Behavioral and psychological symptoms (that is, noncognitive symptoms) are frequently present in patients with Alzheimer’s disease (AD). These symptoms, which include depression, euphoria, agitation, aggression, psychosis, disinhibition, irritability, apathy, and aberrant motor behavior, are a source of distress for caregivers, one of the main reasons for nursing home placement, and one of the main components of the cost of AD. Although noncognitive behavioral changes are ubiquitous in AD, the manifestation of these disturbances is highly heterogeneous across AD patients. Distinct genetic determinants have been hypothesized to be related to several noncognitive symptoms. Association between APOE allele e4, the most important genetic risk factor for AD, and behavior abnormalities has been documented; however, other studies have not found any relation. Besides APOE, other genes such as dopamine (DRD1 and DRD3) and serotonin receptors...
(5-HT2A and 5-HT2C) have been related to neuropsychiatric abnormalities in AD patients. Recently, a study using a large cohort of AD patients and their siblings has showed a familial aggregation of psychotic symptoms in AD. These data support the hypothesis that genetic factors may play a role in the manifestation of noncognitive symptoms in AD; therefore, the behavioral heterogeneity found in AD patients could be related to an individual genetic background.

One crucial event in the pathological process of AD is oxidative stress. Evidences of oxidative damage in AD brains include lipid peroxidation, protein oxidation, and presence of advanced glycation end products, reactive oxygen species, and reactive nitrogen species. One of the cellular physiological strategies to counteract oxidative stress is the production of heat-shock proteins (HSP). These proteins act as molecular chaperones and participate in many biological processes in which protein folding is involved. A large amount of evidence relates HSP to AD pathophysiology: they have been found in senile plaques, they have been related to the protection of tau protein against hyperphosphorylation during heat shock, and mRNA expression of HSP is increased in AD patients, and higher levels of these proteins have been also identified in brains of AD patients compared to healthy subjects.

Within the HSP, the most predominant and highly conserved group is the HSP70 family, whose members have been found to be involved in all the above mentioned processes related to AD pathophysiology.

Association between HSP70 and psychiatric disturbances has been described in schizophrenia. Olney and Farber observed a strong overexpression in corticolumbic neurons after NMDA receptor antagonist administration, which is engaged in the production of schizophrenia symptoms. Other studies have described significant increased levels of anti-HSP70 antibodies in schizophrenic patients. This increase was proportional to the amount of psychopathological symptoms. These results draw attention to the potential relationship between HSPs and noncognitive disturbances.

To establish a possible relationship between oxidative stress and noncognitive alterations presented by AD patients, we performed a cross-sectional study using a genetic variant within the HSP70-2 gene (which is the alias of the approved gene symbol HSPA1B), a member of the HSP70 family, and the information about behavioral alterations assessed with the Neuropsychiatric Inventory (NPI) instrument.

**SUBJECTS AND METHOD**

**Sample**

The subjects consisted of 77 incidental patients (diagnosed from 1999 to 2001) from Catalonia (Spain) recruited and assessed by neurologists and neuropsychologists at the Fundació ACE, Institut Català de Neurociències Aplicades. All individuals met National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria for possible or probable AD. None of the patients had a prior history of psychiatric illness. A large majority of patients were living in the community and had been referred by their general practitioner. After a complete description of all procedures of this study, written informed consent was obtained from all participants, or a legal guardian when patients were not competent to consent.

**Assessment of Noncognitive and Cognitive Status**

To obtain behavioral information from all AD patients, caregivers were interviewed (as a part of the diagnostic assessment) using the NPI. This instrument, which has a high interrater and test-retest reliability, is a powerful tool to assess 10 behavioral disturbances occurring in dementia. These symptoms include delusions, hallucinations, agitation, aggression, depression, anxiety, euphoria, apathy, disinhibition, irritability, and aberrant motor activity. Multiplication of the frequency (rating from 1 to 4) and severity (rating from 1 to 3) for each symptom and a summation of subscale scores produced the total NPI score.

Besides the noncognitive NPI test, the Mini-Mental State Examination (MMSE) was administered to all patients on the same day as the NPI interviews to assess their cognitive status.

**Genotyping**

For all patients, DNA was extracted from fresh blood using standard phenol-chloroform protocols. A 2-allele pentanucleotid tandem duplication polymorphism in the 3' untranslated region of the HSP70-2 gene (GenBank accession number M34269) was determined as described previously. The genotypes were determined by size of DNA fragments: the larger allele (named A1) has a length of 188 base pairs and contains a 5 nucleotide-tandem duplication that is not present in the shorter 183 bp allele (named A2).

**Data Analysis**

To detect any relationship between noncognitive symptoms and the duplication polymorphism at the HSP70-2 gene, NPI mean values were compared according to HSP70-2 genotype with a one-way analysis of the variance (ANOVA). Independently, to clarify if the relation between HSP70-2 and noncognitive alterations was present in all the stages of cognitive decline, the comparison was also performed in each of the 3 groups that resulted from sample stratification. Therefore, patients were divided into 3 groups according to MMSE scores: those with MMSE scores from 0 to 10 were grouped in the severely impaired group, those with MMSE scores from 11 to 20 were grouped in the moderately impaired group, and finally, patients with scores greater than 20 were grouped in the mildly impaired group.
Moreover, we compared the mean NPI score of each behavioral symptom (ie, hallucinations, depression, irritability) between HSP70-2 genotypes.

Student’s t test was used to analyze MMSE and age means with respect to gender and chi-square test to verify Hardy-Weinberg equilibrium.

RESULTS

The sample analyzed in the present study consisted of 77 patients with a mean age of 77 years (range 67-90 years) and a mean MMSE score of 16.4 (range 0-26). Fourteen were males with a mean age of 74.3 years (SD = 5) and a mean MMSE score of 16.8 (SD = 7.3); and 63 were females with a mean age of 78 years (SD = 5.2) and a mean MMSE score of 16.4 (SD = 5.4). No significant differences were found between MMSE mean scores by gender (Student’s t test, t = 0.24, df = 75, P = .81). We classified the patients in 3 groups according to their cognitive deterioration. The mildly (n = 21), moderately (n = 45), and severely impaired (n = 11) groups had mean MMSE scores of 22.9 (SD = 1.42), 16 (SD = 5.59), and 6 (SD = 3.29), respectively.

Descriptive statistics about the patients’ gender, age of onset of cognitive symptoms, MMSE score, and NPI score classified by HSP70-2 genotypes are shown in Table 1. HSP70-2 genotype distribution was in Hardy-Weinberg equilibrium (chi-square = .05, df = 1, P = .8).

Distributions of mean MMSE scores, mean age of onset, and gender did not present any statistical difference between the 3 possible genotypes. Nevertheless, significant differences in noncognitive symptoms, as measured by the mean total NPI scores, were present between HSP70-2 genotypes (F = 4.65, df = 2, P = .012). A nonparametric test also showed significant differences (Kruskal-Wallis test, P = .017). Homozygous patients for the A1 allele presented the lowest NPI score (mean NPI = 7.11), heterozygotes presented an intermediate NPI score (mean NPI = 10.19), and homozygotes for the A2 allele presented the highest NPI total score (mean NPI = 16.22). A linearity test showed a significant (P = .004) linear association between the number of A2 alleles carried and the NPI score. Moreover, when we focused on HSP70-2 alleles, we found a significantly higher mean NPI score for the A2 allele compared to the lower NPI value observed for the A1 allele (14.05 and 9.17, respectively; F = 8.39, df = 1, P = .004).

To clarify the relationship between noncognitive alterations and cognitive decline, we performed an analysis of the total NPI scores mean values for each MMSE group (mild, moderate, and severe). Significant differences were found in the NPI values comparing the 3 groups of patients according to their cognitive status (F = 3.73, df = 2, P = .029). These differences are predominantly due to high NPI scores presented by the severe patients. When a stratification according to the HSP70-2 genotype was performed in each of the 3 groups of cognitive deterioration, NPI score correlated positively with the number of A2 alleles carried by the patient (allele-dosage effect; see Figure 1).

Finally, the averages of NPI scores were compared for each noncognitive symptom (product of severity and frequency of each symptom) according to HSP70-2 genotypes (Figure 2). In all comparisons, patients homozygous for the A2 allele presented the highest scores, whereas patients homozygous for the A1 allele presented the lowest NPI scores in each of the 3 MMSE groups. This increase was statistically significant for those patients with mild dementia (F = 3.85, df = 2, P < .05). Although the trend was also very clear in the cases with severe dementia, it did not reach statistical significance due to the low sample size that resulted from the stratification.

Table 1. Demographic and Clinical Characteristics of Alzheimer’s Disease Patients Sorted by HSP70-2 Genotype Status

<table>
<thead>
<tr>
<th>HSP70-2 Genotype</th>
<th>Total Sample Frequency (%)</th>
<th>Gender</th>
<th>Mean Age of Onset (SD)</th>
<th>Mean MMSE (SD)</th>
<th>Mean Total NPI (SD)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1/A1</td>
<td>9 (12)</td>
<td>Men (%)</td>
<td>72.4 (5.2)</td>
<td>17.6 (5)</td>
<td>7.11 (6.4)</td>
</tr>
<tr>
<td>A1/A2</td>
<td>36 (47)</td>
<td>Women (%)</td>
<td>74.6 (5.8)</td>
<td>15.7 (6)</td>
<td>10.19 (8)</td>
</tr>
<tr>
<td>A2/A2</td>
<td>32 (42)</td>
<td></td>
<td>73.8 (5.1)</td>
<td>17.1 (5.7)</td>
<td>16.22 (12.1)</td>
</tr>
</tbody>
</table>

MMSE, Mini-Mental State Examination; NPI, Neuropsychiatric Inventory.

a One-way ANOVA test, P < .05

Figure 1. Mean Neuropsychiatric Inventory (NPI) scores across the 3 Mini-Mental State Examination groups of patients according to their HSP70-2 genotype.

Differences in the mild dementia subgroup were statistically significant (P < .05).

Figure 2. Distribution of mean NPI scores for each noncognitive symptom according to HSP70-2 genotypes.
In summary, the HSP70-2 A2 allele appears to confer an increased liability to noncognitive symptoms in the dementia of Alzheimer’s type. Since oxidative stress is extensive in AD pathology and an increased synthesis of HSP has been found in heat-shocked cells, the association between HSP70-2 variation with behavioral alterations in AD patients is feasible under such pathophysiological conditions.

As far as we know, these results represent the most clear relationship between a large group of noncognitive alterations presented in AD patients and a genetic polymorphism. Due to the small sample size and the cross-sectional nature of the study, further analyses performed with larger samples and employing longitudinal approaches may provide conclusive evidence of the present results.

References


32. Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry* 1995; 52:998-1007.


