOE ε4 allele differed significantly as a function of age at onset of disease (χ² = 20.17; p < 0.0001). It occurred most commonly in the 60–69 age band, reducing in frequency with both younger and older onset ages. The greater difference for the 60–69 age band was statistically significant when compared to the <50 and 50–59 age bands (χ² = 6.65; p = 0.01 and χ² = 15.14; p < 0.0001, respectively). Comparisons with the 70–79 and >80 age bands did not reach statistical significance. A similar pattern of findings was demonstrated with respect to cases with ε4/ε4 genotype (fig. 1). Relative frequencies differed significantly as a function of age at onset (χ² = 15.91; p = 0.003). They occurred more commonly in the 60–69 age band than in both younger and older age bands: 50–59 (χ² = 7.4; p = 0.006), <50 (χ² = 4.6; p = 0.03) and >80 years (χ² = 7.94; p = 0.005). The comparison with the 70–79 year age band did not reach conventional levels of significance (χ² = 3.34; p = 0.07). Hence, APOE ε4 allele frequency, at 0.44, was highest in the 60–69 age band.

Discussion

One arguable weakness of the present study is that the patients investigated are not a strict community-based sample of patients with AD but are drawn from specialist, referral clinics for both younger and older individuals. Hence, the study does not provide truly epidemiological data in terms of how APOE genotypes might be distributed with age (at onset of disease) within a community sample of persons with AD. However, it was not the purpose of the present study to determine such a measure, but rather to investigate how common APOE genotypes and alleles might be in groups of individuals with AD within increasing age classes by decade. Hence, it was important to include as many persons as possible within each decade class, particularly the younger and older subjects, in order to achieve valid APOE genotype and allele frequency data for that class, even though the actual numbers of individuals falling into each class are unlikely to reflect the proportion of patients of that age group with AD in the community. Therefore, within this present cohort there is likely to be a much greater representation of younger patients with AD than what would be expected if the sample had been drawn from the community. Nevertheless, there is no reason to believe that the EOAD and LOAD patients accessed through referral to CFU and Old Age Psychiatry services are in any way unrepresentative of their peers within each age class within the community as a whole.

A further possible limitation of our study comes from the potential inclusion of some EOAD individuals in the present cohort with APOE ε4 allele who might also be bearers of APP or PSEN1 mutations, and could perhaps bias the outcome of the analysis of these younger age cohorts, at least as far as APOE ε4 allele frequency is concerned. Indeed, within the present cohort, we identified 35 out of 357 (10%) EOAD individuals with AAO within that range usually associated with mutations in these genes (i.e. before 50 years of age). However, we have not been able to perform APP or PSEN1 gene sequencing on these 35 very young EOAD patients, and are therefore unable to say exactly how many might be carriers of mutations in either of these genes. Nonetheless, by invoking strict criteria for autosomal dominant transmission of

<table>
<thead>
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<th>APOE genotype</th>
<th>All AD</th>
<th>EOAD</th>
<th>LOAD</th>
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<tbody>
<tr>
<td>ε2/ε2</td>
<td>59.5 ± 10.6 (2)</td>
<td>52.0 (1)</td>
<td>67.0 (1)</td>
</tr>
<tr>
<td>ε2/ε3</td>
<td>59.8 ± 9.9 (32)</td>
<td>54.6 ± 6.8 (22)</td>
<td>71.3 ± 4.2 (10)</td>
</tr>
<tr>
<td>ε2/ε4</td>
<td>74.3 ± 11.3 (6)</td>
<td>60.0 ± 2.8 (2)</td>
<td>81.5 ± 1.9 (4)</td>
</tr>
<tr>
<td>ε3/ε3</td>
<td>62.2 ± 10.7 (204)</td>
<td>55.7 ± 5.8 (133)</td>
<td>74.6 ± 6.3 (65)</td>
</tr>
<tr>
<td>ε3/ε4</td>
<td>64.7 ± 9.8 (252)</td>
<td>56.9 ± 5.5 (147)</td>
<td>73.0 ± 5.6 (138)</td>
</tr>
<tr>
<td>ε4/ε4</td>
<td>64.1 ± 7.6 (101)</td>
<td>58.3 ± 4.7 (52)</td>
<td>70.2 ± 4.7 (49)</td>
</tr>
<tr>
<td>ε4+</td>
<td>64.6 ± 9.3 (392)</td>
<td>57.3 ± 5.3 (201)</td>
<td>72.4 ± 5.6 (191)</td>
</tr>
<tr>
<td>ε4−</td>
<td>61.9 ± 10.6 (238)</td>
<td>55.5 ± 5.9 (156)</td>
<td>73.9 ± 6.1 (82)</td>
</tr>
<tr>
<td>ε2+</td>
<td>62.0 ± 11.2 (40)</td>
<td>54.9 ± 6.6 (25)</td>
<td>73.7 ± 6.0 (15)</td>
</tr>
<tr>
<td>ε2−</td>
<td>63.7 ± 9.8 (590)</td>
<td>56.6 ± 5.5 (332)</td>
<td>72.8 ± 5.8 (258)</td>
</tr>
</tbody>
</table>

Numbers of cases are shown in parentheses.

Table 2. Age (mean ±SD) at onset of illness according to APOE genotypes, and possession (ε2+ or ε4+) or not (ε2− or ε4−) of at least one APOE ε2 or ε4 allele, respectively, for all cases with AD, and when stratified into EOAD and LOAD cases.
disease (i.e. multiple affected first-degree relatives) we considered that only 5 of these 35 persons had a previous family history of AD consistent with such a mode of inheritance, and therefore very likely candidates for bearing such a mutation. Indeed, one of these individuals was known from previous studies [14, 15] to be a bearer of PSEN1 M139V mutation. We therefore considered that the inclusion of this very low number of (likely and known) EOAD individuals with APP or PSEN1 mutation would not have significantly biased the outcome of present findings, and therefore these were not excluded from the analyses.

Therefore, notwithstanding these potential limitations, the present data clearly show that APOE ε4 allele has its maximum impact between onset ages of between 60 and 70 years, and are consistent with previous findings [3, 7]. The presence of APOE ε4 allele and especially APOE ε4/ε4 genotype is lower in individuals with both younger and older onset age. Most previous studies [for example, see 2] have failed to notice this, presumably because of the ‘conventional’ division of EOAD and LOAD cases at age 65 years, and the separate analysis of cases of EOAD and LOAD accordingly. The finding that APOE ε4 allele frequency in EOAD patients was about 2.3 times higher than that in younger control subjects is consistent with previous findings [3–5], even when allowing for the inclusion of a few individuals potentially or actually bearing APP or PSEN gene mutations. This suggests that possession of APOE ε4 allele may increase the risk of AD before age of 65 years, as well as in older individuals [1–3].

**Fig. 1.** Distribution of APOE ε4 allele bearers and non-bearers across the decades for the whole cohort of 630 patients, expressed both in terms of actual frequencies (a) and percentages (b).
However, present studies suggest that much of this effect is likely to stem from the inclusion of patients with onset ages between 55 and 65 years who are bearers of APOE e4/e4 genotype. In this present cohort, 48 of the total 101 bearers of APOE e4/e4 genotype fell into this particular age band. Indeed, in a meta-analysis of APOE e4 allele frequency based on data from previously published studies of 40 different research groups [3] and other work [6], the odds ratio for developing AD was maximal between 55 and 65 years of age.

The present findings therefore further challenge the validity of using 65 years as the (arbitrary) threshold for EOAD/LOAD borderline, and imply that these boundary should be redefined. One consequence of these observations is that it might be more rational in future genetic studies that AD should be separated, not into EOAD and LOAD with AAO at 65 years being the division point (as is currently commonplace), but into very early (i.e. before 55 years), middle (55–75 years) and late (after 75 years) onset cohorts. Indeed, in the present study, 74% of APOE e4 allele bearers and 83% of APOE e4/e4 homozygotes had AAO between these age points. This middle group ('middle age onset AD') would essentially define such individuals. Such age groupings might make more attractive targets for future studies looking for novel loci.

Hence, it would seem that genetic or non-genetic factors, other than APOE e4 allele, may be of importance in determining the likelihood of developing AD towards the (age) extremes of EOAD and LOAD. In those cases of familial EOAD, where the disease appears to be inherited in an autosomal dominant fashion, mutations in APP or PSEN-1 may be present. It is well known in both APP and PSEN-1 AD that the frequency of APOE e4 allele is not increased [5, 15–20]. In bearers of APP mutations AAO appears to be reduced in a dose-dependent manner according to number of APOE e4 alleles present [16, 18]. Although no such AAO effect has been reported for bearers of PSEN1 mutations in several earlier studies [15, 17, 19, 20], a more recent study, employing a much larger number of individuals all with PSEN1 E280A mutation, has shown AAO to be earlier in APOE e4 allele carriers versus non-e4 allele bearers with the same mutation [21]. Given that APP and PSEN1 mutations generate the same biological effect in promoting γ-secretase-mediated cleavage of APP protein, the finding that in E280A mutation APOE e4 allele modulates AAO is not unexpected, and previous studies [15, 17, 19, 20] probably had insufficient power to detect such an effect.

Interestingly, the APOE e4 allele frequency was lower in the older than in the younger, normal subjects, which may reflect a selective 'drop out' in later life of persons developing AD, or otherwise dying early due to cardiovascular disease or increased atherosclerosis of large cerebral arteries leading to vascular dementia or stroke, all such latter conditions having been associated with increased possession of APOE e4 allele [22–24].

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References


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