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SCIENTIFIC CORRESPONDENCE

Association between the BDNF 196 A/G polymorphism and sporadic Alzheimer's disease

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SIR – Alzheimer's disease (AD) is a chronic brain disorder associated with specific pathological changes resulting in neurodegeneration and in progressive development of dementia. This disease is clinically characterized by memory, reasoning and speech disorders and pathologically by the presence of senile plaques (SP), neurofibrillary tangles, and loss of synapses.[1] There are various hypotheses regarding an involvement of genetic factors in the development of AD. Mutations of genes encoding amyloid precursor protein, presenilin-1 and presenilin-2 cause familial AD,[2,3] and the ϵ4 allele of apolipoprotein E (APOE) gene gives susceptibility to familial and sporadic AD.[4] However, this genetic marker cannot explain the overall genetic susceptibility and additional/other genes may be involved in the development of AD.

Genes involved in the neurodevelopmental process may be considered good candidates to confer susceptibility to AD. Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors which promotes survival, differentiation and maintenance of neurons in peripheral and central nervous system during normal development,[5] influences axonal growth and connectivity[6] and participates in the local responses to various types of neuronal stress or insults.[7]

Several lines of evidence have suggested altered functions of this gene in the pathogenesis of neurodegenerative diseases including Alzheimer's disease: reduced levels of BDNF mRNA in the hippocampus, in the temporal cortex, in brain homogenates and in frontal cortex neurons have been found in individuals with AD.[8]

In order to verify a potential role of the BDNF gene in the neuropathogenesis of Alzheimer's disease, we analyzed allelic distribution of a polymorphism in the coding region of the BDNF gene in a diagnosed AD sample and in a control group.

The polymorphism studied is a A/G (Met/Val) substitution located in the propeptide region, a highly unstable region constituted by 110 aa, in the BDNF gene at position 196 (codon 66) (SWISS.PROT: P23560.VAR 004626).

Genomic DNA was extracted from blood samples obtained, after informed consent, from 130 Alzheimer's patients (mean age 72 ± 3 years; 90 women and 40 men) recruited at the Alzheimer Unit of IRCCS-Fatebenefratelli (Brescia, Italy) and 111 healthy ethnically and age-matched volunteers. Patients and controls were Caucasians living in Northern Italy.

Genotyping for this polymorphism has been done by PCR amplification with suitable primers: (forward: 5′-ACT CTG GAG AGC GTG AAT GG 3′, reverse: 5′-TCC AGG GTG ATG CTC AGT AGT-3′) and enzymatic digestion with restriction enzyme PmaCI, followed by polyacrylamide gel electrophoresis with ethidium bromide staining.

The BDNF genotypes and allele frequencies in patients and controls are shown in Table 1. Allele frequencies were calculated by gene counting and differences between groups were evaluated by the χ^2 test. The genotyping distribution in both patients and con-

Table 1 Allele and genotype frequencies of the BDNF gene polymorphism at position 196 in Alzheimer patients and controls

	Alzheimer patients (n = 130)		*Healthy volunteers (n = 111)*	
BDNF allele frequency				
Allele G		203 (78%)		156 (70.3%)
Allele A		57 (22%)		66 (29.7%)
$\chi^2 = 3.340$ (df = 1) $P = 0.063$				
BDNF genotype	**Total**	**ϵ4 + (65)**	**ϵ4 − (65)**	**Total**
GG	85 (65.4%)	43 (66.2%)	42 (64.6%)	54 (48.7%)
GA	33 (25.4%)	14 (21.5%)	19 (29.2%)	48 (43.2%)
AA	12 (9.2%)	8 (12.3%)	4 (6.2%)	9 (8.1%)
$\chi^2 = 8.68$ (df = 2) $P = 0.013$				
If G allele recessive: $\chi^2 = 6.202$, $P = 0.013$ OR = 1.994 (95% CI 1.149–3.466)				

trols was within Hardy–Weinberg equilibrium. Odds ratio (OD) and their 95% confidence intervals were calculated to evaluate effects of different genotypes. We found a difference in the frequency of A/G alleles between the total amount of patients studied and controls which comes close to statistical significance (χ^2 = 3.440; P = 0.063). The genotype distributions differed significantly in patients with AD compared to the controls (χ^2 = 8.68; P = 0.013). The frequency of individuals who carried two copies of the Val allele was significantly increased in patients than in controls (χ^2 = 6.202; P = 0.013, odds ratio: 1.994, 95% CI 1.15–3.46), suggesting that homozygosity for the Val allele confers an increased risk for AD.

In order to examine the possible interaction between AD and APOE, we genotyped the 130 AD patients and we found 65 carrying the ϵ4 allele of APOE gene. There was no significant difference in the distribution of allele frequencies between the two groups (χ^2 = 0.090, df = 1, P = 0.76) (Table 1), and we concluded that possible effects of Val on susceptibility to AD might be independent from the APOE genotype. These data are in agreement with a recent observation of association of another BDNF gene polymorphism (C270T in the 5′UTR) to late onset AD in the Japanese population.[9] Further studies are needed to confirm the relationship of two polymorphisms including a possible linkage disequilibrium.

In conclusion these results make the BDNF an important candidate gene for susceptibility of AD and might provide a baseline to rationalize the use of neurotrophins as possible therapeutic agents in specific human neurodegenerative diseases.

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Age and the relationship of dopamine D3, serotonin 2C and serotonin 2A receptor genes to abnormal involuntary movements in chronic schizophrenia

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SIR – Tardive dyskinesia (TD) is a serious, treatment-refractory movement disorder which develops in patients chronically exposed to antipsychotic drugs that block dopamine D2 receptors. Since, on average, only about one fifth of patients develop the disorder,[1] there is considerable interest in identifying factors that might underlie individual sensitivity. In a recent issue of *Molecular Psychiatry*, we reported association of TD with the serotonin 2A receptor gene (HTR2A).[2] This receptor is implicated in the mechanism of action of atypical antipsychotic drugs that have a lower propensity to induce TD. Our positive findings were for the silent T102C polymorphism in the coding region and the A-1438G polymorphism in the promoter, which were in complete linkage disequilibrium in our sample. Patients with TD had a significant excess of 102C and –1438G alleles and of 102CC and –1438GG genotypes compared to patients without TD and normal control subjects. Patients carrying the 102CC and –1438GG genotypes had significantly higher scores on the sub-scales of the Abnormal Involuntary Movements Scale (AIMS) that measure dyskinetic trunk movements and degree of incapacitation.

In the same issue of *Molecular Psychiatry*, Basile *et al*,[3] to whom we had communicated our unpublished findings, reported no allelic or genotypic association of the same polymorphisms in HTR2A with TD. Their patients carrying 102CC and –1438GG genotypes did not have higher AIMS total scores than patients homozygous for the wild-type allele. AIMS sub-scale scores were not reported. This disparity contrasts with similar findings of the two groups in the same patient samples in regard to association of the dopamine D3 receptor gene (DRD3) with TD.[4,5]

Among the demographic and clinical factors that have been associated with individual susceptibility to TD, age is the most consistently reported. In a prospective study, Jeste *et al*[6] found the cumulative incidence of TD in patients who initiated treatment with antipsychotic drugs after the age of 45, to be 60% after 3 years. In other studies too, aging appears to be the most predominant patient-related risk factor for TD.[7] We hypothesized that there may be an interaction between genes that predispose to TD and age whereby the effect of such genes may be expressed to a greater extent in older patients. Such an effect could explain the disparity between our findings[2] and those of Basile *et al*[3] in regard to the association of HTR2A with TD. The mean