ApoE genotype as risk factors for Alzheimer’s disease in the population of Lublin region, Poland

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Abstract: There is much data concerning ApoE in the pathology of Alzheimer’s disease. This study examines how ApoE genotype contributes to a risk for Alzheimer’s disease in the population of the Lublin region in Poland. The data were obtained as part of a population based BERCAL (Epidemiologic study of Alzheimer’s diseases and other forms of dementia in the population of Lublin region) study. Odds ratios (ORs) and 95% confidence intervals (CIs) for AD, adjusted for age, were calculated for ApoE genotypes. Epsilon3/epsilon3 genotype was the most prevalent genotype in both examined groups. Epsilon2/epsilon2 and epsilon4/epsilon4 were the least prevalent. Among subjects, the risk of AD was significantly reduced for people with genotypes epsilon2/epsilon2. The same significant result was noticed where results were recalculated so that the presence of at least one epsilon2 allele was taken into account, without any epsilon4 allele in each person. Increased risk with occurrence of epsilon 4 allele is not shown. Increased risk in epsilon4 carriers was observed. The test did not reach the level of power required. This study suggests that the well known genetic factor, namely ApoE, even if of noticeable importance, may not be a main risk factor among the population of the Lublin region in Poland.

Key words: apolipoprotein, Alzheimer’s disease, risk assessment

INTRODUCTION

Alzheimer’s disease (AD) is an irreversible, progressive disorder in which neurons deteriorate, resulting in the loss of cognitive functions, mainly memory, judgment, reasoning, and perception. The disease primarily affects the cerebral cortex and the hippocampus through a number of pathological processes [1, 2]. One such process involves the formation of amyloid plaques. This occurs through the abnormal production and deposition of Amyloid β in the brain and its vasculature [3-5]. Amyloid β is a 42-amino acid polypeptide, believed to be toxic to neurons and nerve terminals [6, 7].

Another pathological feature of AD is the presence of neurofibrillary tangles which are intraneuronal accumulations of paired helical filaments. This process is related to the neuronal loss associated with AD [8].

Over 160 mutations on three genetic loci have been identified as being responsible for most cases of familial early onset autosomal dominant AD (with onset before the age of 65). Chromosome 14, presenilin 1; Chromosome 1, presenilin 2; chromosome 21, β-amyloid precursor protein (β APP). However, these early onset forms are responsible for less than 1-2% of cases [9, 10].

The discovery of these genetic loci linked to AD has led to important insights into the disease. The β APP gene on chromosome 21 is cleaved by β enzymes, known as the α, β and γ secretases, to produce amyloidogenic (Aβ) or nonamyloidogenic breakdown products. The activity of these alternative pathways of β APP catabolism, as well as the rate of β APP expression, is controlled by genotype and the environment [9].

The most important genetic risk factor for sporadic AD is the apolipoprotein E (ApoE) e4 allele located on chromosome 19 [10-15]. The ApoE4 allele is associated with a 2-3-fold increased risk of contracting the disease when one copy is present, and when there are two copies the risk is increased by as much as 12 times (the ApoE gene is co-dominant) [14]. However, the ApoE4 alleles not necessary or sufficient for AD, and perhaps 50% of the inherited risk of contracting the disease is currently unknown. The strong association of ApoE genotype to AD is a potent indicator of the importance of lipid metabolism and diet in the pathogenesis of the disease [15, 16].

Studies have shown that genetics play a role in the extracellular deposition of amyloid. Individuals afflicted with AD carrying the ApoE4 isoforms have shown a greater number of amyloid β plaques when compared to ApoE3 carriers, and inheritance of an ApoE4 allele increases the risk of AD when compared to ApoE2 and ApoE3 carriers [17, 18].

Apolipoprotein E is a polymorphic glycoprotein that plays an essential part in the binding to receptors for the uptake of chylomicrons and VLDL remnants and of LDL. The three major isoforms are ApoE3 (Cys112/Arg158), -E4 (Arg112/Arg158) and -E2 (Cys112/Cys158). The ApoE genetic variation has a great impact. ApoE polymorphism is an essential determinant in the inter-individual variations of lipids in healthy subjects in various populations. Its influence can be significant on the efficacy of nutritional or therapeutic interventions [19, 20]. The allele epsilon 4 appears to be associated with an increased risk of premature atherosclerosis. ApoE polymorphism contributes to the lipid disorders in diabetes and obesity [21]. It has been demonstrated that Epsilon 4 is associated with the lowering...
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of age for the onset of Alzheimer’s disease, but seems to be a major risk factor in late onset AD [18, 19].

In this study, ApoE isoforms were studied as independent risk factors for AD in a group of people selected from the population of the Lublin region in Poland.

METHODS

Population based sampling design. The current study is a part of a large population-based study called BERCAL (Epidemiologic study of Alzheimer’s diseases and other forms of dementia in the population of Lublin region).

The participants of the BERCAL study were randomly selected from a population-based sample within the Lublin Region (2,182,191 inhabitants) in 2005 [22]. The main aim of the project was to assess the prevalence of dementia and its risk factors. As a result of the project, the prevalence of Alzheimer’s disease in the Lublin region of Poland was calculated as 1634.6 /100,000 inhabitants. The screening was repeated three times in order to strengthen the power of the prevalence testing and to obtain more samples for analysis. Since the BERCAL study design has described elsewhere in more detail [22], suffice to mention here that dementia was diagnosed by the Diagnostic and Statistical Manual of Mental Disorders (4th ed.)(DSM-IV) criteria [23], and AD was diagnosed in accordance with the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria [24].

Normal control subjects were recruited from the same population. Control participants had normal cognition, excluding mild cognitive impairment (MCI).

The study was carried out in accordance with the local IRB agreement. Written informed consent was obtained from each patient (if possible), the caregiver, and the patient’s representative (if applicable), before beginning detailed screening activities.

Finally, the AD group (n = 52) and 50 healthy, age and gender matched control were recruited for further genetic analysis.

DNA sampling and analysis methods. A fasting morning blood sample was collected from every subject. Plasma total cholesterol (TC), LDL and HDL was determined. Genomic DNA was extracted fromuffy coat leukocytes by standard method. DNA was amplified by polymerase chain reaction (PCR) in a thermocycler (ABI 9700) (Forward primer 5’-TAAGCTTGGCACGGCTGTCCAAGGA-3’), as described by Hixson and Vernier [25, 26].

Statistical analyses. Descriptive analyses were carried out for all variables. Age, sex, blood pressure, and total cholesterol, HDL and LDL levels, and MMSE, scores were compared among the dementia group and normal controls, using one-way ANOVA. Odds ratios (ORs) and 95% confidence intervals (CIs) for AD, adjusted for age, was the main outcome measurement in this study. Statistical significance was marked when Power of the test was > 0.8 for alpha=0.05.

Sigma Stat 3.0 for Windows was used for statistical analyses. All p values <0.05 were regarded as statistically significant.

RESULTS

Odds ratios (ORs) and 95% confidence intervals (CIs) for AD, adjusted for age, were calculated for ApoE genotypes epsilon2/epsilon2, epsilon2/epsilon3, epsilon2/epsilon4, epsilon3/epsilon3, epsilon4/epsilon4 and epsilon3/epsilon4 group.

Epsilon3/epsilon3 genotype was the most prevalent genotype in both examined groups. Epsilon2/epsilon2 and epsilon4/epsilon4 were the least prevalent (Table 1).

Among BERCAL subjects, the risk of AD was significantly reduced for people with genotypes epsilon2/epsilon2 (Table 2). The same significant result was noticed where the results were recalculated in order that the presence of at least one epsilon2 allele was taken into account, without any epsilon 4 allele, in each person. (Table 3).

Not shown was an increased risk with epsilon 4 allele occurrence. An increased risk in epsilon 4 carriers was observed, but the test did not reach the required level of power, probably because of analyzed sample being too small (Table 4).

![Table 1](https://example.com/table1.png)

<table>
<thead>
<tr>
<th>ApoE genotype</th>
<th>AD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epsilon2/epsilon2</td>
<td>3</td>
<td>5.8%</td>
</tr>
<tr>
<td>Epsilon2/epsilon3</td>
<td>11</td>
<td>21.2%</td>
</tr>
<tr>
<td>Epsilon2/epsilon4</td>
<td>7</td>
<td>13.5%</td>
</tr>
<tr>
<td>Epsilon3/epsilon3</td>
<td>20</td>
<td>38.5%</td>
</tr>
<tr>
<td>Epsilon3/epsilon4</td>
<td>8</td>
<td>15.4%</td>
</tr>
<tr>
<td>Epsilon4/epsilon4</td>
<td>3</td>
<td>5.8%</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>100%</td>
</tr>
</tbody>
</table>

![Table 2](https://example.com/table2.png)

<table>
<thead>
<tr>
<th>ApoE genotype</th>
<th>OR, 95%CI; AD/Control</th>
<th>Power, &gt; 0.08 alpha 0.005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epsilon2/epsilon2</td>
<td>0.1743, 0.0463 to 0.6564*</td>
<td>*0.93</td>
</tr>
<tr>
<td>Epsilon2/epsilon3</td>
<td>0.6899, 0.2783 to 1.7101</td>
<td>0.118</td>
</tr>
<tr>
<td>Epsilon2/epsilon4</td>
<td>3.6556, 1.7207 to 18.5423</td>
<td>0.374</td>
</tr>
<tr>
<td>Epsilon3/epsilon3</td>
<td>1.4583, 0.6401 to 3.3224</td>
<td>0.136</td>
</tr>
<tr>
<td>Epsilon3/epsilon4</td>
<td>2.8485, 0.7101 to 11.4268</td>
<td>0.315</td>
</tr>
<tr>
<td>Epsilon4/epsilon4</td>
<td>3.0301 to 29.8506</td>
<td>0.153</td>
</tr>
</tbody>
</table>

![Table 3](https://example.com/table3.png)

<table>
<thead>
<tr>
<th>Patient with Allele</th>
<th>OR, 95%CI</th>
<th>Power, &gt; 0.08; AD Control alpha 0.005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epsilon2 non 4</td>
<td>0.2668, 0.1162 to 0.6126*</td>
<td>*0.97</td>
</tr>
<tr>
<td>Epsilon4 non 2</td>
<td>3.0854, 0.9115 to 10.4442</td>
<td>0.45</td>
</tr>
</tbody>
</table>

![Table 4](https://example.com/table4.png)

<table>
<thead>
<tr>
<th>Patient with Allele</th>
<th>OR, 95%CI</th>
<th>Power, &gt; 0.08; AD Control alpha 0.005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epsilon4</td>
<td>3.6667, 1.3175 to 10.2049</td>
<td>0.78</td>
</tr>
<tr>
<td>Epsilon2</td>
<td>0.4152, 0.1874 to 0.92</td>
<td>0.58</td>
</tr>
</tbody>
</table>

DISCUSSION

Pinpointing risk factors for Alzheimer’s disease may have a widespread social impact. From a population perspective, it is possible to calculate the attributable risk of a factor if one knows the frequency of the risk factor in the matched, non-affected population, and the relative risk or odds ratio. There is much data concerning ApoE in the pathology of Alzheimer’s disease. In fact, apolipoprotein E epsilon4 allele is the only well confirmed genetic risk factor for sporadic AD. As it contributes to 40-70% of AD cases, a large proportion of genetic variance may be determined by additional loci. Many studies have confirmed that the epsilon4 allele is a dose-response risk factor for AD, and the epsilon4/epsilon4 genotype is associated with a significantly earlier age of onset. Moreover, it has been proved that the epsilon2 allele was a dose-response protective factor for AD, and the epsilon3 allele exerts a weaker dose-response protective effect for risk of AD, compared with epsilon2 [10-12, 20].

ApoE epsilon4 is associated with small vessel arteriolar-sclerosis, microinfarcts of the deep nuclei, neuritic senile plaque density, and amyloid angiopathy in patients with autopsy-proved Alzheimer disease. These results suggest a role for epsilon4 in some of the microvascular changes commonly found in AD, and are consistent with a potential amyloidogenic role for epsilon4 [27].

In the population examined, recruited from the BERCAL study, however, ApoE4 seemed to be the least prevalent, both among AD and control subjects.

On the other hand, multi-centre studies undertaken by Farrer et al. [28] confirmed the high occurrence of ApoE4 in people with AD.

The low percentage of ApoE4 allele in the population examined in the presented study may suggest that factors other than ApoE4 factors may contribute to the occurrence of Alzheimer’s disease in the Lublin region of Poland.

Farrer et al. [28] examined more closely the association between apolipoprotein E genotype and Alzheimer disease by age and sex in populations of various ethnic and racial denominations. Forty research teams contributed data on ApoE genotype, sex, age at disease onset, and ethnic background for 5,930 patients who met criteria for probable or definite AD, and 8,607 controls without dementia who were recruited from clinical, community, and brain bank sources.

Similar to the presented study, Farrer et al. [27] studied odds ratios and 95% confidence intervals for AD, adjusted for age, and stratified by major ethnic group (Caucasian, African American, Hispanic, and Japanese, for ApoE genotypes epsilon2/epsilon2, epsilon2/epsilon3, epsilon2/epsilon4, epsilon3/epsilon3, epsilon3/epsilon4, and epsilon4/epsilon4 relative to the epsilon3/epsilon3 group. For the purpose of this discussion, only the results from Caucasian subjects have been taken into consideration, among whom the risk of AD was significantly increased for people with genotypes epsilon2/epsilon4 (OR=2.6, 95% CI=1.6-4.0), epsilon3/epsilon4 (OR=3.2, 95% CI=2.8-3.8), and epsilon4/ epsilon4 (OR=14.9, 95% CI=10.8-20.6).

In the population under study, increased risk in epsilon 4 carriers was observed, but the test did not reach the required level of test power, probably because the sample analyzed was too small. Even if had been powerful enough, the test would not, however, have the same influence. In the population of the Lublin region, the carrier state of epsilon 4 would not increase the risk by even 4 times and the occurrence of E4/E4 by even 3 times, as opposed to the 15 times reported by Farrer’s metha-analysis [27].

In Farrer’s study, ORs were decreased for people with genotypes epsilon2/epsilon2 (OR=0.6, 95% CI=0.2-2.0) and epsilon3/epsilon3 (OR=0.6, 95% CI=0.5-0.8).

In the presented study, the same effect was observed in the AD group for epsilon2/epsilon2. In the case of epsilon2/ epsilon3 genotype, however, the data were not significant. Despite the fact that the results of OR seemed to grant protection, the test was insufficiently powerful. This effect may be explained simply by the small sample size.

Farrer et al. [28] concluded that the ApoE epsilon4 allele represents a major risk factor for AD in all ethnic groups studied, across all ages from 40-90 years, and in both genders.

In the presented study, however, ApoE epsilon 4 alleles did not fulfill the criteria for a major AD risk factor, and population analysis should be continued to recognize more factors contributing to a risk. If these risk factors are simply additive at the population level, family history/ApoE4 would account for over 30% of the attributable risk for AD. For example, Mortimer [29] calculated the attributable risk due to family history as 26%. A similar calculation for ApoE4 suggested that over 30% of cases between the ages of 65-80 may be attributable to the E4 allele.

Verification of these risk factors for Alzheimer’s disease obviously has important sociological implications. This study suggested that the well known genetic factor, mainly ApoE, even if of noticeable importance, may not be a main risk factor in the population of the Lublin region in Poland.

Alzheimer’s disease needs analysis other then the genetic risk factors in its epidemiological characteristic.

REFERENCES


