Apolipoprotein E genotype and schizophrenia: further negative evidence


Objective: To investigate the association between apolipoprotein E (ApoE) genotype and schizophrenia.

Method: We genotyped 106 schizophrenic out-patients [Diagnostic Statistic Manual IV (DSM-IV) criteria] and 250 healthy volunteers (hospital staff and blood donors) from Asturias (Northern Spain). The ApoE genotypes (ε2, ε3, ε4 – alleles) were determined after polymerase chain reaction (PCR) amplification, followed by digestion with the restriction enzyme CfoI and electrophoresis on a 4% agarose gel.

Results: No significant differences in ApoE-allele frequencies between patients and controls was found, although an increased ε4-frequency was recorded in patients compared with controls [9.0% vs. 6.2%, P = 0.124; odds ratio (OR) = 1.49; 95% confidence interval (CI) = 0.82–2.70]. ApoE-genotype frequencies did not differ between both groups. The mean age of onset for schizophrenic patients that carried the ε4-allele was not significantly different from that of patients without this allele.

Conclusion: Variation in the ApoE gene was not associated with the development of schizophrenia in our population. ApoE-genotypes did not modify the age of onset of the disease.

Introduction

Apolipoprotein E (ApoE) is a 34-kDa protein encoded by a gene on human chromosome 19. ApoE is a major component of lipoproteins and plays an important role in cholesterol transport. ApoE may be involved in cholesterol transport into neurons, thus playing an important role in neuronal growth and in the central nervous system response to injury, particularly in the hippocampal region (1).

The ApoE gene is polymorphic, and three alleles (ε2, ε3, ε4) that differ in amino acids at positions 112 and 158 have been described. The ε4 allele has been linked to an increased risk of developing late-onset Alzheimer’s disease (AD) (2–8). In addition, ε4-carriers would have an earlier onset of AD, compared with ε3ε3 (9, 10), while the ε2 allele would confer a protective role, delaying age of onset of AD (11). In addition to its role in AD, ApoE-variation has been associated with vascular dementia and Lewy body dementia (12, 13), a fact that supports an important role for ApoE in neuropsychiatric disorders.

Because the ApoE- genotypes may influence the phenotype of several neuropsychiatric disorders, it has been proposed as a non-specific risk factor that would modify the risk conferred by other more specific factors (14, 15). Schizophrenia is a complex genetic disorder that would involve variation at several genes of small effect (16). The genetic association between the ε4 allele and AD and other neuropsychiatric diseases, plus the clinical similarities between chronic schizophrenic patients and those with AD (cognitive impairment, psychotic symptoms, and changes of behavioral and affective patterns) have led several authors to investigate the role of the ApoE genotypes and schizophrenia.

A role for ApoE-alleles in schizophrenia has been reported by some authors, but not others. Thus, an increased ε4-frequency in patients compared with controls has been described (14), while others found a decreased ε4-frequency (17, 18). In addition, ApoE ε4-frequency was lower in schizophrenics with progressive dementia and late paraphrenia (19, 20), and at least one study has reported an earlier age of onset for adult...
schizophrenics who were ε4-carriers (15). However, this association between ApoE ε4 and the age of onset was not found by others (21). Most of the studies did not support a role for the variation at the ApoE and schizophrenia (15, 21–31).

A relationship between ApoE-genotypes and the clinical course of the disease has been reported. Thus, Pickar et al. (32) found significantly lower psychosis scores among ε4-carriers (although the distribution of the genotypes was consistent with a lack of association between ApoE and the risk of developing the disease). In opposition to these authors, Ohara et al. (33) reported no difference in clinical characteristics between patients with and without the ε4-allele. Finally, Rietschel et al. (34) suggested that positive symptoms of ‘incoherence’, ‘speech difficult to understand’, and ‘persecutory delusions’ are preferentially displayed by ApoE ε4-carriers but who manifested less severe positive symptoms of schizophrenia than patients who do not carry this allele.

In order to clarify the possible association between the variation at the ApoE-gene and schizophrenia, we analysed a group of schizophrenic patients and matched controls. The possible association between ApoE-genotypes and age of onset of the disease has also been investigated.

Material and methods

Subjects

One hundred and six schizophrenic out-patients from the region of Asturias (Northern Spain, total population 1 million) were enrolled in the study. All patients had a diagnosis of schizophrenia according to Diagnostic Statistic Manual IV (DSM-IV) criteria, and the age of onset was the age at which symptoms were of sufficient degree to meet the DSM-IV criteria for schizophrenia (15).

Two hundred and fifty healthy volunteers (hospital staff and blood donors), matched with the patients for age and ethnicity, were also genotyped. The study was conducted according to the provisions of the World Medical Association Declaration of Helsinki, and ethical approval of the study was granted (35).

ApoE genotyping

Genomic DNA was extracted from leukocytes in 10 ml of peripheral blood. ApoE-genotyping was performed as previously described (36). Briefly, 100 ng of genomic DNA were polymerase chain reaction (PCR)-amplified with primers TCCAAG-GAGCTGCAGGCGGCGCA and ACAGAATT-GCCCCGGCCTGGTACACTGCCA. After 35 cycles of amplification, reactions were digested with the restriction enzyme CfoI, and the digested-fragments were separated by electrophoresis on a 4% ethidium-bromide-stained agarose gel. Allele assignment was based on the presence or absence of CfoI cutting sites in the amplified sequence.

Statistical analysis

Differences between allele and genotype frequencies were assessed using a χ² test or Fisher’s exact test, and P ≤ 0.05 was considered as significant. Odds ratios (ORs) and their confidence intervals (95% CIs) were also calculated. The relationship between ApoE-genotypes and age of onset was explored in two ways (15). First, the cohort was divided into two groups based on the presence or absence of at least one ε4-allele, and these groups were compared using Student’s t-test. Secondly, in order to define a possible dose–effect relationship, we graded each genotype on a five-point scale, with the ε2ε2 representing the lowest point and ApoE ε4ε4 the highest point (1 = ε2ε2, 2 = ε2ε3, 3 = ε3ε3 or ε2ε4, 4 = ε3ε4, 5 = ε4ε4). This allowed us to account for a possible protective effect of the ε2 allele when added against the presumed neutral effect of ε3 and the negative effect of ε4 (15). The SPSS/PC+ sofware was used for the statistical analyses.

The sample-size required to reach a power of 80, at a significance level (α) of 0.05, was also calculated.

Results

We genotyped 106 schizophrenic out-patients (DSM-IV criteria) [mean age (SD): 35.1 (10.3) years; males: 55.6% (60 patients)] and 250 healthy volunteers [mean age (SD): 46 (15.0) years; males 48% (120 subjects)]. The ApoE-genotypes distribution in schizophrenics and controls is summarized in Table 1. Genotype frequencies did not differ between patients and controls (P = 0.399). However, an increased frequency of ε4-carriers (ε3ε4 + ε4ε4) was found in patients compared with controls (P = 0.096; OR = 1.60; 95% CI = 0.86–2.99) (Table 2). To reach a power of 80 (at a significance level α = 0.05), a total of 441 patients and 1041 controls should be analysed. Allele frequencies did not differ between the two groups (P = 0.124; OR = 1.49; 95% CI = 0.82–2.70) (Table 3).

The mean age of onset for ε4-carriers (ε3ε4, ε4ε4) was 26.62 (11.77), compared with 25.56 (8.70) among the ε4-negative patients. This difference between groups was not significant.
Apolipoprotein E genotype and schizophrenia

Table 1. Distribution of the ApoE genotypes in schizophrenic patients and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Schizophrenics</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2/ε2</td>
<td>0 (0)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>ε2/ε3</td>
<td>7 (6.6)</td>
<td>25 (10.0)</td>
</tr>
<tr>
<td>ε3/ε3</td>
<td>80 (75.5)</td>
<td>194 (77.6)</td>
</tr>
<tr>
<td>ε3/ε4</td>
<td>19 (17.9)</td>
<td>29 (11.6)</td>
</tr>
<tr>
<td>ε4/ε4</td>
<td>0 (0)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
<td>250</td>
</tr>
</tbody>
</table>

χ² test, P = 0.399. Values in parentheses are in percentages.

Table 2. Distribution of ApoE ε4 carriers in schizophrenic patients and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Schizophrenics</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2/ε2/ε3/ε3/ε3</td>
<td>87 (82.1)</td>
<td>220 (88.0)</td>
</tr>
<tr>
<td>ε3/ε4/ε4/ε4</td>
<td>19 (17.9)</td>
<td>30 (12.0)</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
<td>250</td>
</tr>
</tbody>
</table>

Fisher’s exact test, P = 0.096; OR = 1.60; 95% CI = 0.86–2.99. Values in parentheses are in percentages.

Table 3. Allele frequencies of ApoE in schizophrenic patients and controls

<table>
<thead>
<tr>
<th>ApoE allele</th>
<th>Schizophrenics</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2/ε3</td>
<td>193 (91.0)</td>
<td>469 (83.8)</td>
</tr>
<tr>
<td>ε4</td>
<td>19 (9.0)</td>
<td>31 (6.2)</td>
</tr>
<tr>
<td>Total</td>
<td>312</td>
<td>500</td>
</tr>
</tbody>
</table>

Fisher’s exact test, P = 0.124; OR = 1.49; 95% CI = 0.82–2.70. Values in parentheses are in percentages.

(t = −0.343, P = 0.736). Age of onset did not correlate with ApoE grade (r = 0.088, P = 0.407).

Discussion

To our knowledge this is one of the first reports analysing the association between the ApoE-polymorphism and schizophrenia in the Spanish population. Our data do not support a role for ApoE-genotypes in schizophrenia, a result that is in agreement with previous reports (15, 21–31). Although carriers of the ε4-allele were at a higher frequency among the patients, the difference did not reach a statistical significance (P = 0.096), and the size of the patients and controls required to reach a power of 80 (at a significance level α = 0.05) was too high. In opposition to these authors, Harrington et al. (14) described an increased frequency of the ε4-allele among schizophrenics, while Igata-Yi et al. (17) and Chen et al. (18) found a decreased ε4-frequency in patients.

There are several explanations for this discrepancy (25). First, the association between ApoE-genotypes and schizophrenia could differ between different populations (37). Secondly, positive findings could be because of selection bias or type II errors as a consequence of a reduced sample-size. To further confirm previous results, Town et al. (27) combined the data of three studies (14, 23, 27), increasing the power to 93 (thus reducing the likelihood of a type II error), and again found no association between ε4-carriers status and schizophrenia diagnosis (χ² = 0.376, P = 0.540). Thirdly, the criteria for diagnosis of schizophrenia in the postmortem cohort studied by Harrington et al. (14) were not specified. Arnold et al. (38) described wrong diagnostic classification in as high as 50% of the cases, when only listed chart diagnosis were used without application of more rigorous criteria in chart review or patient examination. Fourthly, because the diagnosis of schizophrenia is based on symptom-oriented criteria, the selection of cases according to the same criteria does not assure etiologically homogeneous patients in different studies (25). Fifthly, patients recruited from a out-patient service could have a less severe psychopathology, and the discrepancies between the studies could be a consequence of the differences in the nature of the sample source.

No patient in our sample has the ε4ε4 genotype. This is in agreement with previous reports (18, 21, 25, 33). As the ε4 allele is significantly associated with the development of AD, this finding may partially explain why the AD neuropathology is infrequent in demented schizophrenic subjects (39). In addition, ε4-carriers have higher plasma cholesterol levels compared with non-carriers (40), a finding that is in agreement with previous reports that described lower cholesterol levels in schizophrenics compared with healthy controls (41). The lack of association with ApoE expression found in our study may provide evidence for alternative molecular mechanisms underlying the dementia in schizophrenia and AD.

Our results are similar to those of Kimura et al. (21) and do not support an involvement of the ε4-allele in the age of onset of schizophrenia (15). The cause of such discrepancy is uncertain, but could be the result of ethnic or environmental differences. This explanation is supported by the fact that the association between the ApoE-genotype and AD would be race-dependent (42). Another important factor could be the differences in the methodology used for determining the age of onset. However, our method was similar to that used by Arnold et al. (15). Finally, as Arnold et al. (15) suggested, it is also possible that their result is a false positive.
In conclusion, the results of our study are in accordance with previous results reports, and do not support a role for the ApoE ε4 allele in schizophrenia. However, further studies in schizophrenic patients with severe cognitive dysfunction may help to clarify the relationship, if any, between ApoE and schizophrenia.

References

35. WORLD MEDICAL ASSOCIATION. Declaration of Helsinki. Recommendations guiding physicians in biomedical research involving human subjects. Amended by the 41st World Medical Assembly, Hong Kong, September 1989.
38. ARNOLD SE, GUR RE, SHAPIRO RM et al. Prospective clinicopathologic studies of schizophrenia: accrual and...


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