Association of IL-1 RN*2 allele and methionine synthase 2756 AA genotype with dementia severity of sporadic Alzheimer’s disease

P Bosco, R-M Guéant-Rodríguez, G Anello, A Romano, B Namour, R S Spada, F Caraci, G Tringali, R Ferri, J-L Guéant

Background: Genetic polymorphisms of APO-E, homocysteine, and the IL-1 gene cluster (IL-1A, IL-1B, receptor antagonist IL-1RN) are associated with sporadic Alzheimer’s disease and may involve interdependent pathways of neuronal toxicity.

Objective: To determine whether these polymorphisms and the genetic determinants of homocysteine (methylene tetrahydrofolate reductase, MTHFR; methionine synthase, MTR; transcobalamin, TC) are associated with an increased risk of severe dementia in Alzheimer’s disease.

Methods: 152 patients with Alzheimer’s disease and 136 controls were studied. The association of occurrence and dementia severity (Reisberg score <6 and ≥6) of Alzheimer’s disease with APO-E, IL-1A, IL-1B, IL-1RN, MTHFR 677 C→T and 1298 A→C, MTR 2756 A→G, and TC 776 C→G polymorphisms was evaluated by multivariate logistic regression analysis after adjustment for age, sex, and age of onset of Alzheimer’s disease.

Results: IL-1A TT and IL-1B CT/TT associated genotypes were at risk of Alzheimer’s disease (odds ratio 4.80 (95% confidence interval, 1.32 to 17.40), p = 0.017); the MTR 2756 AA genotype was at risk of severe dementia (OR 2.97 (1.23 to 7.21), p = 0.016); IL-1 RN*2 was protective (OR 0.28, (0.11 to 0.69), p = 0.006). Allele ε4 of the APO-E and IL-1B CC genotypes increased the risk of severe Alzheimer’s disease associated with the MTR 2756 AA genotype by 3.3-fold and 1.5-fold, respectively.

Conclusions: Distinct determinants of the IL-1 gene cluster are related to the generation and progression of Alzheimer’s disease. MTR only influences progression of the disease, which may be enhanced by carriage of allele ε4 of APO-E.

Sporadic Alzheimer’s disease is the major cause of dementia in elderly individuals, the pathogenesis of which is influenced by ε4 allele of apolipoprotein E (APO-E) and possibly by other genetic factors. Both the ε4 allele of apolipoprotein E and homocysteine may modulate the neurotoxicity of β amyloid fragment. The proinflammatory interleukin 1 (IL-1) cytokine has been shown to upregulate the expression and processing of the β amyloid precursor protein, and may therefore contribute to the pathogenic effect of β amyloid fragment. Recently, an association with Alzheimer’s disease of polymorphisms in the chromosome 1 cluster of genes coding for IL-1α (IL-1A), IL-1β (IL-1B), and IL-1 receptor antagonist (IL-1 RN) has been described, and related to the occurrence and age of onset of sporadic Alzheimer’s disease, but this finding has not been confirmed by others.

The association observed between homocysteine and the occurrence of Alzheimer’s disease is not related to vitamin B-12 or folate, suggesting that it may depend on genetic determinants rather than on nutritional factors. The cellular metabolism of homocysteine is affected by genetic polymorphisms of methylene tetrahydrofolate reductase (MTHFR 677 C→T) and methionine synthase (MTR 2756 A→G), but several studies have failed to find any association of MTHFR polymorphism with Alzheimer’s disease. We also showed recently that polymorphism of transcobalamin (TC 776 C→G) is a weak genetic determinant of homocysteine.

Because polymorphisms of APO-E and the IL-1 gene cluster, and genetic determinants of homocysteine may involve interdependent pathways leading to neurotoxicity and progression of Alzheimer’s disease, we evaluated the association of these polymorphisms with the occurrence and dementia severity in cases of sporadic Alzheimer’s disease in a case-control series from southern Italy.

METHODS

Patients

We recruited 152 ambulatory patients with Alzheimer’s disease (mean age 74.8 years, range 47 to 99; male to female sex ratio 0.85) in the specialised centre of Troina, which receives only patients from Sicily for clinical follow up during short periods of one to two weeks. Institutional review board approval was obtained from the ethics committee of the hospital centre, and informed consent from the subjects or their families.

The diagnosis was made on the basis of established criteria. “Sporadic” was defined as absence of any first degree relative with dementia. The severity of the dementia was assessed using the Reisberg scale. Patients were classified into two groups with either mild (n = 101) or severe (n = 47) dementia, with respective Reisberg scores of <6 and ≥6. Four patients could not be scored. Early onset was defined as before 65 years (n = 41) and late onset as 65 years or later (n = 111).

The 136 controls (mean age 69.3 years, range 55 to 99; sex ratio 0.81) were unrelated ambulatory individuals randomly selected on the criterion of a normal neurological and medical examination. They originated from the same geographical area of Sicily and were attending the centre for preventive care.

Genetic analyses

DNA was isolated from a lymphocyte enriched fraction of whole blood using a Nucleon BACC3 kit for extraction of genomic DNA (Amersham Pharmacia Biotech, Milan, Italy).

Abbreviations: MTHFR, methylene tetrahydrofolate reductase; MTR, methionine synthase; TC, transcobalamin

The procedures for detecting APO-E alleles, 677 C→T, and 1298A→C polymorphisms of MTHFR and the MTR 2756 A→G polymorphisms were based on polymerase chain reaction (PCR) amplification, restriction cleavage, and separation of the DNA fragments by electrophoresis, as previously described. TC 776 C→G polymorphism, IL-1A–889C→T and IL-1B–511 C→T biallelic polymorphisms, and the variable number of tandem repeat polymorphism of IL-1 RN (four repeats = allele *1; two repeats = allele *2) were genotyped as described previously.

**Statistical analyses**

Differences of categorical variables were assessed by χ² test. The significance and odds ratio of independent categorical determinants on the severity of dementia were determined by backward stepwise multivariate logistic regression analysis using a model including all variables with a probability (p) value of <0.10, and with adjustment for age, sex, and age onset of the disease. A p value less than 0.05 was considered significant. A weak association was found with IL-1A TT (OR = 2.32 (0.92 to 5.95), p = 0.075), becoming more significant when this genotype was combined with IL-1B CT/TT (OR = 4.80 (1.32 to 17.40), p = 0.017). The MTR 2756 AA genotype was associated with risk for severe dementia (OR = 2.97 (1.23 to 7.21), p = 0.016), while IL-1 RN*2 was protective (OR = 0.28 (0.11 to 0.69), p <0.006). Allele e4 of APO-E and IL-1B CC genotype increased the risk of severe Alzheimer dementia associated with the MTR 2756 AA genotype by 3.3-fold and 1.5-fold, with respective odds ratios of 9.81 (1.20 to 80.05), p = 0.033, and 4.38 (1.12 to 17.05), p = 0.033.

**DISCUSSION**

The association of IL-1A T and IL-1B T alleles with the risk of occurrence of Alzheimer’s disease has been reported in several studies, but not all studies. In the present study, we found a weak association of the IL-1A TT genotype with Alzheimer’s disease, which was strengthened by allele T of controls (14.5% v 6.9%, p = 0.0456). The allele distribution of polymorphisms was considered as a function of a Reisberg scale score of <6 or ≥6. The allele *2 frequency of IL-1 RN was significantly lower in the patients with dementia grade ≥6 (24.2% v 9.6%, p = 0.003). The difference in MTR 2756 G allele frequency between the two groups was at the limit of statistical significance (19.4% v 10.7%, p = 0.069). The MTR 2756 AA genotype distribution did not differ between patients and controls, but was more common in the patients with severe dementia than in those with a Reisberg score of <6 (80.8% v 63.4%, respectively; p = 0.032).

**Table 1** Allele frequencies and percentage of the genetic polymorphisms in Alzheimer’s patients and controls

<table>
<thead>
<tr>
<th>Allele</th>
<th>Controls</th>
<th>Alzheimer’s disease</th>
<th>χ²</th>
<th>p Value</th>
</tr>
</thead>
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<tr>
<td>IL-1A– 889</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele C</td>
<td>178 (70.1)</td>
<td>197 (64.8)</td>
<td>1.750</td>
<td>0.186</td>
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<tr>
<td>Allele T</td>
<td>76 (29.9)</td>
<td>107 (35.2)</td>
<td></td>
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<tr>
<td>IL-1B–511</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele C</td>
<td>164 (63.6)</td>
<td>197 (64.8)</td>
<td>0.093</td>
<td>0.760</td>
</tr>
<tr>
<td>Allele T</td>
<td>94 (36.4)</td>
<td>107 (35.2)</td>
<td></td>
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<tr>
<td>IL-1 RN</td>
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<td></td>
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<tr>
<td>Allele *1</td>
<td>192 (75.5)</td>
<td>235 (77.8)</td>
<td>0.856</td>
<td>0.652</td>
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<tr>
<td>Allele *2</td>
<td>52 (20.4)</td>
<td>59 (19.5)</td>
<td></td>
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</tr>
<tr>
<td>Others†</td>
<td>10 (4.1)</td>
<td>8 (2.7)</td>
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<tr>
<td>APO-E</td>
<td></td>
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<tr>
<td>Allele e2</td>
<td>16 (5.9)</td>
<td>5 (1.7)</td>
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<tr>
<td>Allele e3</td>
<td>240 (88.2)</td>
<td>223 (73.3)</td>
<td>49.871</td>
<td>&lt;0.0001</td>
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<tr>
<td>Allele e4</td>
<td>16 (5.9)</td>
<td>76 (25.0)</td>
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<td></td>
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<tr>
<td>MTHFR 677</td>
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<tr>
<td>Allele C</td>
<td>157 (57.7)</td>
<td>182 (59.8)</td>
<td>2.261</td>
<td>0.133</td>
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<tr>
<td>Allele T</td>
<td>115 (42.3)</td>
<td>122 (40.2)</td>
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<tr>
<td>MTHFR 1298</td>
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<tr>
<td>Allele A</td>
<td>178 (65.9)</td>
<td>205 (67.4)</td>
<td>2.319</td>
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<tr>
<td>Allele C</td>
<td>92 (34.1)</td>
<td>99 (32.6)</td>
<td></td>
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<tr>
<td>MTR 2756</td>
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<tr>
<td>Allele A</td>
<td>216 (81.2)</td>
<td>254 (83.5)</td>
<td>0.541</td>
<td>0.462</td>
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<tr>
<td>Allele G</td>
<td>50 (18.8)</td>
<td>50 (16.5)</td>
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<td>TC 776</td>
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<tr>
<td>Allele C</td>
<td>179 (66.2)</td>
<td>196 (64.4)</td>
<td>0.210</td>
<td>0.647</td>
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<tr>
<td>Allele G</td>
<td>91 (33.8)</td>
<td>108 (35.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are n (%). †Three, five, and six repeat alleles.

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IL-1 B. In contrast, there has been no previous evaluation of an association with progression of the disease, except for a case-control study which showed an accelerated rate of cognitive decline in IL-1A CC carriers.12 We observed that the IL-1RN*2 allele was protective for dementia severity, independent of age. This may be explained by the influence of this polymorphism on the expression level of IL-1Ra, assuming that the corresponding phenotype in glial cells is the same as in peripheralic leucocytes. Indeed, carriers of the IL1-RN*2 allele have a higher blood level of IL-1Ra than non-carriers.21 This is in agreement with a previous report of a decreased IL-1Ra level in cerebrospinal fluid from patients with Alzheimer’s disease compared with controls.22 IL-1RN is also the main determinant of IL-1β bioactivity within the IL-1 gene cluster. IL-1RN*2 allele carriage is associated with lower IL-1β release in culture of peripheral blood mononuclear cells.7,8 Our results are therefore consistent with the previously observed association of IL-1 CC genotype with cognitive decline, as this genotype is also associated to a reduced production of IL-1β.23 Finally, previously published reports and our present results may indicate that the genetic determinants of the initiation of Alzheimer’s disease differ from those that sustain it.

We showed a weak but significant association of MTR 2756 A→G polymorphism with the dementia severity of sporadic Alzheimer’s disease. While carrying out our study, another group reported an association of MTR 2756 AA with Alzheimer’s disease.24 However, dementia was not scored in that study. The effect of this polymorphism on the activity of MTR is not known. The MTR AA genotype has been found to be a risk factor for secondary adverse events in coronary artery disease, while the MTR G allele increases the risk of having a child with Down’s syndrome and neural tube defects.19 20

Allele e4 of APO-E increased the risk of severe Alzheimer’s disease dementia associated with the MTR 2756 AA genotype by 3.3-fold, suggesting a gene–gene interaction. This finding is in accord with the in vitro enhancing effect of homocysteine on the neurone toxicity of amyloid β peptide. Both homocysteine and amyloid β peptide increase cellular calcium influx and oxidative stress, leading to apoptosis.20 We also observed a significant but weaker influence of IL-1B polymorphism on the MTR risk associated with Alzheimer’s disease dementia grade. Inflammation is a cause of oxidative stress and this may influence homocysteine metabolism by modifying the reduction of vitamin B-12, the co-factor of MTR.21

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