**Molecular Psychiatry (1998) 3,** 438–441


## ORIGINAL RESEARCH ARTICLE

# The short variant of the polymorphism within the promoter region of the serotonin transporter gene is a risk factor for late onset Alzheimer's disease

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**Keywords:** late onset Alzheimer's disease; 5-HTT serotonin polymorphism; allelic association

**We analyzed a deletion/insertion polymorphism within the promoter region of the serotonin transporter gene (5-HTTPLR) in 81 patients with late onset Alzheimer's (AD) disease (mean age 70.02 ± 8.13 years). Control groups included 81 normal subjects with comparable age (mean age 75.6 ± 10.2) and 82 younger normal subjects (mean age 37.4 ± 9.1). Statistical analysis showed a significant difference in the genotype and gene frequencies between the AD group and normal controls ($\chi^2$ = 9.021; 2 d.f. and $\chi^2$ = 5.59, 1 d.f., respectively, $P<0.05$) due to the higher frequency of the L allele and the lower frequency of the s allele in controls than among AD patients. However, no differences were found in the genotype frequencies in older as compared to younger normal control groups ($\chi^2$ = 0.337, 2 d.f. and $P>0.05$). The present study confirms, in a different population, that the short variant of the 5-HTTPLR polymorphism may be a risk factor for late onset AD.**

Alzheimer's disease (AD), the most common form of dementia in the elderly, is characterized by a progressive deterioration in memory, language and other cognitive functions. The ethiology of AD is complex with involvement of genetic and environmental factors. Three relatively rare genes associated with early onset AD have been identified: the amyloid precursor protein gene (APP), the presenilin 1 gene (PS1) and the presenilin 2 gene (PS2), chromosomes 21, 14 and 1,[1–4] respectively.

The ϵ4 allele of the apoliprotein E (APOE) gene, at chromosome 19, has been reported in numerous studies worldwide as a risk factor associated with the disease, mainly in the late onset form,[5] and this was also confirmed in the Brazilian population.[6] More recently a new susceptibility locus for late-onset familial AD has been identified on chromosome 12.[7]

Serotonin (5-hydroxytryptamine or 5-HT) is a key neurotransmitter in the central and peripheral nervous systems that is implicated in the control of mood, sleep, appetite and a variety of traits and behaviors. Its potential role in psychiatric conditions such as depression, obsessive-compulsive disorder, schizophrenia and AD has been the subject of considerable study.[8] The 5-HT transporter (5-HTT), by regulating the magnitude and duration of serotonergic responses, is central to the regulation of brain serotonergic neurotransmission and of the peripheral actions of 5-HT.[9] The 5-HTT may therefore be involved in the pathogenesis of several psychiatric disorders.[10–13] The 5-HTT is encoded by a gene at 17q11.1–q12, with 14 exons which spans approximately 35 kb.[14–17]

Recently, a polymorphism in the transcriptional control region upstream of the 5-HTT coding sequence has been reported. It has been demonstrated that the long (L) and short (s) variants of this 5-HTT gene-linked polymorphic region (5-HTTLPR) have different transcriptional activities. The short variant of the polymorphism reduces the transcriptional efficiency of the 5-HTT gene promoter resulting in decreased 5-HTT expression and 5-HT uptake in lymphocytes.[18,19]

An association between the 5-HTTLPR and anxiety-related traits has been recently reported.[20] It has been suggested that this polymorphism accounts for 3–4% of total variation and 7–9% of inherited variance in anxiety-related personality traits in individuals as well as sibships. Furthermore, in a recent study, Collier *et al*[13] found that the frequency of the low activity allele (s) was higher in patients with affective disorders than in normal controls. However, discordant results were found by us and other investigators for affective disorders and other psychiatric conditions.[21–23] Subsequently, Li *et al*[24] also reported an excess of the low activity allele (s) in patients with late onset AD as compared to normal controls.

We are not aware of any similar study in the Brazilian population, which is characterized by a high racial admixture. In order to evaluate if there is an association between the 5-HTTLPR and AD in a different ethnic group, we analyzed 81 patients with a probable/possible diagnosis of AD as compared to 81 control individuals of comparable age. In addition, to further evaluate if there is an age effect related to this polymorphism, we also analyzed a younger group of normal control individuals ($n$ = 82).

The diagnosis of probable/possible Alzheimer disease in the 81 patients, who were evaluated in a private clinic and at the University Hospital (University of Pernambuco) was based on NINCDS-ADRDA.[25] Their mean age was 70.02 ± 8.13 years (ranging from 56 to 85 years old). The control group ($n$ = 81) was evaluated in a nursing home and was selected based on the Mini Mental State Exam and/or Blessed scale[26,27] or

**Table 1** Genotype and allele frequencies in 81 patients with Alzheimer disease (AD), 81 older controls and 163 total controls

| *Genotypes* | *AD (n = 81)* | *Normal controls Older group (n = 81)* | *Normal controls Total (n = 163)* |
|---|---|---|---|
| LL | 18 (21.22%) | 36 (44.44%) | 69 (42.33%) |
| Ls | 47 (58.02%) | 33 (40.74%) | 68 (41.71%) |
| ss | 16 (19.75%) | 12 (14.81%) | 26 (15.95%) |
| *Alleles* | | | |
| L | 83/162 | 105/162 | 206/326 |
| | 0.512 ± 0.039 | 0.648 ± 0.038 | 0.631 ± 0.026 |
| s | 79/162 | 57/162 | 120/326 |
| | 0.488 ± 0.039 | 0.352 ± 0.038 | 0.368 ± 0.026 |

depending on their educational level through familial interviews. Their mean age was 75.6 ± 10.2 (ranging from 50 to 98 years old). In addition, a younger control group (mean age 37.4 ± 9.1) was also analyzed and compared with patients and older controls. This younger group (which was not submitted to the Mini Mental State Exam and/or Blessed scale) included students and volunteers who worked at the University or normal relatives of Duchenne patients.

DNA was extracted from blood after informed consent, and the 5-HTTLPR was analyzed by polymerase chain reaction (PCR) according to the method reported previously.[20] According to this method, two fragments are generated: the short variant (s) with 484 bp and the long one (L) with 528 bp.

Genotype frequencies (Alzheimer patients *vs* control groups) were compared by the usual $\chi^2$ analysis on a $2 \times 3$ contingency table with two degrees of freedom; in order to locate the categories responsible for significant statistics values (bicaudal testing), the procedure described by Haberman[28] (see appendix) was applied. The observed genotype and allele frequencies in AD patients and normal controls are shown in Table 1 and the $\chi^2$ analyses for Hardy–Weinberg equilibrium are shown in Table 2.

Statistical analysis ($2 \times 3$ table) showed a statistically significant difference in the genotype frequencies between AD patients and the 81 normal controls of comparable age ($\chi^2 = 9.021$; 2 d.f.; $P < 0.05$). This difference is due to a significantly higher frequency of the LL genotype and a significantly lower frequency of the Ls genotype among normal controls than in the AD group, as shown by Haberman's test (reduced normal deviations) performed on the data shown in Table 1. The gene frequencies at this locus were also significantly different between patients and controls ($\chi^2 = 5.59$; 1 d.f.; $P<0.05$), due to the higher frequency of the L allele and the lower frequency of the s allele in controls than in AD patients.

**Table 2** Hardy–Weinberg proportions in controls and Alzheimer patients

| *Groups* | *P (L)* | *P (s)* | *$\chi^2$ (1 d.f.)* | *Significance* |
|---|---|---|---|---|
| Older controls (o) | 0.648 | 0.352 | 0.923 | $P>0.05$ |
| Young controls (y) | 0.616 | 0.384 | 0.786 | $P>0.05$ |
| All controls (o+y) | 0.632 | 0.368 | 1.737 | $P>0.05$ |
| Alzheimer patients | 0.512 | 0.488 | 2.105 | $P>0.05$ |

In addition, in order to verify if the genotype and allele frequencies were related to age we compared the sample of 81 elderly normal controls with a younger control group of 82 individuals. No statistically significant differences were observed for genotype frequencies ($\chi^2 = 0.337$; 2 d.f.; $P>0.05$). Therefore the two control groups were assembled and analyzed together for comparison with the AD group. This second analysis confirmed the significant differences between the Alzheimer and normal control groups ($\chi^2 = 9.64$; 2 d.f.; and $\chi^2 = 5.92$, 1 d.f., respectively for genotype and gene frequencies, $P<0.05$). As seen in Table 2, all genotypes were in Hardy–Weinberg equilibrium.

The role of the serotonin-transporter (5-HTT) as well as the association of the 5-HTT deletion/insertion polymorphism (5-HTTLPR) has been recently investigated in several psychiatric disorders. It has been suggested that the low activity allele of 5-HTTLPR could be a susceptibility factor for affective disorders[13] and for autism.[23] However, no association between this polymorphism and bipolar disorders or schizophrenia was observed by us in Brazilian patients[21] or by other authors in schizophrenia,[22] panic disorder,[29] autism[23] and obsessive compulsive disorder.[30]

For AD, results from the present study confirmed in a different population that the low activity allele of the serotonin transporter is a risk factor for AD, as recently reported by Li *et al*.[24] Interestingly, however, the genotype distributions differed in the two studies which could be explained by the different genetic background of our population. In addition, we also observed that the genotype and gene frequencies for the 5-HTTLPR do not vary with age in normal subjects. However, the fact that the low activity allele is also found in normal control subjects suggests that other factors may contribute to serotonin metabolism and transport.

The cause of depressive manifestations, which are frequently observed in AD patients, is still unknown. It is possible that the depressive symptoms shown by AD subjects might be related to other still unknown biological factors or might represent the patient's reaction to the diagnosis and to the limitations imposed by the disease.[31] In any case, since drugs currently used to treat the depressive symptoms in AD patients inhibit serotonin uptake, it would be interesting to compare the effects of those drugs in patients with different genotypes regarding the 5-HTTLPR polymorphism.

In summary, the present study supports the data of Li *et al*[24] and suggests that in addition to the $\epsilon$4 apoE polymorphism, the low-activity 5-HTTLPR allele s is also a risk factor for AD, in accordance with the

hypothesis of a multifactorial ethiology for the late onset form of this disorder. However, it is still unknown whether the association of 5-HTTLPR and AD is due to linkage desequilibrium or if this polymorphism acts directly on AD. The replication of the present study in other populations will be important in answering these questions.

## Acknowledgments

We would like to thank the following individuals for their help and suggestions: Carlos Mitsuo Shimokomaki, Marcelo Okuma, Antonia Cerqueira, Marta Canovas, Mariz Vainzof, Dr Francisco Jose Trindade Barreto, Dr Salustiano Lins, Dr Homero Vallada, Alessandra Kupper, Daniel Rocha, Dr Jose Luis de Lima Filho, Dr Luis Bezerra de Carvalho Junior, Constancia Urbani. Special thanks also to Dr Valentim Gentil-Filho for his constant support and encouragement. This work was supported with grants from FAPESP, CNPq, PRONEX, LIKA-JIKA, FINEP and IAEA.

## Appendix

Genotype frequencies (Alzheimer's patients *vs* control groups) were compared by the usual $\chi^2$ analysis on a $2 \times 3$ contingency table with two degrees of freedom. In order to locate the categories responsible for significant statistics values the procedure described by Haberman[28] was applied. The following explanation was adapted from Everitt, 1977[32]: since the contribution of each cell to the final $\chi^2$ figure is $(n_{ij} - E_{ij})^2/E_{ij}$, the procedure involves the examination of adjusted standardized residuals $d_{ij} = e_{ij}/(v_{ij})^{1/2}$, where $e_{ij} = (n_{ij} - E_{ij})/(E_{ij})^{1/2}$, $v_{ij} = (1 - n_i/N)(1 - n_j/N)$, $n_{ij}$ is the observed quantity on the {i-th,j-th} cell, and $E_{ij} = n_i n_j/N$ is the expected number ($n_i$ = row total, $n_j$ = column total, N = total number of observations). When the variables forming the table are independent, the distribution of $d_{ij}$ is expected to be normal with mean 0 and s.d. 1; therefore, if the modulus (absolute value) of a given $d_{ij}$ is larger than the 5% standard normal deviation in a bicaudal testing, namely 1.96, one concludes that the corresponding cell contributes significantly to the $\chi^2$ value.

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Received 2 February 1998; revised and accepted 26 March 1998