The impact of apolipoprotein E genotypes on age at onset of symptoms and phenotypic expression in Wilson’s disease

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Summary

Wilson’s disease is a disorder of biliary copper excretion that may result in severe neurological symptoms and advanced liver disease. The wide variation of phenotypic disease expression cannot be fully explained by the different mutations of the Wilson disease gene. In neurological disorders, such as Alzheimer’s disease, temporal lobe epilepsy and cerebral trauma, the presence of the apolipoprotein E (ApoE) allele ε4 is associated with an increased vulnerability of the brain to the effects of the disease, whereas the presence of the ApoE genotype ε3/3 appears to provide moderate neuroprotection. We examined whether this hypothesis holds true for the development of neurological symptoms in patients with Wilson’s disease. The ApoE genotype and the H1069Q mutation (the most common in Wilson’s disease) status were determined by polymerase chain reaction-based mutation assays in 121 well-characterized, symptomatic index patients with Wilson’s disease. An investigation profile was established in which the patients were grouped according to the clinical symptoms at presentation, the ApoE genotypes and the status of the H1069Q mutation. Fifty-nine per cent of the 121 patients had the allele combination ApoE ε3/3 (21% ApoE ε3/4, 19% ApoE ε3/2, 1% ApoE ε4/2). The distribution of ApoE genotypes did not deviate from known distributions in healthy European subjects. Within the group of 40 H1069Q-homozygous patients, the onset of symptoms was significantly delayed in patients with the ApoE ε3/3 genotype (25 ± 6 years at presentation) compared with patients with the ApoE ε3/4 genotype (20 ± 3 years at presentation). In this study, the ApoE genotype was established as an important factor delaying the onset of neurological and hepatic symptoms, but not modifying phenotypic disease expression in a homogenous group of patients with Wilson’s disease (all H1069Q-homozygotes, similar genetic background). The presence of ApoE ε3/3 attenuates clinical manifestations in Wilson’s disease by mechanisms which might involve the antioxidant and membrane-stabilizing properties of the ApoE 3 protein.

Keywords: Wilson’s disease; H1069Q mutation; apolipoprotein E; clinical manifestations

Abbreviations: ApoE = apolipoprotein E; HSD = honestly significant difference; LDL = low density lipoprotein

Introduction

Wilson’s disease is a disorder of biliary copper excretion due to mutations in the ATP7B gene located on chromosome 13 (Petrukhin et al., 1994). The prevalence of Wilson’s disease is ~1 in 30 000 live births (Gollan and Gollan, 1998). Patients homozygous for the H1069Q mutation present more frequently with neurological than hepatic symptoms compared with patients heterozygous for H1069Q, or patients with other or unknown mutations (Maier-Dobersberger et al., 1997). Various defects of the ATP7B gene product, a copper-transporting P-type ATPase, cause an accumulation of copper in the liver and other organs, such as kidneys, cornea and brain. The most common mutation in Wilson disease patients of Northern and Eastern European ancestry is the H1069Q mutation, with a frequency ranging from 36% in Sweden to 93% in Poland (Thomas et al., 1995; Czlonkowska et al., 1997; Gollan and Gollan, 1998). In Austria, the H1069Q mutation is present in 61% of Wilson disease patients (21% homozygous, 40% compound heterozygous).

The wide spectrum of symptoms and the difference in age at onset of clinical manifestations in Wilson’s disease raise the question of whether these features are determined exclusively by the type of the ATP7B mutation or by other genetic factors, such as apolipoprotein E (ApoE) genotypes. There is evidence that ApoE genotypes containing the ε4
allele are over-represented in patients with rapidly progressing dementia in Alzheimer’s disease (Welsh-Bohmer et al., 1997). In a similar vein, several working groups have reported a less favourable neuropsychiatric outcome after traumatic, epileptic or haemorrhagic brain injury in patients with the ApoE ε4 allele compared with patients with other ApoE alleles (Alberts et al., 1995; Gouras et al., 1997; Teasdale et al., 1997). The mechanisms by which the ApoE 3 protein attenuates neuronal vulnerability in vivo are unknown. However, in vitro investigations have shown that ApoE 3 stimulates the branching of growing neurons and prevents neuronal death in conditions of oxidative stress (Nathan et al., 1994; Miyata and Smith, 1996). High copper concentrations, as seen in Wilson’s disease, induce tissue damage via the production of oxygen radicals (Petrukhin et al., 1994). ApoE 3 could therefore provide neuroprotection in patients with Wilson’s disease.

To determine whether the type of symptoms and the age of patients at presentation are influenced by the ApoE genotype, 121 patients with symptomatic Wilson’s disease were investigated with the intention of answering the following questions: (i) do patients with the ApoE ε3/3 genotype develop initial symptoms later than patients with other ApoE genotypes? and (ii) do ApoE genotypes determine phenotypic disease expression?

**Patients and methods**

Between 1988 and 1999, 121 patients with Wilson’s disease were investigated clinically and characterized according to symptoms at presentation as either neurological or hepatic. The ApoE genotype and the H1069Q mutation status were determined in each patient. Seventy-eight index patients were recruited from the Department of Internal Medicine IV, Gastroenterology and Hepatology, University of Vienna, Austria. Thirty-seven were diagnosed and treated at the Departments of Neurology and Internal Medicine II, University of Leipzig, Germany, three at the Semmelweis University, Budapest, Hungary and three at the University of Zagreb, Croatia.

From an original total of 155 subjects with laboratory findings suggestive of Wilson’s disease, we excluded 24 siblings, four patients with symptoms that could not be attributed unequivocally to Wilson’s disease and six patients whose medical history contained contradictory information on symptoms at presentation. The distributions of gender, ApoE genotypes and the H1069Q mutation in the group of 34 patients excluded were not different from those of the group of 121 patients whose data were used for statistical analysis.

Wilson’s disease was diagnosed on the basis of typical symptoms and the presence of conventional biochemical indicators (plasma ceruloplasmin <200 mg/l, serum copper <10 μmol/l, 24 h urine copper excretion ≥2 μmol/day and/or liver copper concentrations >250 mg/g on needle biopsy). All patients were examined by slit-lamp for the presence of Kayser–Fleischer rings around the iris. In 43% of our patients, an MRI examination of the brain was performed, and in 45% of the patients somatosensory evoked potentials were measured (Grimm et al., 1990).

Each patient was examined by two experienced, independent neurologists. Neuropsychiatric symptoms, such as tremor, dysarthria, ataxia, rigidity, dyskinesia, cognitive impairment and mood disturbances, were assessed in a semiquantitative fashion and ranged from 0 (completely normal) to 3 (severely impaired). Patients without major hepatic symptoms and with scores ≥ 2 for one or more neurological symptoms at the time of diagnosis, at any time before diagnosis (only if data were obtained by neurological specialists) or 1 year after diagnosis/start of therapy, were classified as patients presenting with neurological symptoms. Patients with scores from 0 to 1 (normal or slight neurological signs) and with major hepatic symptoms were classified as patients presenting with hepatic symptoms (Oder et al., 1993).

The ApoE genotype was determined by polymerase chain reaction using the commercially available ApoE Amplification Kit (Innogenetics, Zwijndrecht, Belgium). After amplification of the mutated region of the ApoE ε alleles, the presence of the amplified product was verified by agarose electrophoresis with a single band of 228 bp. Genotyping was performed by a reverse hybridization technique using the Inno LiPA ApoE Kit (Innogenetics, Zwijndrecht, Belgium).

H1069Q was identified as homozygous, compound heterozygous or negative by a polymerase chain reaction based assay (Maier-Dobersberger et al., 1997).

The software SPSS for Windows version 8.0 was used for statistical analysis. We tested for differences in distribution of nominal variables (H1069Q, ApoE, symptoms at presentation) using χ² analysis-of-contingency tables (with Yate’s corrections for continuity or Fisher’s exact test where appropriate). Differences in age within groups were tested after confirmation of normal distribution (Shapiro Wilk’s test) using Student’s t-test (two groups) or factorial analysis of variance with subsequent multiple post hoc comparisons according to Tukey’s honestly significant difference (HSD). Parametric values were expressed as mean ± standard deviation. Statistical significance was assumed if P < 0.05.

**Results**

Of the 121 patients (70 women, 51 men) with symptomatic Wilson’s disease, 60 patients presented with neurological symptoms, 61 patients with hepatic symptoms, and 88 patients exhibited the H1069Q mutation (41 homozygous, 47 compound heterozygous). Patients homozygous for the H1069Q mutation were classified more often as neurological than compound heterozygous and H1069Q-negative patients (Table 1). Our 61 hepatic patients were younger at the onset of symptoms than our 60 neurological patients (19 ± 7 versus 23 ± 7 years; t = 3.39, P = 0.001). No gender-specific differences were found with respect to clinical
Table 1  Clinical phenotype, H1069Q mutation status and ApoE genotypes in patients with Wilson’s disease

<table>
<thead>
<tr>
<th>Genetic determination</th>
<th>Neurological patients (n)</th>
<th>Hepatic patients (n)</th>
<th>Σn</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1069Q-homozygous</td>
<td>30*</td>
<td>11</td>
<td>41</td>
</tr>
<tr>
<td>H1069Q-heterozygous</td>
<td>20</td>
<td>27</td>
<td>47</td>
</tr>
<tr>
<td>H1069Q-negative</td>
<td>10</td>
<td>23</td>
<td>33</td>
</tr>
<tr>
<td>ApoE ε3/3 genotype</td>
<td>34</td>
<td>37</td>
<td>71</td>
</tr>
<tr>
<td>ApoE ε3/4 genotype</td>
<td>12</td>
<td>14</td>
<td>26</td>
</tr>
<tr>
<td>ApoE ε3/2 genotype</td>
<td>13</td>
<td>10</td>
<td>23</td>
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* H1069Q-homozygous patients developed neurological symptoms more often than H1069Q-heterozygous ($\chi^2 = 7.17$, $P = 0.007$, Yates correction for continuity) and H1069Q-negative patients ($\chi^2 = 11.86$, $P = 0.001$, Yates correction for continuity).

Symptoms (neurological versus hepatic), the H1069Q mutation status and ApoE genotypes.

The distribution of ApoE genotypes is given in Table 1 (ApoE ε3/3 = 59%, ε3/4 = 21%, ε3/2 = 19%). One H1069Q-homozygous, neurological patient exhibiting the genotype ApoE ε4/2 was excluded from statistical analysis with respect to ApoE genotypes for uniqueness. None of our patients had the extremely rare allele combinations ApoE ε4/4 or ε2/2. The overall ApoE genotype distribution in our patients was similar to that in healthy European subjects (de Knijff et al., 1994; Siest et al., 1995; Schmidt et al., 1997) and was homogenous within the two subgroups classified according to the H1069Q mutation status and clinical symptoms at presentation (Table 1).

Since the age of onset of symptoms and phenotypic disease expression depend on the type of defect of the Wilson disease gene (Thomas et al., 1995; Maier-Dobersberger et al., 1997; Shah et al., 1997; Gollan and Gollan, 1998), we established three subgroups (H1069Q-homozygous, H1069Q-heterozygous and H1069Q-negative) for further statistical analysis. Other unknown mutations in H1069Q-heterozygous and H1069Q-negative patients influence phenotypic disease expression and may abolish the positive effect of the ApoE ε3/3 genotype with respect to a delayed onset of symptoms. This was indeed the case in our patients, where disease onset was early irrespective of the ApoE genotypes present (47 H1069Q-heterozygous patients: 20 ± 9 years; 33 H1069Q-negative patients: 20 ± 8 years).

The genetic defect is well characterized in H1069Q-homozygous patients, therefore the investigation of additional genetic factors is easier. By stratifying all patients who were homozygous for the H1069Q mutation (n = 40) into three ApoE groups (ApoE ε3/3, ApoE ε3/4 and ApoE ε3/2) and two groups according to clinical symptoms (neurological or hepatic), we found that neurological patients were older at presentation than hepatic patients and that the age at onset of symptoms is determined by the ApoE genotype (Fig. 1). No interaction between the clinical symptoms and the ApoE genotypes was found and neither was synergistic. Post hoc comparison showed that H1069Q-homozygous patients with the ApoE ε3/3 genotype were significantly older at onset of symptoms than H1069Q-homozygous patients with the ApoE ε3/4 genotype (Tukey’s HSD, $P = 0.04$) but not significantly older than patients with the ApoE ε3/2 genotype, although statistical significance was nearly reached (Tukey’s HSD, $P = 0.07$).

Discussion

The results of this study indicate that patients with the ApoE ε3/3 genotype develop initial symptoms later than patients with other ApoE genotypes. However, ApoE genotypes do not determine phenotypic disease expression.

Any attempt to correlate phenotypes with genotypes in inherited diseases faces a variety of problems. Selection bias is the most important variable. The diagnosis of Wilson’s disease is made most commonly when neurological symptoms are present. Wilson disease patients with liver disease frequently do not develop all diagnostic features listed in textbooks and are thus missed (Steindl et al., 1997). In this study, most patients were recruited in a single centre where they underwent a standardized neurological and hepatological evaluation. The 78 Austrian index patients in this study represent 90% of all known Wilson disease families in this country. The second largest group of patients enrolled in this study was diagnosed at the University of Leipzig, a known referral centre for Wilson’s disease, according to the same principles as in the Austrian centre. In addition, definition of disease onset is quite difficult. Early diagnosis is missed in a substantial proportion of patients because of neurological or hepatic symptoms unrelated to Wilson’s disease. In some patients, birth trauma or head injury result in neurological symptoms similar to those in Wilson’s disease. Likewise, severe alcohol abuse may mimic neurological and hepatic symptoms otherwise characteristic of Wilson’s disease. In some primarily hepatic cases, Wilson’s disease is diagnosed only after the onset of Wilson disease-specific neurological symptoms. Finally, there are >50 known mutations of the Wilson disease gene, most of which are rare and occur in single patients only. Moreover, a large proportion of Wilson disease patients have two different mutations (compound heterozygotes). Some mutations are associated with severe dysfunctions of the Wilson disease gene product and frequently cause serious liver disease in early childhood; other mutations are less severe and are associated with late-onset neurological disease. Of the known mutations, only a few occur in a larger number of families. Sufficient clinical information is available only for the H1069Q mutation. This mutation occurs in 30–90% of patients of Northern European (Waldenstrom et al., 1996), Central European (Maier-Dobersberger et al., 1997) and Eastern European (Czlonkowska et al., 1997) origin. Most patients who are homozygous for the H1069Q mutation present with neurological symptoms with a late onset (Houwen et al., 1995; Maier-Dobersberger et al., 1997). The H1069Q mutation is regarded as a ‘mild’ mutation which does not completely disrupt biliary copper transport and thus delays the
manifestation of symptoms (Thomas et al., 1995; Czlonkowska et al., 1997). Our results are in agreement with previous findings on H1069Q homozygosity and late-onset neurological Wilson’s disease (Petrukhin et al., 1994; Houwen et al., 1995; Czlonkowska et al., 1997; Maier-Dobersberger et al., 1997). However, this genotype–phenotype correlation was not found in a study on 109 North American and Canadian patients (30 of whom were H1069Q-homozygous) mainly because of selection bias and the availability of clinical data in only ~60% of the patients (Shah et al., 1997). Larger population-based studies should address the question of whether additional genetic factors influence the age at onset of symptoms and phenotypic disease expression in Wilson’s disease.

The influence of ApoE genotypes on disease onset and the severity of symptoms has been investigated mainly in several neuropsychiatric disorders and in patients with impaired lipoprotein lipid metabolism. ApoE 4 has been identified as a risk factor in Alzheimer’s disease and worsens neurological outcome after traumatic brain damage (Teasdale et al., 1997; Welsh-Bohmer et al., 1997). The ApoE 2 protein appears to protect patients against coronary arteriosclerosis (Siest et al., 1995).

ApoE is a key protein for the transport of cholesterol esters and lipids in the CNS. In contrast to the brain where ApoE is the major apolipoprotein, the liver synthesizes a greater amount and several different apolipoproteins, resulting in a comparatively low plasma concentration of ApoE. On the other hand, low density lipoprotein (LDL) receptor expression and apolipoprotein synthesis (including ApoE synthesis) are decreased in several hepatic disorders, such as hepatitis and toxin-induced liver failure, whereas regenerating liver tissue requires a high cholesterol supply (Fukushima et al., 1993). Different ApoE isoforms have different affinities for the LDL receptor. ApoE 2 shows a defective binding activity leading to an upregulation of the LDL receptor, thereby inducing hypocholesterinaemia, whereas ApoE 4 induces a downregulation of the LDL receptor leading to hypercholesterinaemia (Siest et al., 1995). Furthermore, the plasma concentrations of ApoE itself, of other apolipoproteins and of triglycerides vary within individuals with different ApoE allelic combinations (Siest et al., 1995).

Several biochemical mechanisms may explain why the onset of neurological as well as hepatic symptoms occurs later in Wilson disease patients with the ApoE ε3/3 genotype compared with patients with other ApoE genotypes. The cytotoxic effect of copper is mediated by the production of highly reactive radicals that might damage cell membranes by lipid peroxidation. It is not clear whether copper itself, accumulating in the liver and in the brain, is cytotoxic, or whether peroxidation is caused by the interaction of copper and iron. However, it is known that ApoE is able to bind metal ions, with the highest affinity for copper (Miyata and Smith, 1996). Moreover, it has been suggested that the sequestering of copper might be the most important mechanism responsible for the antioxidative effect of the ApoE protein. In addition, it has been speculated that the affinity of ApoE proteins for copper and other metal ions varies depending on the ApoE isoform (Miyata and Smith, 1996). However, copper toxicity may not be restricted to
direct cell damage. In vitro, copper impedes the transcription of the gene for ApoE synthesis (Jo et al., 1995). Thus, the gradual accumulation of copper might reduce the concentrations of ApoE proteins and thereby induce the early manifestation of symptoms, particularly in patients with the ApoE ε3 genotype on one allele only.

ApoE, as the main carrier of lipids in the brain, is thought to be especially important for repair mechanisms in the CNS. After neuronal tissue damage, the synthesis of the ApoE protein is upregulated in reactive astrocytes (Snipes et al., 1986; Nathan et al., 1994). The ApoE 3 protein acts as an antioxidant and is able to promote axonal growth (Nathan et al., 1994). However, ApoE isoforms vary in their neuroprotective properties. ApoE 3, in contrast to ApoE 4, enhances neuronal growth (Nathan et al., 1994) and increases the resistance of cell cultures to oxidative stress (Miyata and Smith, 1996). Furthermore, ApoE-deficient mice are more prone to oxidative tissue damage after ischaemia than mice with normal ApoE production (Hayek et al., 1994; Laskowitz et al., 1997). Similarly, transgenic mice with the ApoE ε3/3 genotype have a better neurological outcome and survival rate after ischaemic stress than mice with the ApoE ε4/4 genotype (Sheng et al., 1998). Taken together, these results clearly indicate a neuroprotective effect of ApoE by preserving the integrity of neuronal membranes, ApoE 3 being more effective than ApoE 4.

In conclusion, this study provides evidence that Wilson disease patients homozygous for the most common mutation, i.e. the H1069Q mutation, and also exhibiting the ApoE genotype ε3/3 develop initial symptoms between 5 and 11 years later than patients with genotypes other than ApoE ε3/3. No relationship was found between the ApoE genotype distribution and the type of clinical symptoms at presentation.

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