The 5-HTTPR*S/*L Polymorphism and Aggressive Behavior in Alzheimer Disease

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Background: Aggressive behavior in Alzheimer disease (AD) has been linked to dysfunction of serotonin neurotransmission. Homozygosity for the long variant (*L) of an identified biallelic polymorphism of the serotonin transporter promoter region (5-HTTPR) is associated with increased expression of the transporter protein and increased speed of response to serotonin reuptake inhibitor treatment.

Objective: To determine whether the *L/*L genotype and the *L allele are associated with an increased risk of aggressive symptoms in patients with AD.

Design: Case-control study.

Setting: University hospital geriatric psychiatry inpatient program and Alzheimer disease research center.

Results: The *L/*L genotype was significantly associated with aggression in patients with AD (odds ratio, 2.8; 95% confidence interval, 1.2-6.5). Similar results were obtained for *L allele frequency.

Conclusion: The 5-HTTPR*L allele and *L/*L genotype may predispose patients with AD to develop aggressive behavior.

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Alzheimer disease (AD) is frequently complicated by psychiatric symptoms including verbal and physical aggression. Altered function of the serotonin (5-hydroxytryptamine or 5-HT) neurotransmitter system has been implicated in impulsive aggressive behavior and in aggressive behavior in patients diagnosed as having AD. Post-mortem and biopsy studies of brains from patients with AD show a decrease in levels of 5-HT, 5-HT receptors, and the 5-HT transporter (5-HTT). The concentration of 5-hydroxyindoleacetic acid, a metabolite of 5-HT, has been shown to be decreased in the cerebrospinal fluid and cerebral cortex of patients with AD. A few studies have demonstrated a specific association between aggressive behavior in AD and greatly diminished cortical levels of 5-HT. Therapeutic agents that act on the 5-HT system have been shown to be helpful in the treatment of aggression in patients diagnosed as having AD. Consistent with the findings of excess 5-HT loss in aggressive subjects with AD, fentanyl challenge studies have found a hypersensitive postsynaptic response in agitated subjects with AD compared with nonagitated control subjects with AD.

In 1996, a 44–base pair (bp) insertion/deletion polymorphism (5HTTPR) was discovered in the 5′ promoter region of the 5-HTT gene (HTT, SLC6A4). The 5HTTPR alleles are defined by differing numbers of a 44-bp GC-rich repetitive sequence. The basal transcriptional activity of the long variant (*L) is about 2.5- to 3-fold higher than that of the short variant (*S). This differential rate of transcription results in a reduction of 5-HT reuptake sites of approximately 40% in *S/*S homozygotes and a reduction of approximately 30% for heterozygotes (*S/*L), leading some to suggest that the *S allele is functionally dominant.

We hypothesized that the *L/*L genotype would lead to a depletion of extraneuronal 5-HT and might contribute to the risk of aggression in AD. We examined this hypothesis in 137 subjects diagnosed as having possible or probable AD, 58 of whom demonstrated aggressive behavior.
PATIENTS AND METHODS

PATIENTS

All patients were examined at the Geriatric Psychiatry Inpatient Program and the Alzheimer Disease Research Center of the University of Pittsburgh Medical Center, Pittsburgh, Pa, between December 5, 1991, and October 13, 1999. Subjects underwent an extensive diagnostic and behavioral assessment, which has been described in detail elsewhere, and received a diagnosis of possible or probable AD by means of the criteria of the National Institute of Neurologic and Communicative Disorders and Stroke–Alzheimer Disease and Related Disorders Association.

Aggressive subjects, as defined by the presence of physical or verbal aggression rated with the Empirical Behavioral Pathology in Alzheimer Disease scale, were identified among participants in a clinical trial for treatment of behavioral disturbances in dementia conducted at the Geriatric Psychiatry Inpatient Program. Because of evidence that frequency of aggression in patients with AD may increase with severity of dementia, a group of never-aggressive patients with AD, with similar dementia severity as rated on the Mini-Mental State Examination (MMSE), was also identified from subjects participating in the Alzheimer Disease Research Center. Never aggressive was defined as the absence, on initial and annual follow-up examinations, of verbal and physical aggression as defined by the Behavior Rating Scale for Dementia of the Consortium to Establish a Registry for Alzheimer Disease. All data collected in this study were obtained with protocols approved by the Institutional Review Board of the University of Pittsburgh.

GENOTYPING

Lymphocytes were harvested from whole blood and DNA was extracted from lymphocytes by means of a DNA blood kit (QIAamp; Qiagen Inc, Valencia, Calif). The *S and *L alleles were determined by using DNA amplification (polymerase chain reaction) and establishing flanking primers. Amplification products were resolved by electrophoresis and visualized with ethidium bromide staining and UV transillumination, according to the method of Edenberg and Reynolds. Samples from all 137 patients were analyzed for the 5-HTTPLS*/*L polymorphism.

STATISTICAL ANALYSIS

Pearson $\chi^2$ test (exact method) was used to compare the groups with respect to race, sex, and allele and genotype frequencies (StatXact4, version 4.0.1; Cytel Software Corp, Cambridge, Mass). Intergroup differences in age and MMSE score were assessed by $t$ tests (SAS, version 8.0; SAS Institute Inc, Cary, NC). Equality of variance was assessed before the $t$ tests were performed. All tests were 2-tailed, and a significance level of .05 was used.

Table 1. Demographic and Clinical Data for Aggressive and Never-Aggressive Patients With Alzheimer Disease

<table>
<thead>
<tr>
<th></th>
<th>Aggressive (n = 58)</th>
<th>Never Aggressive (n = 79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD, y</td>
<td>79.0 ± 8.0</td>
<td>73.1 ± 8.0</td>
</tr>
<tr>
<td>Sex, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>26 (44.8)</td>
<td>29 (36.7)</td>
</tr>
<tr>
<td>F</td>
<td>32 (55.2)</td>
<td>50 (63.3)</td>
</tr>
<tr>
<td>Race, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>52 (88.7)</td>
<td>70 (88.6)</td>
</tr>
<tr>
<td>Black</td>
<td>6 (10.3)</td>
<td>9 (11.4)</td>
</tr>
<tr>
<td>MMSE† score, mean ± SD</td>
<td>7.4 ± 7.5</td>
<td>8.1 ± 4.2</td>
</tr>
</tbody>
</table>

*P < .001.
†MMSE indicates Mini-Mental State Examination.
‡n = 57 for this variable.

Table 2. Genotype and Allele Frequency Data for Aggressive and Never-Aggressive Patients With Alzheimer Disease

<table>
<thead>
<tr>
<th>Genotype*</th>
<th>Aggressive No. (%)</th>
<th>Never Aggressive No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*L/*L</td>
<td>23 (39.7)</td>
<td>15 (18.1)</td>
</tr>
<tr>
<td>*S/*L</td>
<td>34 (58.6)</td>
<td>38 (48.1)</td>
</tr>
<tr>
<td>*S/*S</td>
<td>1 (1.7)</td>
<td>26 (32.9)</td>
</tr>
<tr>
<td>Allele†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*L</td>
<td>80 (69.0)</td>
<td>68 (43.0)</td>
</tr>
<tr>
<td>*S</td>
<td>36 (31.0)</td>
<td>90 (57.0)</td>
</tr>
</tbody>
</table>

*P < .01 for *L/*L vs all others.
†P < .001.

RESULTS

Demographic and clinical characteristics of patients with AD exhibiting aggressive behavior (n=58) and those with no history of aggressive behavior (n=79) are shown in Table 1. There were no significant differences with regard to sex and race. The MMSE scores also did not differ between groups, confirming adequate matching on this variable. Mean age was significantly higher, however, in the aggressive subjects ($t=4.3, P < .001$).

Genotype and allele frequencies of the 5-HTTPLS polymorphism among aggressive and never-aggressive patients with AD are shown in Table 2. The increased frequency of *L alleles in the aggressive patients was highly significant ($\chi^2 = 18.0, P < .001$). There was a corresponding significant difference in genotype distribution between the aggressive and never-aggressive patients ($\chi^2 = 7.1, P < .01$). Aggressive patients with AD had a significantly higher frequency of *L/*L genotypes than the never-aggressive patients with AD. The odds ratio (95% confidence interval) for aggression associated with the *L/*L genotype was 2.8 (1.2-6.3). When genotype, age, sex, and MMSE score were entered into a stepwise logistic regression model with aggression as the dependent variable, both *L/*L genotype and age, but not sex or MMSE, demonstrated significant associations with aggression (genotype: $\chi^2 = 6.2, P = .01$; age: $\chi^2 = 15.8, P < .001$).
To our knowledge, this is the first study to relate aggressive behavior in AD to the 5-HTTPR polymorphism. As hypothesized, there was a significantly greater frequency of the *L/*L genotype in the aggressive subjects. We also found aggressive patients with AD to have a significantly higher frequency of *L alleles than never-aggressive patients with AD.

We predicted that the *L/*L genotype would be more commonly found in patients with AD with aggressive behavior because of the established relationship between 5-HT depletion and aggression.2-7 There is reason to believe that subjects who are of the *L/*L genotype would have less 5-HT available at the synapse because of the higher density of 5-HTT that this genotype confers.10,37 The low level of synaptic 5-HT caused by increased reuptake would therefore approximate the reduced 5-HT levels seen in subjects with impulsive aggressive behavior associated with other neuropsychiatric conditions,2,37 and in aggressive patients with AD.4 While reports of increased prolactin response to fenfluramine challenge in agitated patients with AD may initially seem to contradict these findings, they may be readily understood as an up-regulated postsynaptic state that develops in response to tonically reduced intrasynaptic 5-HT.

In addition to the hypothesized association of aggression with the *L/*L genotype, we found a significant elevation of *L allele frequency. Inspection of the rates of aggression in the genotype groups in Table 2 shows a progressive increase in aggression frequency from *S/*S to *S/*L to *L/*L subjects: aggression was present in 1 of 27 *S/*S subjects, 34 of 72 *S/*L subjects, and 23 of 38 *L/*L subjects. This pattern is not consistent with reports of *S allele dominance.18,28 The relationship between 5HTTPR genotype and 5-HTT expression in regions of human brain25,36 may differ, however, from that observed in in vitro expression systems or in peripheral tissues. In fact the 5-HTT promoter region is subject to regulation by multiple transcription factors33; thus, the effect of the *L and *S alleles on 5-HTT expression may vary in accordance with the specific transcription factors expressed in a specific tissue, region of tissue, or disease state. It is worth noting that, in some previous studies that found an association of 5HTTPR genotype with AD risk, a pattern like that of the current study, ie, more consistent with codominance than with a dominant-recessive system, has been observed.12,33

The preclinical neurochemical findings of reduced 5-HTT levels in aggressive patients with AD have suggested a role for 5-HT-enhancing drugs in the treatment of aggressive behaviors in AD. Emerging clinical data suggest this may indeed be the case.12,13,34 The current genetic findings are consistent with these earlier observations. Moreover, we previously reported that, among older subjects treated for major depressive illness with the selective 5-HT reuptake inhibitor paroxetine, *L/*L subjects had a significantly earlier antidepressant response than their *S/*S and *S/*L counterparts.35 It remains to be determined whether the *L/*L genotype will similarly predict response of aggressive behaviors in AD to treatment with 5-HT reuptake inhibitors.

Previous research on the 5HTTPR polymorphism and AD found an association between the *S allele and the development of late-onset AD in European and Brazilian subjects,2,33,36 although a subsequent study failed to replicate this association.37 Our findings suggest that these inconsistent associations could result from variability in the extent to which aggressive patients with AD were included. Behavioral characterization of subjects should be considered in future 5-HTTPR research in patients with AD.

Because this is the first study relating aggression in patients with AD to the 5-HTTPR polymorphism, independent replication will be necessary to confirm the association of the *L allele with aggressive behavioral disturbance. The major potential limitation of the study is the differing referral sources for aggressive subjects (Geriatric Psychiatry Inpatient Program) and nonaggressive subjects (Alzheimer Disease Research Center), also rated by different behavioral measures (Empirical Behavioral Pathology in Alzheimer Disease and Behavior Rating Scale, respectively). If referral source is a surrogate for a subject characteristic (other than aggression) that was associated with the 5HTTPR, a false-positive association with aggression could have resulted. The current study was also limited by incomplete matching for subject age, with the aggressive AD group being 6 years older on average than the never-aggressive group. Nevertheless, the association of genotype with aggression remained significant after controlling for the effect of age. Finally, as in all genetic association studies, a type I error might result from population stratification effects.

In summary, we found the *L allele and the *L/*L genotype of the 5HTTPR polymorphism to be increased in frequency in aggressive patients with AD when compared with patients with AD who were never aggressive. This study is the first of its kind, to our knowledge, and therefore must be considered preliminary, pending independent replication. Future studies examining the association between 5-TTPR genotype and response to serotonergic treatment of aggression in AD are also warranted.

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