Age of onset in Huntington disease: sex specific influence of apolipoprotein E genotype and normal CAG repeat length

Patrick Kehoe, Michael Krawczak, Peter S Harper, Michael J Owen, A Lesley Jones

Abstract
Age of onset (AO) of Huntington disease (HD) is known to be correlated with the length of an expanded CAG repeat in the HD gene. Apolipoprotein E (APOE) genotype, in turn, is known to influence AO in Alzheimer disease, rendering the APOE gene a likely candidate to affect AO in other neurological diseases too. We therefore determined APOE genotype and normal CAG repeat length in the HD gene for 138 HD patients who were previously analysed with respect to CAG repeat length. Genotyping for APOE was performed blind to clinical information. In addition to highlighting the effect of the normal repeat length upon AO in maternally inherited HD and in male patients, we show that the APOE ε2/3 genotype is associated with significantly earlier AO in males than in females. Such a sex difference in AO was not apparent for any of the other APOE genotypes. Our findings suggest that subtle differences in the course of the neurodegeneration in HD may allow interacting genes to exert gender specific effects upon AO.

Keywords: Huntington disease; APOE; age of onset

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Age of onset.12 More recent evidence indicates that ApoE exerts an isoform specific antioxidant role. Thus, E2 gives most protection and E4 least,13 a finding not inconsistent with the substantially later AO of AD observed among ε2 carriers as compared with ε4 carriers.4

The association of APOE genotype with several neurodegenerative disorders has been examined, with inconclusive results,14 but an analysis of APOE genotypes in HD patients has not been reported so far. We therefore studied a series of DNA samples from patients previously analysed with respect to their CAG repeat number and AO1 to see whether APOE genotype could explain a significant proportion of AO variation.

Materials and methods
A total of 138 Welsh HD patients who had had their CAG repeat numbers determined before4 were analysed for their APOE genotype, blind to repeat number and AO. For 121 of these patients, the sex of the transmitting parent was also known. Polymerase chain reaction and CfoI digestion were carried out as described by Wenh et al.15 followed by polyacrylamide gel electrophoresis of fragments on 6% non-denaturing gels with ethidium bromide staining.

Statistical analysis was performed using the SAS/STAT® software package (version 6). Comparison of two Pearson correlation co-effi-
Table 1  Age of onset (AO) and APOE genotype in Welsh Huntington disease patients. AO is given as mean (SD), pathological repeat number is given as mean (range)

<table>
<thead>
<tr>
<th>APOE genotype</th>
<th>Male AO</th>
<th>Female AO</th>
<th>Pathological repeat No</th>
<th>Male AO</th>
<th>Female AO</th>
<th>Pathological repeat No</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2ε3</td>
<td>31.2 (6.3)</td>
<td>31.2 (6.3)</td>
<td>48.1 (12.9)</td>
<td>41.8 (6.3)</td>
<td>41.8 (6.3)</td>
<td>48.1 (12.9)</td>
</tr>
<tr>
<td>n=5</td>
<td>(41–52)</td>
<td>(41–52)</td>
<td>(38–54)</td>
<td>(38–54)</td>
<td>(38–54)</td>
<td>(38–54)</td>
</tr>
<tr>
<td>ε3ε3</td>
<td>42.5 (10.9)</td>
<td>42.5 (10.9)</td>
<td>43.2 (11.5)</td>
<td>42.8 (11.5)</td>
<td>42.8 (11.5)</td>
<td>43.2 (11.5)</td>
</tr>
<tr>
<td>n=34</td>
<td>(38–58)</td>
<td>(38–58)</td>
<td>(38–60)</td>
<td>(38–60)</td>
<td>(38–60)</td>
<td>(38–60)</td>
</tr>
<tr>
<td>ε3ε4</td>
<td>42.9 (11.6)</td>
<td>42.9 (11.6)</td>
<td>40.0 (13.9)</td>
<td>44.8 (13.9)</td>
<td>44.8 (13.9)</td>
<td>40.0 (13.9)</td>
</tr>
</tbody>
</table>

Table 2  Logistical survival analysis of age of onset in 17 HD patients with APOE genotype ε2ε3

<table>
<thead>
<tr>
<th>Model parameter</th>
<th>Model I ML estimates</th>
<th>Model II ML estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>β</td>
<td>−22.200</td>
<td>−19.141</td>
</tr>
<tr>
<td>b_a</td>
<td>0.138</td>
<td>0.121</td>
</tr>
<tr>
<td>b_b</td>
<td>0.201</td>
<td>0.265</td>
</tr>
<tr>
<td>b_c</td>
<td>2.145</td>
<td>NC</td>
</tr>
<tr>
<td>ln(ML)</td>
<td>−58.367</td>
<td>−62.024</td>
</tr>
</tbody>
</table>

ML: maximum likelihood; NC: not considered.

Table 3  Logistical survival analysis of age of onset in 56 male HD patients

<table>
<thead>
<tr>
<th>Model parameter</th>
<th>Model III ML estimates</th>
<th>Model IV ML estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>β</td>
<td>−21.348</td>
<td>−20.739</td>
</tr>
<tr>
<td>b_a</td>
<td>0.154</td>
<td>0.147</td>
</tr>
<tr>
<td>b_b</td>
<td>0.292</td>
<td>0.287</td>
</tr>
<tr>
<td>b_c</td>
<td>1.309</td>
<td>NC</td>
</tr>
<tr>
<td>ln(ML)</td>
<td>−191.482</td>
<td>−193.890</td>
</tr>
</tbody>
</table>

ML: maximum likelihood; NC: not considered.
significant ($\chi^2 = 7.314, 1 \text{ df}, p=0.007$). Similarly, the annual hazard rate of male $\varepsilon_{2 \varepsilon 3}$ carriers was 1.3 times higher on the logit scale than that of male HD patients with other APOE genotypes (table 3). This difference was also found to be statistically significant when tested by means of twice the log likelihood difference between the respective models, III and IV ($\chi^2 = 4.816, 1 \text{ df}, p=0.028$). Logistic survival analysis did not, however, show any significant difference between the annual hazard rates of female $\varepsilon_{2 \varepsilon 3}$ carriers and non-carriers ($\chi^2 = 0.464, 1 \text{ df}, p>0.4$) which implies that their differences in AO may be explicable in terms of different pathological repeat length.

**Discussion**

In trinucleotide repeat disorders where the expanded repeat is expressed within a protein, the extent of the pathological expansion shows consistent negative correlation with AO $^{2,4}$ and, at least for Machado-Joseph disease, the length of the normal allele is also critical for AO. $^{5}$ The length effect of the normal allele in HD has been assessed by a number of different groups. $^{2,4}$ An association of the normal repeat with AO has been reported previously in the Welsh population where an effect was found in maternally inherited HD, that is, when the normal repeat came from the father. The present study reproduces this finding and reports further data highlighting the importance of the normal repeat for AO. Undoubtedly, the phenomenon of anticipation in HD is related to the progressive expansion of the pathological CAG repeat upon transmission from parent to child, with greater expansions occurring in the paternal germline. In the 37–52 repeat range, Duyao et al. found an additional sex transmitted parental effect on AO which was independent of repeat length. It was observed that, on average, maternally inherited HD has AO two years later than paternally inherited HD with the same pathological repeat length, but no significant effect of the normal allele was apparent. $^{1}$ Our finding of a negative correlation between AO and the length of the normal repeat in paternally derived cases, however, appears to imply an additional effect of the normal allele reducing AO.

The ratio of pathological to normal repeat length may be as important for AO as the length of the normal repeat itself; the larger the length, the greater binding affinity or higher dissociation constants preventing the release of interacting proteins from any complexes formed with the pathologically expanded polyglutamine tracts, then a longer normal polyglutamine tract (that is, a smaller ratio of normal to expanded CAG repeat) may compete better with the expanded protein, allowing fewer aberrant interactions and thus delaying AO. The observed sex specificity may reflect gender differences in the availability of various proteins for interaction. Alternatively, an imprinted modifier gene in linkage disequilibrium with HD might drive the interaction, thereby rendering the effect dependent upon sex of the transmitting parent.

While no sex difference in AO is observed for patients with APOE genotypes $\varepsilon_{3 \varepsilon 3}$ or $\varepsilon_{3 \varepsilon 4}$, the $\varepsilon_{2 \varepsilon 3}$ genotype is associated with significantly earlier onset of HD in men than in women. Also, $\varepsilon_{2 \varepsilon 3}$ males had significantly earlier onset than other male patients while for female patients this relationship appeared to be reversed. It cannot be excluded that this is because of linkage disequilibrium with a nearby gene, but APOE genotype itself is known to affect pathology in disease and traumatic injury. In AD and in head injury, for example, ApoE is associated with the deposition of $\beta$-amyloid and such deposition is more prevalent in carriers of the $\varepsilon 4$ allele. $^{22}$ Furthermore, a sex difference has been shown in prevalence and age of onset for AD: women with the $\varepsilon_{3 \varepsilon 4}$ genotype have AO as early as $\varepsilon_{4 \varepsilon 4}$ homozygotes while in men the $\varepsilon_{3 \varepsilon 4}$ genotype gives the same (late) onset as $\varepsilon_{3 \varepsilon 3}$. $^{27}$ Carriership of $\varepsilon 4$ has also been implicated in Pick disease, corticobasal degeneration, and progressive supranuclear palsy $^{15}$ and motor neurone disease. $^{22}$ These findings suggest that ApoE is likely to affect neuronal function in the brain over and above the formation of neurofibrillary tangles and neuritic plaques, characteristic of AD. Expression of ApoE rises dramatically in response to brain injury and damage $^{16}$ and it may be expressed in the damaged areas of the brains of HD patients or be associated with the observed inclusions. $^{22}$. Although ApoE genotype is associated with late onset Alzheimer disease, the mechanism for this is unknown and thus it is difficult to assess what the mechanism behind this observed effect in HD might be.

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