

# Effect of a Triallelic Functional Polymorphism of the Serotonin-Transporter-Linked Promoter Region on Expression of Serotonin Transporter in the Human Brain

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**Objective:** The authors examined effects of a triallelic functional polymorphism of the human serotonin-transporter-linked promoter region (5-HTTLPR) on in vivo expression of serotonin transporter in the brain in healthy volunteers and subjects with major depressive disorder.

**Method:** Twenty-five medication-free subjects with DSM-IV major depressive disorder during a major depressive episode and 42 healthy volunteers were clinically evaluated

and genotyped. Serotonin transporter binding potential ( $f_1B_{max}/K_d$ ) was determined by using positron emission tomography with the radiotracer [ $^{11}C$ ]McN 5652 and metabolite-corrected arterial input functions.

**Results:** There was no difference in serotonin transporter binding potential by genotype in healthy volunteers or in subjects with major depressive disorder. Allelic frequencies did not differ between subjects with major depressive disorder and healthy volunteers.

**Conclusions:** Associations of the 5-HTTLPR polymorphism to clinical phenotypes appear to be due to developmental effects of 5-HTTLPR on expression and not due to its direct effect on serotonin transporter binding in adulthood.

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Alterations of serotonin (5-HT) neurotransmission and transporter binding have been implicated in major depressive disorder (1). A 44-base-pair insertion/deletion polymorphism in the serotonin-transporter-linked promoter region (5-HTTLPR) regulates serotonin transporter gene expression in cell lines wherein the short (S) allele was originally reported to be associated with lower expression levels, compared with the long (L) allele. Subsequently, functional variants were identified in the L allele and designated as  $L_A$  and  $L_G$  (2). Thus, 5-HTTLPR is a triallelic locus with alleles designated as  $L_G$ ,  $L_A$ , and S (2). Because the  $L_G$  and S alleles have comparable levels of serotonin transporter expression, both of which are lower than that of  $L_A$ , we reclassified the alleles on the basis of lower and higher levels of expression.  $L_G$  and S were reclassified as  $S'$ , and  $L_A$  was reclassified as  $L'$ . Associations between 5-HTTLPR and psychiatric illnesses have been reported (3). However, serotonin transporter expression in humans in vivo and postmortem has not been clearly related to the genotype (4–9). In previous studies, a biallelic 5-HTTLPR genotype classification has been used. Use of this system could lead to a functional misclassification of cases with the  $L_G$  allele, and some of the disagreement in the literature could be explained by the effects of this allele.

We used positron emission tomography (PET) with the radiotracer [ $^{11}C$ ](+)-6 $\beta$ -(4-methylthiophenyl)-1,2,3,5,6 $\alpha$ ,10 $\beta$ -hexahydropyrrolo[2,1-a]isoquinoline ([ $^{11}C$ ]McN 5652) to examine the relationship of the triallelic polymorphism to serotonin transporter binding in the amygdala, hippocampus, thalamus, dorsal putamen, anterior cingulate, and midbrain in healthy volunteers and in subjects with major depressive disorder during a major depressive episode. A companion article describes differences in serotonin transporter binding potential between subjects with major depressive disorder and healthy comparison subjects (see the next article in this issue of the *Journal*).

## Method

Twenty-five subjects with major depressive disorder with a current major depressive episode and 42 healthy comparison subjects (Table 1) were evaluated by clinical history, Structured Clinical Interview for DSM-IV (10), physical examination, and laboratory analyses. Detailed inclusion criteria are reported in the companion article. The Institutional Review Boards of the New York State Psychiatric Institute and Columbia University approved the protocol. All subjects gave written informed consent after explanation of the study.

Compound preparation (11), PET and magnetic resonance imaging data acquisition, region-of-interest determination, image

**TABLE 1. Demographic Characteristics and Serotonin-Transporter-Linked Promoter Region (5-HTTLPR) Genotypes of Healthy Comparison Subjects and Subjects With Major Depressive Disorder**

Characteristic	Healthy Comparison Subjects (N=42)		Subjects With Major Depressive Disorder (N=25)		p <sup>a</sup>
	N	%	N	%	
Male	22	52	7	28	
Female	20	48	18	72	
Inpatient			14	56	
Outpatient			11	44	
	Mean	SD	Mean	SD	
Age (years)	38.8	15.88	38.0	13.4	
24-item Hamilton Depression Rating Scale score	0.68	0.91	24.36	7.26	
Beck Depression Inventory score	1.7	2.4	23.4	11.9	
Global Assessment Scale score	89.8	4.5	52.5	11.0	
Age at first major depressive episode (years)			21.4	11.4	
Number of major depressive episodes			4.4	3.0	
	N	%	N	%	
Triallelic genotype					0.15
L <sub>A</sub> /L <sub>A</sub>	10	24	6	24	
L <sub>A</sub> /L <sub>G</sub>	3	7	7	28	
L <sub>G</sub> /L <sub>G</sub>	0	0	1	4	
L <sub>A</sub> /S	14	33	6	24	
L <sub>G</sub> /S	4	10	1	4	
S/S	11	26	4	16	
Biallelic genotype reclassification <sup>b</sup>					0.56
L'L'	10	24	6	24	
L'S'	17	40	13	52	
S'S'	15	36	6	24	
Triallelic allele frequency					0.05
L <sub>A</sub>	37	44	25	50	
L <sub>G</sub>	7	8	10	20	
S	40	48	15	30	
Biallelic reclassification allele frequency					0.59
L'	37	44	25	50	
S'	47	56	25	50	

<sup>a</sup> Chi-square test.

<sup>b</sup> The alleles in the triallelic genotypes were reclassified on the basis of lower and higher levels of expression. L<sub>G</sub> and S, which had lower levels of expression than L<sub>A</sub>, were reclassified as S', and L<sub>A</sub> was reclassified as L'.

analysis, and arterial input indices were as previously described (12). After a 10-minute transmission scan, [<sup>11</sup>C]McN 5652 was injected intravenously, and emission data were acquired for 130 minutes. The derivation of [<sup>11</sup>C]McN 5652 regional distribution volumes (V<sub>T</sub>) was performed by using likelihood estimation in a graphical approach (13). V<sub>T</sub> is the sum of specific (V<sub>3</sub>) and nondisplaceable (free plus nonspecific binding) (V<sub>2</sub>) distribution volumes. Binding potential (BP') was calculated as follows: V<sub>T</sub> - V<sub>2</sub>, equivalent to f<sub>1</sub>B<sub>max</sub>/K<sub>d</sub>, where f<sub>1</sub> is the free concentration of the radiotracer in plasma, B<sub>max</sub> is the maximum number of binding sites, and K<sub>d</sub> is the dissociation constant. The specific-to-nonspecific equilibrium partition coefficient (V<sub>3</sub>') was calculated as follows: BP'/V<sub>2</sub>.

Data were analyzed by using linear mixed-effects models with six regions and all subjects or with subjects segregated into two diagnostic groups (healthy comparison group and major depressive disorder patients) as fixed effects and subject as the random effect. To stabilize variance and satisfy modeling assumptions, natural log-transformed data were used in the analysis after a quantity was added to all measures to ensure positive values.

Subjects were genotyped with the biallelic classification (9) and with the triallelic classification of the same polymorphism in which the alleles act co-dominantly (2). We report the results of the triallelic classification, which were comparable with results

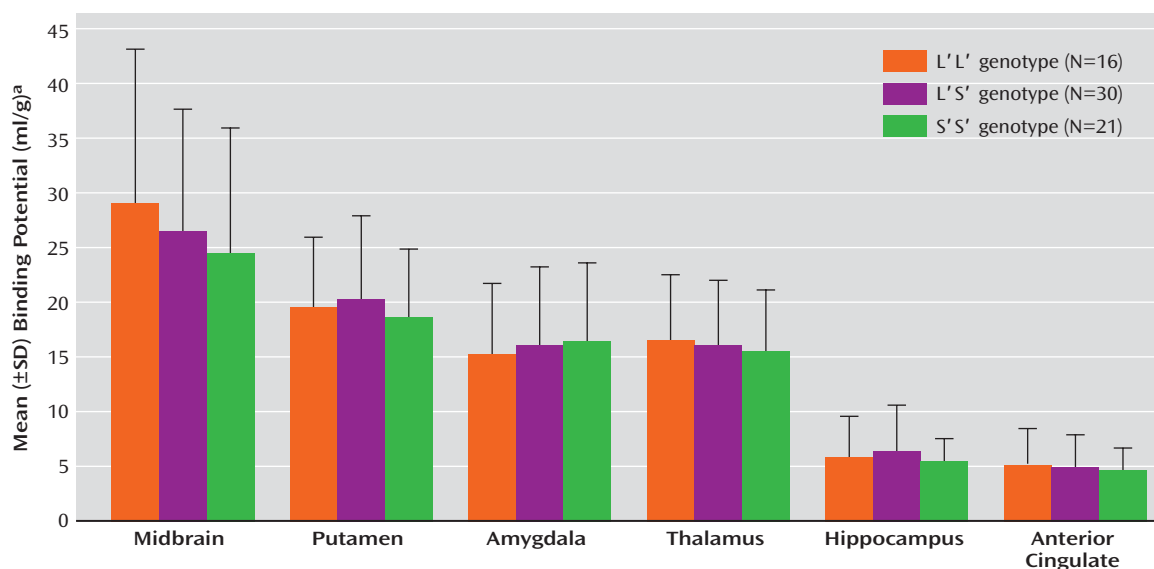
obtained with the biallelic classification (data not shown). The triallelic genotypes were reclassified into a biallelic model by their level of expression as follows: L<sub>G</sub>/S, L<sub>G</sub>/L<sub>G</sub>, and S/S were reclassified as S'S', L<sub>A</sub>/S and L<sub>A</sub>/L<sub>G</sub> were reclassified as L'S', and L<sub>A</sub>/L<sub>A</sub> was reclassified as L'L'.

## Results

Genotype distribution was in Hardy-Weinberg equilibrium in the healthy volunteers ( $\chi^2=0.38$ , df=1, p=0.54) and in the depressed subjects ( $\chi^2=1.75$ , df=1, p=0.19). Age (subjects with the L'L' genotype: mean=37.05 years, SD=13.92; subjects with the L'S' genotype: mean=38.96, SD=15.96; subjects with the S'S' genotype: mean=40.61, SD=15.39) (F=0.27, df=2, 64, p=0.76) and sex ( $\chi^2=0.87$ , df=2, p=0.65) did not differ between genotype groups.

Genotype was not associated with diagnosis ( $\chi^2=3.71$ , df=2, p=0.16) (Table 1). There was no effect of genotype on BP' and no effect of the interaction of genotype and brain region on BP' in the healthy comparison subjects (F=0.14, df=2, 39, p=0.87), the subjects with major depressive disorder

FIGURE 1. [ $^{11}\text{C}$ ]McN 5652 Binding Potential in Multiple Brain Regions in Healthy Comparison Subjects and Medication-Free Subjects With Major Depressive Disorder, by Serotonin-Transporter-Linked Promoter Region (5-HTTLPR) Genotype<sup>a</sup>



<sup>a</sup> No significant difference in binding potential between brain regions.

der ( $F=0.47$ ,  $df=2$ ,  $22$ ,  $p=0.63$ ), or the two groups combined ( $F=0.27$ ,  $df=2$ ,  $63$ ,  $p=0.77$ ). No genotype effects were found in an analysis of the brain regions separately (Figure 1). No difference was found in the cerebellar  $V_T$  between genotypes ( $F=0.89$ ,  $df=2$ ,  $65$ ,  $p=0.42$ ). In analyses in which diagnostic group and genotype were independent variables, there was no main effect of genotype on  $BP'$  ( $F=0.19$ ,  $df=2$ ,  $58$ ,  $p=0.83$ ) and no effect of the genotype-by-group interaction on  $BP'$  ( $F=0.37$ ,  $df=4$ ,  $58$ ,  $p=0.83$ ). Adding the variables of sex and age to the model did not alter the results.

## Discussion

We found no effect of 5-HTTLPR genotype on serotonin transporter binding potential in medication-free patients with major depressive disorder or in healthy volunteers. Previous *in vivo* imaging studies do not agree on the effect of 5-HTTLPR genotype on expression of serotonin transporter (4–8). Some of the previous studies demonstrated more (8) or less (4) binding with the S allele, and others found no differences in binding potential between genotypes (5–7). The newer triallelic genotype classification (2) was not used in these previous studies, which may have resulted in the classification of some L alleles as having higher levels of expression. In previous studies, the outcome measure was  $V_3''$  ( $BP'/V_2$ ). Our results with this outcome measure were identical to those in which  $BP'$  was calculated, which suggests that the lack of differences between the genotypes is not due to alterations in the free fraction of the tracer in the plasma or cerebrospinal fluid.

5-HTTLPR variants have been associated with clinical illness and with the function of the amygdala (14). If these findings are not due to altered serotonin transporter bind-

ing, they may reflect changes in maximum velocity in the enzymatic reaction ( $V_{max}$ ), effects of other polymorphisms (15), or developmental effects on neurogenesis or methylation patterns, perhaps in interaction with early-life stress (16). In our study, the error associated with determination of  $BP'$  in the midbrain was greater than in other regions of interest (Figure 1), which increases the risk of a type II error. At least 10 allelic variants have been identified for 5-HTTLPR (17), and the range of variants in this important susceptibility gene should be examined more fully in future studies. Finally, [ $^{11}\text{C}$ ]N,N-dimethyl-2-(2-amino-4-cyanophenylthio)benzylamine ([ $^{11}\text{C}$ ]DASB) has a higher specific-to-nonspecific binding ratio and measurable concentration of free radioligand in plasma, compared to [ $^{11}\text{C}$ ]McN 5652, and future studies could use [ $^{11}\text{C}$ ]DASB to determine binding potential in some cortical regions (11).

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This article is the first of two on serotonin transporters in the human brain in this issue of the *Journal*. The second article immediately follows this one.

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