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**Previous studies have provided conflicting evidence regarding the association of the serotonin transporter (5-HTT) gene with autism. Two polymorphisms have been identified in the human 5-HTT gene, a VNTR in intron 2[1] and a functional deletion/insertion in the promoter region (5-HTTLPR) with short and long variants.[2] Positive associations of the 5-HTTLPR polymorphism with autism have been reported by two family-based studies, but one found preferential transmission of the short allele[3] and the other of the long allele.[4] Two subsequent studies failed to find evidence of transmission disequilibrium at the 5-HTTLPR locus.[5,6] These conflicting results could be due to heterogeneity of clinical samples with regard to serotonin (5-HT) blood levels, which have been found to be elevated in some autistic subjects.[7-9] Thus, we examined the association of the 5-HTTLPR and VNTR polymorphisms of the 5-HTT gene with autism, and we investigated the relationship between 5-HTT variants and whole-blood 5-HT. The transmission/disequilibrium test (TDT) revealed no linkage disequilibrium at either loci in a sample of 96 families comprising 43 trios and 53 sib pairs. Furthermore, no significant relationship between 5-HT blood levels and 5-HTT gene polymorphisms was found. Our results suggest that the 5-HTT gene is unlikely to play a major role as a susceptibility factor in autism.**

Family and twin studies indicate that autism is one of the most strongly genetic neuropsychiatric disorders.[10,11] The pattern of recurrence risk among relatives suggests that several interacting genes are likely to underlie susceptibility to autism.[11] Genetic factors predisposing to autism may also confer a risk for a broader phenotype that extends beyond strictly defined autism to include a range of related but milder behavioral deficits. Indeed, cognitive, social, and language impairments are more frequently observed among relatives of autistic probands than among relatives of controls.[12] Similarly, elevated levels of whole blood or platelet serotonin (5-hydroxytryptamine, 5-HT) have been consistently observed in about one third of autistic subjects[7] and in their first-degree relatives,[8,9,13,14] suggesting that hyperserotonemia may be a marker of genetic susceptibility to autism. Other lines of evidence also suggest that a dysregulation in serotonergic neurotransmission might be involved in the pathogenesis of autism. Short-term dietary depletion of the 5-HT precursor tryptophan results in an exacerbation of behavioral symptoms in autistic subjects.[15] Conversely, 5-HT reuptake inhibitors, which block the reuptake of 5-HT into the presynaptic neuron by inhibiting the 5-HT transporter (5-HTT), appear to be of some benefit in the treatment of autistic symptoms such

as ritualistic behavior and aggression.[16,17] These data suggest that the 5-HTT is a compelling candidate gene for autism.

Two common polymorphisms of the 5-HTT gene have been described, a variable number of tandem repeats (VNTR) in intron 2 (serotonin transporter intron 2, STin2),[1] and a deletion/insertion in the 5'-flanking regulatory region (5-HTT gene-linked polymorphic region, 5-HTTLPR).[2] The 5-HTTLPR polymorphism consists of varying lengths of a repetitive sequence comprising 20-23-bp-long repeat elements; the deletion/insertion results in two major alleles, long and short, corresponding to 14 and 16 copies of the repeat element. The short variant reduces the transcriptional efficiency of the 5-HTT gene promoter, resulting in decreased 5-HTT expression and lower 5-HT uptake activity.[2,18,19]

Two recent family-based studies suggested an association of the 5-HTTLPR polymorphism with autism, but their findings were conflicting. Cook *et al*[3] reported preferential transmission of the short variant in a sample of families from the United States, whereas Klauck *et al*[4] observed preferential transmission of the long allele in a German sample. Neither study found evidence for an association with the VNTR polymorphism. Two subsequent family-based association studies failed to find linkage disequilibrium of either allele of the 5-HTTLPR polymorphism with autism.[5,6] Moreover, a case-control study also reported lack of association of 5-HTTLPR variants with autistic disorder.[20]

These discrepant findings might be explained by heterogeneity in the patient populations studied, particularly regarding 5-HT blood levels. Indeed, hyperserotonemia in autistic patients appears to result from an increased rate ($V_{max}$) of 5-HT uptake by platelets.[21] Given that 5-HTTLPR variants have been shown to affect the $V_{max}$ of 5-HT uptake in human blood platelets,[19] it would be interesting to evaluate the possible association between 5-HTT gene polymorphisms and 5-HT blood levels in autistic subjects. Thus, the purpose of the present study was to further investigate the association between 5-HTT gene polymorphisms and autism in a sample of trios and sib pairs recruited by the Paris Autism Research International Sibpair (PARIS) study, by using whole blood 5-HT levels as an endophenotype in addition to clinical classification. Use of quantitative traits, such as concentrations of neurotransmitters, in addition to dichotomous qualitative traits classically employed, could improve phenotypic characterization and contribute to the identification of more genetically homogeneous subgroups.

The results of the TDT analysis for the alleles of the 5-HTTLPR and intron 2 VNTR polymorphisms are presented in Table 1. The TDT analysis in sib pair families was performed by counting transmissions to both affected siblings. No preferential transmission of either short

or long alleles of the 5-HTTLPR locus was detected when analyzing the samples of trios and sib pairs separately or combined. Similarly, analysis of the intronic VNTR polymorphism revealed no evidence for association in the total sample or in the subset of trios or sib-pair families.

The haplotype analysis of the two markers in the total sample and in the sib-pair families yielded non-significant results (Table 2). However, in the trio sample, TDT analysis of the haplotypes revealed a significant transmission distortion (TDT $\chi^2$ = 6.42, df = 4, p<0.02). In these families, the long/STin2.12 haplotype was less transmitted than expected by chance ($\chi^2$ = 5.85, df = 1, p<0.02).

Linkage analysis in the affected sib pairs revealed no increased allele-sharing for either locus. Two-point NPL scores for the 5-HTTLPR and intron 2 VNTR polymorphisms were 1.36 (p<0.09) and –0.24 (p<0.58), respectively. Multipoint analysis yielded similar, non-significant results (maximum NPL score = 1.00, p = 0.14).

Table 3 shows 5-HTT genotypes for the promoter and the intronic VNTR polymorphisms and whole blood 5-HT levels in autistic probands. Blood 5-HT concentration in 45 autistic patients was 1.07 ± 0.69 μmol/l (mean ± SD). With hyperserotonemia defined as a 5-HT level greater than 0.9 μmol/l, 55% (25/45) affected individuals were hyperserotonemic. ANOVA revealed no significant effect of 5-HTTLPR or intron 2 VNTR genotypes on 5-HT levels (F(2,44) = 2.29, p = 0.11; and F(3,43) = 1.11, p = 0.35, respectively). Blood 5-HT levels were higher in patients homozygous for the long allele of the 5-HTTLPR polymorphism (1.48 ± 1.31 μmol/l) than in individuals with one or two copies of the short allele (0.96 ± 0.34 μmol/l), although this difference was not significant (Student's t-test, separate variances, t = 1.25; p = 0.24).

Our results revealed no evidence of association between autistic disorder and 5-HTT promoter variants in 43 trios and 53 sib-pair families. Similarly, two other TDT studies failed to reveal an association of the 5-HTTLPR locus with autism in their data sets: Maestrini *et al*[5] studied 82 multiplex families and 8 trios and Persico *et al*[6] tested 86 trios and 5 multiplex families. These negative findings are in contrast with the initial reports by Cook *et al*[3] and Klauck *et al.*[4] Cook *et al*[3] observed a preferential transmission of the short allele of the 5-HTTLPR in a sample of 86 trios, whereas Klauck *et al*[4] reported a preferential transmission of the long allele in a sample of 65 trios. A meta-analysis of the 5-HTTLPR polymorphism in the four family-based association studies published to date[3-6] and our results, comprising 504 heterozygote parents, indicated no association of either allele with autistic disorder; the short allele was transmitted 262 times versus 242 times not transmitted ($\chi^2$ = 0.79, df = 1, p = 0.37).

Thus, it is possible that the conflicting findings concerning the 5-HTTLPR result from spurious association to the 5-HTT gene, due to the relatively small patient samples tested by Cook *et al*[3] and Klauck *et al.*[4] Alternatively, the contradictory data might be a consequence of the genetic heterogeneity underlying autism, suggesting that the 5-HTT gene could be involved in the susceptibility to autism only in a small subset of patients.

The 5-HTT VNTR in intron 2 has been found to be associated with affective disorders and has recently been shown to act as a transcriptional regulator in an allele-dependent fashion,[22] thus providing a potential mechanism for its contribution to disease susceptibility. In agreement with previous studies,[3-5] our results did not reveal an association between the STin2 VNTR polymorphism and autism in the total sample of trios and sib-pair families. However, in the subsample of trios, the long/STin2.12 haplotype was less transmitted to autistic patients than what would be expected by chance. This finding is unlikely to represent a true association, given that the linkage disequilibrium disappears when analyzing the trios and sib-pairs together.

In the present study we also tested the association between the two 5-HTT gene polymorphisms and blood 5-HT levels. Hyperserotonemia, observed in patients with autism as well as in their first-degree relatives,[8,9,13,14] is one of the potential endophenotypes that could be used to identify more homogenous groups of subjects carrying a vulnerable genotype, thereby facilitating the detection of genes involved in autism. Neither the 5-HTTLPR nor the intron 2 VNTR polymorphism were associated with whole blood 5-HT concentration. Given the limited sample size used to measure 5-HT levels, the lack of significant findings do not completely dispel the potential relationship between blood 5-HT levels and 5-HTT polymorphisms in autistic subjects and further studies in other populations are required.

Although the mechanisms responsible for hyperserotonemia in autistic subjects remain unclear, converging evidence suggests that high 5-HT levels result from increased platelet uptake.[14,21] In vitro functional studies have shown that the long allele of the 5-HTTLPR polymorphism displays increased transcriptional activity, resulting in enhanced 5-HTT protein expression and higher 5-HT uptake in transfection assays[2] and human lymphoblasts.[18] Furthermore, the long allele is associated with a more rapid 5-HT uptake ($V_{max}$) in human blood platelets.[19] The short variant appears to exert a dominant influence, since 5-HTT expression and uptake are indistinguishable in lymphoblasts and platelets from individuals homozygous or heterozygous for the short allele.[18-19] In agreement with the present results in autistic probands, Greenberg *et al*[19] reported that the 5-HTTLPR polymorphism did not influence

platelet 5-HT levels in healthy male volunteers. Indeed, the only index of platelet 5-HTT function affected by the 5-HTTLPR genotype was the $V_{max}$ of 5-HT uptake, thus supporting a previous finding showing that the $V_{max}$, but not the affinity ($K_m$), is highly heritable.[23]

In conclusion, the present findings do not support the existence of association between the 5-HTTLPR or the VNTR polymorphism in intron 2 and autism in our set of families, suggesting that genetic variability of the 5-HTT gene is not a major risk factor for autism. Furthermore, our results do not support an association between 5-HTT gene polymorphisms and 5-HT blood levels in autistic patients. In view of the heterogeneity of the genetic mechanisms underlying complex psychiatric disorders such as autism, further studies of the 5-HTT gene in subgroups of patients with particular phenotypic characteristics are indicated.

## Materials and methods

### *Families*

Trios were recruited at an outpatient university clinic in Paris (Hôpital Robert Debré). Families with at least two children with autistic disorder were recruited by the PARIS study at specialized clinical centers in seven countries (Austria, Belgium, France, Italy, Norway, Sweden, and the United States). All patients fulfilled the DSM-IV criteria for autistic disorder and the Autism Diagnostic Interview-Revised (ADI-R) algorithm for ICD-10 childhood autism.[24] Subjects were included after a complete clinical and neuropsychological evaluation described previously.[25] Patients diagnosed with associated organic conditions or other established chromosomal disorders were excluded. Informed consent was obtained from all families participating in the study. The study was approved by the ethical committees of the collaborating institutions.

Fifty-three families with at least two affected siblings (including one family with three autistic children) and 43 trios were included in the study. All the sib-pairs were Caucasians; among the trios, 40 were Caucasians, 2 Caucasian/African Caribbean, and 1 Caucasian/African. The mean age of the probands was 14.5 years (age range, 4-44); 109 subjects were male and 41 were female.

### *Genotyping*

DNA was prepared from peripheral blood leukocytes using standard procedures. The 5-HTTLPR was amplified by polymerase chain reaction (PCR) using primers stpr5 (5'-GGCGTTGCCGCTCTGAATGC-3') and stpr3 (5'-GAGGGACTGAGCTGGACAACCAC-3'), which yielded short (484 bp) and long (528 bp) fragments. The PCR products were separated by electrophoresis on 2% agarose gels and stained with ethidium bromide. The 5-

HTT VNTR polymorphism in the second intron was amplified using primers hst1 (5'-GCTGTGGACCTGGGCAATGT-3') and hst2 (5'-GACTGAGACTGAAAAGACATAATC-3'). These primers amplified three alleles, containing 9 (STin2.9), 10 (STin2.10) or 12 (STin2.12) copies of the 17-bp repeat element. The PCR products were resolved by electrophoresis on 8% polyacrylamide gels and silver staining.

***Whole blood 5-HT***

Blood 5-HT levels were measured in 45 autistic probands recruited in Paris, belonging mostly to singleton families (42/45). Blood was drawn between 9 and 11 a.m. after two days of a diet low in tryptophan and 5-HT. Whole blood 5-HT content was measured using a radioenzymatic assay.[26] Because more than 99% of blood 5-HT is in the platelet fraction, this assay is a reliable measure of platelet content of 5-HT.

***Statistical analysis***

Standard biallelic TDT,[27] allele-wise TDT and genotype wise-TDT for multiallelic markers were performed using the ETDT program.[28] Haplotype frequencies were estimated using the program TRANSMIT[29] which performs estimation from the data by maximum likelihood. Haplotype transmission was tested using the same program. When transmission or parental haplotype assignments are uncertain, TRANSMIT averages observed transmission frequency over all the possible assignments consistent with the observed data (thus resulting in observations with decimal values). In addition to the global test of deviation of the random transmission frequencies, TDT was carried out for each specific haplotype. Non parametric linkage analysis was performed using the program GENEHUNTER ($NPL_{all}$ statistic).[30] Multipoint NPL scores were computed assuming a 0.0001 cM recombination fraction between the two polymorphisms. The association between 5-HTT gene polymorphisms and blood 5-HT levels was tested using ANOVA and Student's t-test.

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## References


1 Lesch KP, Balling U, Gross J, Strauss K, Wolozin BL, Murphy DL *et al*. Organization of the human serotonin transporter gene. *J Neural Transm* 1994; **95**: 157-162.

2 Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D *et al*. Allelic variation of human serotonin transporter gene expression. *J Neurochem* 1996; **66**: 2621-2624.

3 Cook EH Jr, Courchesne R, Lord C, Cox NJ, Yan S, Lincoln A *et al*. Evidence of linkage between the serotonin transporter and autistic disorder. *Mol Psychiatry* 1997; **2**: 247-250.

4 Klauck SM, Poustka F, Benner A, Lesch KP, Poustka A. Serotonin transporter (5-HTT) gene variants associated with autism? *Hum Mol Genet* 1997; **6**: 2233-2238.

5 Maestrini E, Lai C, Marlow A, Matthews N, Wallace S, Bailey A *et al*. Serotonin transporter (*5-HTT*) and γ-aminobutyric acid receptor subunit β3 (*GABRB3*) gene polymorphisms are not associated with autism in the IMGSA families. The International Molecular Genetic Study of Autism Consortium. *Am J Med Genet* 1999; **88**: 492-496.

6 Persico AM, Militerni R, Bravaccio C, Schneider C, Melmed R, Conciatori M *et al*. Lack of association between serotonin transporter gene promoter variants and autistic disorder in two ethnically distinct samples. *Am J Med Genet* 2000; **96**: 123-127.

7 Anderson GM, Freedman DX, Cohen DJ, Volkmar FR, Hoder EL, McPhedran P *et al*. Whole blood serotonin in autistic and normal subjects. *J Child Psychol Psychiatry* 1987; **28**: 885-900.

8 Abramson RK, Wright HH, Carpenter R, Brennan W, Lumpuy O, Cole E *et al*. Elevated blood serotonin in autistic probands and their first-degree relatives. *J Autism Dev Disord* 1989; **19**: 397-407.

9 Leboyer M, Philippe A, Bouvard M, Guilloud-Bataille M, Bondoux D, Tabuteau F *et al*. Whole blood serotonin and plasma beta-endorphin in autistic probands and their first-degree relatives. *Biol Psychiatry* 1999; **45**: 158-163.

10 Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E *et al*. Autism as a strongly genetic disorder: evidence from a British twin study. *Psychol Med* 1995; **25**: 63-77.

11 Pickles A, Bolton P, Macdonald H, Bailey A, Le Couteur A, Sim CH *et al*. Latent-class analysis of recurrence risks for complex phenotypes with selection and measurement error: a twin and family history study of autism. *Am J Hum Genet* 1995; **57**: 717-726.

12 Bailey A, Palferman S, Heavey L, Le Couteur A. Autism: the phenotype in relatives. *J Autism Dev Disord* 1998; **28**: 369-392.

13 Leventhal BL, Cook EH Jr, Morford M, Ravitz A, Freedman DX. Relationships of whole blood serotonin and plasma norepinephrine within families of autistic children. *J Autism Dev Disord* 1990; **20**: 499-511.

14 Cook EH Jr, Arora RC, Anderson GM, Berry-Kravis EM, Yan SY, Yeoh HC *et al*. Platelet serotonin studies in hyperserotonemic relatives of children with autistic disorder. *Life Sci* 1993; **52**: 2005-2015.

15 McDougle CJ, Naylor ST, Cohen DJ, Aghajanian GK, Heninger GR, Price LH. Effects of tryptophan depletion in drug-free adults with autistic disorder. *Arch Gen Psychiatry* 1996; **53**: 993-1000.

16 Gordon CT, State RC, Nelson JE, Hamburger SD, Rapoport JL. A double-blind comparison of clomipramine, desipramine, and placebo in the treatment of autistic disorder. *Arch Gen Psychiatry* 1993; **50**: 441-447.

17 McDougle CJ, Naylor ST, Cohen DJ, Volkmar FR, Heninger GR, Price LH. A double-blind, placebo-controlled study of fluvoxamine in adults with autistic disorder. *Arch Gen Psychiatry* 1996; **53**: 1001-1008.

18 Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S *et al*. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 1996; **274**: 1527-1531.

19 Greenberg BD, Tolliver TJ, Huang SJ, Li Q, Bengel D, Murphy DL. Genetic variation in the serotonin transporter promoter region affects serotonin uptake in human blood platelets. *Am J Med Genet* 1999; **88**: 83-87.

20 Zhong N, Ye L, Ju W, Tsiouris J, Cohen I, Brown WT. 5-HTTLPR variants not associated with autistic spectrum disorders. *Neurogenetics* 1999; **2**: 129-131.

21 Katsui T, Okuda M, Usuda S, Koizumi T. Kinetics of $^{3}$H-serotonin uptake by platelets in infantile autism and developmental language disorder (including five pairs of twins). *J Autism Dev Disord* 1986; **16**: 69-76.

22 MacKenzie A, Quinn J. A serotonin transporter gene intron 2 polymorphic region, correlated with affective disorders, has allele-dependent differential enhancer-like properties in the mouse embryo. *Proc Natl Acad Sci USA* 1999; **96**: 15251-15255.

23 Meltzer HY, Arora RC. Genetic control of serotonin uptake in blood platelets: a twin study. *Psychiatry Res* 1988; **24**: 263-269.

24 Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord* 1994; **24**: 659-685.

25 Philippe A, Martinez M, Guilloud-Bataille M, Gillberg C, Råstam M, Sponheim E *et al*. Genome-wide scan for autism susceptibility genes. Paris Autism Research International Sibpair Study. *Hum Mol Genet* 1999; **8**: 805-812.

26 Walker RF, Friedman DW, Jimenez A. A modified enzymatic-isotopic microassay for serotonin (5HT) using 5HT-N-acetyltransferase partially purified from Drosophila. *Life Sci* 1983; **33**: 1915-1924.

27 Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 1993; **52**: 506-516.

28 Sham PC, Curtis D. An extended transmission/disequilibrium test (TDT) for multi-allele marker loci. *Ann Hum Genet* 1995; **59**: 323-336.

29 Clayton D, Jones H. Transmission/disequilibrium tests for extended marker haplotypes. *Am J Hum Genet* 1999; **65**: 1161-1169.

30 Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES. Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 1996; **58**: 1347-1363.

**Table 1** Transmission/disequilibrium test of two 5-HTT gene polymorphisms and autistic disorder

| *Allele* | *Trios* | | *Sib pairs* | | *Total sample* | |
|---|---|---|---|---|---|---|
| | *T* | *NT* | *T* | *NT* | *T* | *NT* |
| **5-HTTLPR** | | | | | | |
| Short | 28 | 19 | 43 | 36 | 71 | 55 |
| Long | 19 | 28 | 36 | 43 | 55 | 71 |
| | TDT $\chi^2$ = 1.73, df = 1, p < 0.19 | | TDT $\chi^2$ = 0.62, df = 1, p < 0.43 | | TDT $\chi^2$ = 2.03, df = 1, p < 0.15 | |
| **5-HTT intron 2 VNTR** | | | | | | |
| Stin2.9 | 2 | 2 | 4 | 2 | 6 | 4 |
| Stin2.10 | 21 | 12 | 29 | 36 | 50 | 48 |
| Stin2.12 | 13 | 22 | 36 | 31 | 49 | 53 |
| | TDT $\chi^2$ = 2.50, df = 2, p < 0.28 | | TDT $\chi^2$ = 1.30, df = 2, p < 0.52 | | TDT $\chi^2$ = 0.47, df = 2, p < 0.78 | |

T = transmitted, NT = not transmitted.

**Table 2** Transmission/disequilibrium test of the haplotypes of the 5-HTTLPR and intron 2 VNTR polymorphisms of the 5-HTT gene and autistic disorder

| *Haplotype* | *Trios* | | *Sib pairs* | | *Total sample* | |
|---|---|---|---|---|---|---|
| | *Obs* | *Exp* | *Obs* | *Exp* | *Obs* | *Exp* |
| Long/Stin2.9 | 2.00 | 2.07 | 4.08 | 3.12 | 6.10 | 5.21 |
| Long/Stin2.10 | 23.82 | 21.57 | 62.40 | 66.36 | 86.12 | 87.69 |
| Short/Stin2.10 | 6.17 | 4.27 | 16.98 | 14.91 | 23.07 | 18.97 |
| Long/Stin2.12 | 17.17 | 23.86 | 47.60 | 48.40 | 64.79 | 72.27 |
| Short/Stin2.12 | 36.82 | 34.22 | 62.92 | 61.20 | 99.91 | 95.85 |
| | TDT $\chi^2$ = 6.42, df = 4, p < 0.02 | | TDT $\chi^2$ = 1.92, df = 4, p < 0.75 | | TDT $\chi^2$ = 4.01, df = 4, p < 0.45 | |

Obs = observed, Exp = expected.

**Table 3** Whole blood 5-HT levels and 5-HTT gene polymorphisms in autistic patients

| *Genotype* | *n* | *Blood 5-HT (μmol/l, mean ± SD)* |
|---|---|---|
| **5-HTTLPR** | | |
| Short/short | 8 | 0.99 ± 0.46 |
| Short/long | 27 | 0.95 ± 0.31 |
| Long/long | 10 | 1.48 ± 1.31 |
| **5-HTT intron 2 VNTR** | | |
| Stin2.9/Stin2.10 | 3 | 0.89 ± 0.06 |
| Stin2.10/Stin2.10 | 8 | 1.42 ± 1.48 |
| Stin2.10/Stin2.12 | 17 | 1.15 ± 0.39 |
| Stin2.12/Stin2.12 | 16 | 0.89 ± 0.35 |