Association of the Serotonin Transporter and Receptor Gene Polymorphisms in Neuropsychiatric Symptoms in Alzheimer Disease

Frédéric Assal, MD; Maricela Alarcón, PhD; Esther C. Solomon, BS; Donna Masterman, MD; Daniel H. Geschwind, MD, PhD; Jeffrey L. Cummings, MD

Background: Serotonin has been linked to neuropsychiatric symptoms in Alzheimer disease, mainly agitation/aggression, depression, and psychosis. Neuropsychiatric symptoms have been associated with polymorphisms of the promoter region (5-HTTPR) and intron 2 of the serotonin transporter gene (5-HTTVNTR) or the 5-HT2A and 5-HT2C receptor genes in some but not all studies.

Objective: To examine the association of the serotonin promoter, transporter, and receptor genes with neuropsychiatric symptoms in patients with Alzheimer disease.

Methods: The sample included 96 patients with Alzheimer disease from the outpatient clinic of the University of California Los Angeles Alzheimer's Disease Research Center, Los Angeles. The Neuropsychiatric Inventory was used to measure neuropsychiatric symptoms, and blood samples were available for genetic analysis. Based on the literature, we hypothesized that the 5-HT2A and 5-HT2C receptor polymorphisms would be associated with agitation/aggression and psychosis, and the 5-HTTPR or 5-HTTVNTR polymorphisms, with agitation/aggression or depression and anxiety. One-way analyses of variance were performed with age, ethnicity, sex, or education as covariates.

Results: The 102T genotype of the 5-HT2A receptor was significantly associated with delusions ($P = .045$) and agitation/aggression ($P = .002$). We did not replicate previous associations of the 5-HT2C receptor polymorphism with psychosis or of the 5-HTTPR polymorphism with agitation/aggression, psychosis, or depression. We did not find any associations with the 5-HTTVNTR polymorphism and agitation/aggression, depression, or anxiety.

Conclusions: The 5-HT2A receptor polymorphism may contribute to the expression of psychosis and agitation/aggression in patients with Alzheimer disease. Absence of other positive associations may be due to the relatively small sample size and/or potentially small effect size of the polymorphisms and requires further study.

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SEROTONIN HAS BEEN ASSOCIATED with various neuropsychiatric symptoms in Alzheimer disease (AD), such as agitation/aggression, depression, or psychosis.¹ These noncognitive symptoms, and in particular agitation, constitute a major source of distress for the patient’s caregiver and increase the likelihood of nursing home placement.²³ Recent genetic studies in AD showed associations between several polymorphisms of the serotonin neurotransmitter genes and some neuropsychiatric symptoms. A 102T/C polymorphism in the 5-HT2A receptor (5-HT2AR) gene was associated with visual and auditory hallucinations⁴ or psychosis⁵ in patients with AD. The Cys23Ser polymorphism in the 5-HT2C receptor (5-HT2CR) gene was significantly associated with visual hallucinations and hyperphagia in another sample of patients with AD.⁶ A 44–base pair (bp) insertion (the long form or l form) of the 5-HTT promoter region (5-HTTPR) was reported to be associated with psychosis and aggression.⁷ A variable number tandem repeat (VNTR) polymorphism in intron 2 of the 5-HTT gene was also associated with susceptibility to unipolar or bipolar depression.⁸ but this association was not found in patients with AD with depression.⁹ The purpose of this brief study was to examine whether variations in serotonin genes were related to neuropsychiatric symptoms in a sample of patients with AD. Based on the literature, we addressed the following questions: (1) Are 5-HT2AR and
5-HT2CR polymorphisms associated with psychosis (delusions, hallucinations)? (2) Are these polymorphisms related to agitation/aggression? and (3) Are 5-HTTPR and 5-HTTVNTR polymorphisms associated with anxiety, depression, or agitation/aggression in patients with AD?

METHODS

PATIENTS

The sample included 96 patients with AD from the Alzheimer’s Disease Research Center of the University of California in Los Angeles evaluated between 1996 and 2000. Since our aim was to test the association of abnormal behaviors with candidate genes in patients with AD, we did not include healthy controls in the study. Inclusion criteria were (1) diagnosis of probable or possible AD according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Associations; (2) evaluation of the neuropsychiatric symptoms with the Neuropsychiatric Inventory (NPI)11; and (3) presence of a blood sample stored in the genetic database. Patients with a major psychiatric disorder or a history of alcohol or drug abuse were excluded. We used the total NPI subscale scores as measures of each symptom including delusions, hallucinations, agitation/aggression, depression, and anxiety. Demographic data also were collected, and subjects were assessed for the severity of cognitive impairment using the Mini-Mental State Examination (MMSE).12 All clinical and genetic data were obtained with protocols approved by the University of California Los Angeles institutional review board.

GENOTYPING

DNA was extracted from peripheral blood using the EasyDNA kit (Invitrogen, Carlsbad, Calif), and coded samples were stored in a –70°C freezer. An MJ Research PTC-200 Periher thermocycler (Watertor, Mass) was used for all polymerase chain reaction (PCR) amplifications. All PCR reactions included 30 to 80 ng of genomic DNA, 1 U Taq polymerase (Qiagen, Valencia, Calif), 0.3X corresponding Qiagen PCR buffer, 3.75 µg of sense and antisense primers, and 2.5mM dinucleoside triphosphate (dNTP) in a final volume of 50 µL. All PCR products were electrophoresed in a 3% MetaPhor agarose gel (Cambrex, Rockland, Me) in Tris-borate/ethylenediaminetetraacetic acid buffer (TBE), stained with ethidium bromide, and viewed by UV light. Four variants were genotyped: 5-HTTPR in the promoter region, 5-HTTVNTR in intron 2 of the transporter gene, and 5-HT2AR and 5-HT2CR in the receptor gene.

5-HTTPR POLYMORPHISM

The 5-HTTPR polymorphism had a long allele that was 529 bp and a short allele with a 44-bp deletion.13 The following primer pairs were designed for PCR amplification: 5’-GGGTTTGCCGCCTCTGAATGC-3’ and 5’-AGGGGACTGACTGGAACACCA-3’. The PCR reaction conditions were 94°C for 5 minutes, 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 60 seconds followed by a final extension at 72°C for 10 minutes.

5-HTTVNTR POLYMORPHISM

The 5-HTTVNTR had 3 alleles that were 250 bp, 267 bp, and 300 bp that corresponded to 9, 10, and 12 repeats, respectively. The following primer pairs were modified from Li et al14: 5’-GTCAAGTACACAGCTGGCGA-3’ and 5’-TGTTTCCTAGCTTACGCGAGT-3’. The cycling conditions were 94°C for 5 minutes, 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 60 seconds followed by a final extension at 72°C for 10 minutes.

5-HT2AR POLYMORPHISM

The 5-HT2AR polymorphism, 102T/C, allowed theMspI restriction enzyme to cleave a 342-bp product and produced 2 short fragments (126 and 216 bp in length). The primer pairs were adapted from Warren et al13: 5’-TCTGCTCAAGTTCGTGGCTI-3’ and 5’-CTCGACCTTTTCTCTCAGGGG-3’. The initial cycles had the following conditions: 94°C for 3 minutes, 55°C for 45 seconds, and 72°C for 1.5 minutes. These were followed by 35 cycles of 94°C for 1 minute, 55°C for 45 seconds, 72°C for 1.5 minutes, and a final 10-minute extension step at 72°C. The PCR product was digested with MspI.

5-HT2CR POLYMORPHISM

The 5-HT2CR Cys23Ser polymorphism, C/G, was detectable by the introduction of a restriction site upstream of the change. If the polymorphism was unchanged (ie, C), then the PCR product was digested producing 2 small fragments of 18 bp and 86 bp. If the polymorphism was changed, then the PCR product remained uncut and was 104 bp long. The following primer pairs were modified from Sodhi et al15: 5’-TGGCCCTATTGGTTTGGGAATGTG-3’ and 5’-GTCTGGGAAATTGGAAGC-GTCCAC-3’. The first primer introduced a C-to-G substitution 4 bp upstream from the polymorphism. The cycling conditions were 95°C for 5 minutes, 35 cycles of 93°C for 1 minute, 55°C for 2 minutes, and 72°C for 3 minutes. The PCR product was digested with HinfI.

STATISTICAL ANALYSIS

Data were analyzed using the SPSS computer software program.16 To test our hypotheses, we used 1-way analysis of variance (ANOVA) for each NPI subscale score. Prior to the ANOVAs, independent t tests were performed to compare the patient groups (neuropsychiatric symptoms present vs neuropsychiatric symptoms absent) by age, sex, education, and severity of disease (using the MMSE). Then, variables with statistically significant differences (age, sex, ethnicity, or education) were used as covariates in the 1-way ANOVA. All tests were 2-tailed, and we chose a significance level of P= .05.

RESULTS

The total sample consisted of 96 subjects with AD, of whom 59 (61.5%) were women and 37 (38.5%) were men. The average age was 76.7± 7.4 years, and the average educational level was 13.8 ± 3.3 years. The ethnic composition was 70 white non-Hispanic subjects (72.9%), 17 African American subjects (17.7%), 5 Asian/Pacific Islander subjects (5.2%), 1 Hispanic/Latino subject (1.0%), and 2 subjects of “other” race (2.1%); there was 1 subject missing data (1%). The allele frequencies for the sample are shown in Table 1. They did not vary as a function of ethnicity.

The number of patients with the neuropsychiatric symptoms that we tested were: 24 (25%) with delusions, 12 (12.6%) with hallucinations, 39 (40.6%) with agitation, 49 (51%) with depression, and 38 (39.6%) with...
Table 1. Genotypic Distribution and Allele Frequencies of the Serotonin Transporter and Receptor Gene Polymorphisms

<table>
<thead>
<tr>
<th>Gene Polymorphisms</th>
<th>n (%) of patients</th>
<th>Genotype, No. (%) of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT2 Promoter Region (n = 85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype, No. (%) of patients</td>
<td>Long/long</td>
<td>Long/short</td>
</tr>
<tr>
<td>f(long)</td>
<td>28 (33)</td>
<td>25 (29)</td>
</tr>
<tr>
<td>f(short)</td>
<td>0.48</td>
<td>0.52</td>
</tr>
<tr>
<td>5-HT2A Receptor Gene (n = 93)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype, No. (%) of patients</td>
<td>C/C</td>
<td>T/C</td>
</tr>
<tr>
<td>f(T)</td>
<td>40 (44.1)</td>
<td>30 (32.3)</td>
</tr>
<tr>
<td>5-HT2C Receptor Gene (n = 88)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype, No. (%) of patients</td>
<td>C/C</td>
<td>C/S</td>
</tr>
<tr>
<td>f(S)</td>
<td>60 (68.2)</td>
<td>18 (20.5)</td>
</tr>
<tr>
<td>f(C)</td>
<td>0.78</td>
<td>0.22</td>
</tr>
<tr>
<td>5-HTT Variable Number Tandem Repeat (n = 71)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype, No. (%) of patients</td>
<td>9/12</td>
<td>10/10</td>
</tr>
<tr>
<td>f(9)</td>
<td>2 (2.8)</td>
<td>15 (21.1)</td>
</tr>
<tr>
<td>f(10)</td>
<td>0.01</td>
<td>0.40</td>
</tr>
</tbody>
</table>

*Patients homozygous for the long genotype vs patients with either 1 or 2 copies of the short genotype. 

The average MMSE score was 19.1 ± 6.9, indicating that our sample was in the moderate stage of dementia severity. Differences of MMSE scores as a function of the presence (or absence) of neuropsychiatric symptoms were not significant using independent t tests (results not shown). Age, sex, or ethnicity were used as covariates in the 1-way ANOVA as appropriate based on the preliminary results. Initial power calculations for a 1-way ANOVA assuming equal sample sizes for the 3 genotype groups (N=25), an α=.05, and a fixed-effects model suggested that we had between 61% and 88% power to detect a medium effect size and at least 98% power to detect a large effect size. These power estimates varied according to the assumed within-group standard deviations and sample sizes.

Significant associations between neuropsychiatric symptoms and genotypes are shown in Table 2. In our sample, there were significant associations between the 5-HT2AR polymorphism and NPI subscale score for agitation/aggression (F3 = 6.34; P = .002) and for delusions (F3 = 2.55; P = .045). Bonferroni correction of the initial P = .05 for the 12 tests that we performed suggested that a computed P < .004 was significant. The association between the 5-HT2AR polymorphism and agitation/aggression remained significant, although the corresponding association with delusions was not. However, we consider the Bonferroni method too conservative in our case because of its assumption of the independence of the variables. Moreover, post hoc power for the association test of the polymorphism with agitation/aggression was 90%, as opposed to the corresponding value of 60% for the test of delusions. Thus, the lack of power to test the association of the 5-HT2AR polymorphism with delusions in this sample suggests that our result is not conclusive and further investigation is warranted in a larger AD sample. Patients with 1 or 2 copies of the 102T polymorphism had higher NPI subscale scores than those homozygous for 102C.

Associations were not significant for other hypotheses tested using 1-way ANOVA: hallucinations and the 5-HT2AR polymorphisms; delusions and hallucinations and the 5-HT2C polymorphisms; affective symptoms (depression, anxiety) or agitation/aggression and the 5-HTTPR polymorphisms. Nonsignificant associations are presented in Table 3. Post hoc analyses showed that we had less than 80% power to test these associations in our study. Since preliminary power calculations showed we had enough power to detect an association with a moderate to high effect size, the modest observed power estimates suggest that the effect sizes were small and a larger sample is necessary to address our hypotheses.

**COMMENT**

We found positive associations with the 5-HT2AR 102T/C polymorphism and patients with AD with agitation/aggression and delusions. Although the Bonferroni correction for multiple comparisons is not appropriate in our case because of nonindependence of the neuropsychiatric symptoms, the association with agitation/aggression remained significant after the excessively stringent correction. Interestingly, the patients with AD who were homozygous for the 102T genotype had higher agitation/aggression or delusions scores than those who were heterozygous. The latter had higher agitation scores than those who were homozygous for the 102C form. A type...
I error is possible with such a sample, but indirect evidence points to a possible involvement of 5-HT2AR and agitation even if it is still not known if the 5-HT2AR 102T/C polymorphism is functional or silent. Postmortem studies of patients with AD showed that lower serotonin levels were correlated with aggression in the orbitofrontal cortex. Randomized, placebo-controlled clinical trials demonstrated that risperidone and olanzapine, which are atypical antipsychotic agents acting on the 5-HT2AR receptor, decreased agitation and psychosis in patients with AD. Indirectly, in patients with schizophrenia, a decreased agitation and psychosis in patients with the 5-HT2AR polymorphism may predispose to the clinical expression of agitation in AD. The 5-HT2AR polymorphism was significantly associated with delusions but not with hallucinations. That may be because hallucinations were not frequently observed in our sample. Moreover, delusions are more common than hallucinations in AD, and the latter are usually more common in more advanced stages of the disease. We did not find associations of the 5-HT2CR polymorphism with hallucinations or with delusions and, thus, did not confirm previous findings.

For the 5-HTT promoter region (II, ls, ss) genotypes, the polymorphism was not significantly associated with delusions or hallucinations. However, results of our post hoc calculations suggest that we had less than 80% power to detect these associations given the unequal sample sizes of our groups, our large neuropsychiatric symptoms standard deviations, and, possibly, the small effect sizes of the polymorphisms. Thus, a larger sample is needed to examine these associations more definitively. The NPI has the advantage to screen for a 10 different behaviors and may be particularly accurate in revealing neuropsychiatric symptoms in a given patient. Finally, although our patients did not have significant differences in their cognitive impairment and we did not need to covary for the MMSE, our sample was a cross-sectional assessment and did not follow behaviors across time. Since neuropsychiatric symptoms are expressed preferentially for a given patient at a critical window during the evolution of the disease, such a genetic predisposition could be missed because the neuropsychiatric symptoms would not be expressed during the time of assessment. Future association studies of neuropsychiatric symptoms should measure these repeatedly across the course of the disease. Combining behavioral neurogenetics and pharmacogenomics with pharmacoimaging may allow greater specificity in understanding the pathophysiological features of neuropsychiatric symptoms in AD and optimizing treatment.

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Correspondence: Jeffrey L. Cummings, MD, Reed Neurological Research Center 2-238, UCLA Alzheimer’s Disease Center, 710 Westwood Plaza, Los Angeles, CA 90095-1769 (cummings@ucla.edu).

Author Contributions: Study concept and design: Assal, Alarcón, Geschwind, and Cummings. Acquisition of data: Assal, Solomon, and Masterman. Analysis and interpretation of data: Assal and Alarcón. Drafting of the manuscript: Assal. Critical revision of the manuscript for important intellectual content: Alarcón, Solomon, Masterman, Geschwind, and Cummings. Statistical expertise: Assal and Alarcón. Obtained funding: Cummings. Administrative, technical, and material support: Geschwind and Cummings. Study supervision: Geschwind and Cummings.

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