SHORT REPORT

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

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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 (methylenetetrahydrofolate 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 $\geqslant 6$) of Alzheimer's disease with *APO-E*, *IL-1A*, *IL-1B*, *IL-1RN*, *MTHFR677 C→T* and *1298A→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 $\epsilon 4$ 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 $\epsilon 4$ 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*)[1] and possibly by other genetic factors.[2 3] Both the ε4 allele of apolipoprotein E and homocysteine may modulate the neurotoxicity of β amyloid fragment.[4] 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,[5–8] but this finding has not been confirmed by others.[9–12]

The association observed between homocysteine and the occurrence of Alzheimer's disease is not related to vitamin B-12 or folate,[2] 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 C→G*), but several studies have failed to find any association of *MTHFR* polymorphism with Alzheimer's disease.[13–15] We also showed recently that polymorphism of *transcobalamin* (*TC 776 C→G*) is a weak genetic determinant of homocysteine.[16]

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.[17] "Sporadic" was defined as absence of any first degree relative with dementia. The severity of the dementia was assessed using the Reisberg scale.[18] Patients were classified into two groups with either mild (n = 101) or severe (n = 47) dementia, with respective Reisberg scores of <6 and $\geqslant 6$. 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, methylenetetrahydrofolate 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.[19 20] *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.[11 16]

Statistical analyses

Differences of categorical variables were assessed by χ^2 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. Data were collected and analysed prospectively using Statview 5 software for Windows (SAS Institute, Berkeley, California, USA) and the SPSS 10.0 software for Windows (SPSS Inc, Chicago, Illinois, USA).

RESULTS

The allele frequencies of the genetic polymorphisms in Alzheimer's disease cases and controls are shown in table 1. All the genotype distributions were in Hardy–Weinberg equilibrium. The allele distribution of *MTHFR*, *MTR*, *TC*, and polymorphisms of the *IL-1* cluster did not differ between the two groups (table1). The allele *ϵ4* of *APO-E* was significantly more common in the individuals with Alzheimer's disease, as recorded before in many studies.[1] The frequency of the IL-1 TT genotype was also significantly higher in the patients than in the 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).

Allele *ϵ4* of *APO-E* was the single genetic determinant that was significantly associated with Alzheimer's disease risk after adjustment for age and sex (odds ratio (OR) = 8.0 (95% confidence interval, 4.06 to 15.95), p<0.0001), as previously observed.[1] 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 *ϵ4* 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[5–9] but not all studies.[9–12] 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

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

	Controls	Alzheimer's disease	χ^2	p Value
IL-1α−889				
Allele *C*	178 (70.1)	197 (64.8)	1.750	0.186
Allele *T*	76 (29.9)	107 (35.2)		
IL-1−511				
Allele *C*	164 (63.6)	197 (64.8)	0.093	0.760
Allele *T*	94 (36.4)	107 (35.2)		
IL-1 RN				
Allele **1*	192 (75.5)	235 (77.8)	0.856	0.652
Allele **2*	52 (20.4)	59 (19.5)		
Others†	10 (4.1)	8 (2.7)		
APO-E				
Allele *ϵ2*	16 (5.9)	5 (1.7)		
Allele *ϵ3*	240 (88.2)	223 (73.3)		
Allele *ϵ4*	16 (5.9)	76 (25.0)	49.871	**<0.0001**
MTHFR 677				
Allele *C*	157 (57.7)	182 (59.8)	2.261	0.133
Allele *T*	115 (42.3)	122 (40.2)		
MTHFR 1298				
Allele *A*	178 (65.9)	205 (67.4)	2.319	0.128
Allele *C*	92 (34.1)	99 (32.6)		
MTR 2756				
Allele *A*	216 (81.2)	254 (83.5)	0.541	0.462
Allele *G*	50 (18.8)	50 (16.5)		
TC 776				
Allele *C*	179 (66.2)	196 (64.4)	0.210	0.647
Allele *G*	91 (33.8)	108 (35.6)		

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

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 *IL1-RN*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 peripheral 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-1 Ra level and an undetectable level of IL-1β in cerebrospinal fluid from patients with Alzheimer's disease compared with controls.[22] *IL-1 RN* 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.[23] 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.[12] 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.[13 19 20]

Allele *ϵ4* 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.[4] Both homocysteine and amyloid β peptide increase cellular calcium influx and oxidative stress, leading to apoptosis.[4] 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.[25]

Authors' affiliations

P Bosco, G Anello, R S Spada, F Caraci, R Ferri, IRCCS Associazione Oasi Maria SS, Institute for Research on Mental Retardation and Brain Aging, Troina, Italy
R-M Guéant-Rodríguez, B Namour, J-L Guéant, Cellular and Molecular Pathology in Nutrition, EMI–INSERM 0014, Vandoeuvre lès Nancy, France
A Romano, Department of Internal Medicine and Geriatrics, UCSC, CI Columbus, Rome, Italy
G Tringali, Istituto Ricerca Medica ed Ambientale, Catania, Italy

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P Bosco, R-M Guéant-Rodríguez, and G Anello contributed equally to this work

Correspondence to: Professor J L Guéant, EMI–INSERM 0014, Faculty of Medicine, BP 184, 54500 Vandoeuvre lès Nancy, France; jean-louis.gueant@medecine.uhp-nancy.fr

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