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Susceptibility for schizophrenia is not influenced by a functional insertion/deletion variant in the promoter of the serotonin transporter gene

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Abstract A possible dysregulation of serotonergic neurotransmission has been implicated in the aetiology of schizophrenic psychoses. In the present study we analysed allelic and genotypic variations of a recently described functional polymorphic region in the promoter of the human serotonin transporter gene (5-HTTLPR) and a variable tandem repeat (VNTR) in intron 2 of the 5-HTT gene. We investigated 413 unrelated individuals, 180 schizophrenic patients and 233 blood donors as controls. With regard to the 5-HTTLPR, both the schizophrenic and the control group did not significantly differ between genotype frequencies (χ^2, p = 0.920) and allele frequencies (χ^2, p = 0.836). The odds ratio for subjects with schizophrenia who were homozygous for the short allele was 1.04 (95% CI 0.59–1.84). No evidence of allelic association to specific schizophrenia subtypes was found. The 5-HTT associated VNTR also showed no significant differences between either the allelic or the genotypic distributions. Haplotype analysis revealed a significant overall linkage disequilibrium at a level of p = 0.00004. Our findings indicate that both polymorphisms are unlikely to play a substantial role in the genetic predisposition to schizophrenic disorders.

Key words 5-HTT · Serotonin transporter · Gene · Promoter · Allelic variation · Repeat element polymorphism · Schizophrenia

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Introduction

The human serotonin transporter (5-HTT) is the high affinity and substrate-specific cellular uptake site for serotonin to reaccumulate and recycle the released neurotransmitter into presynaptic terminals aiding termination of synaptic transmission. The gene encoding the human 5-HTT was mapped to chromosome 17q12 (Gelernter et al. 1995). It consists of 14 exons spanning ~35 kilobases and predicts a protein of 630 amino acids with 12–13 putative membrane spanning domains (Lesch et al. 1994). The 5-HTT gene promoter is defined by a TATA-like motif and several potential binding sites for transcription factors (Heils et al. 1996). Within the promoter we identified a tandemly repeated sequence containing GC-rich, 20- to 23-bp long repeat elements. Within this repeat the 5-HTT-linked polymorphic region (5-HTTLPR) is generated by a 44-bp deletion (at bp –1212 to –1255). Functional analyses demonstrated that the 5-HTT gene transcription is influenced by this insertion/deletion variant. The short allelic variant results in a reduction in 5-HTT gene transcription and, as a consequence, a decrease in 5-HT reuptake. In an association study of the 5-HTT gene Lesch et al. (1996) reported that in anxiety-related personality traits the 5-HTTLPR accounts for 3–4% of total variation and 7–9% of genetic variance based on the assumption that these personality characteristics are 40–60% heritable. Furthermore, the low-activity variant of the 5-HTT was found to be associated with susceptibility to affective disorders in a large sample derived from three European centres (Collier et al. 1996b). The population-attributable risk resulting from the low-activity 5-HTT gene was calculated to be approximately 29%. These findings supported previous data demonstrating a decrease in 5-HTT function in brains of depressed and suicide subjects (Routledge and Middlemiss 1996). Analysis of the coding region of the gene and its adjacent exon-intron junctions for polymorphic variants revealed, in addition to some rare polymorphisms which were not associated with major psychiatric disorders, a 17-bp variable tandem repeat (VNTR) located in intron 2 (Lesch et al. 1994, 1995;

DiBella et al. 1996). The importance of this VNTR with 9, 10 or 12 repeat elements for the development of affective disorders is equivocal. Ogilvie et al. (1996) reported that the rare allele 9 of the VNTR was associated with major depression, and on the contrary, Collier et al. (1996a) found the allele 12 associated with bipolar disorder. In our German sample we observed no allelic associations with both unipolar and bipolar affective disorder compared with controls (Stöber et al. 1996).

The serotonin hypothesis of schizophrenia originated in the observation of alterations of serotonergic neurotransmission in schizophrenia which is thought to be mediated via 5-HT receptors and the 5-HT transporter (Roth and Meltzer 1995). Among the various 5-HT receptors, however, no consistent association with schizophrenia was detectable on the molecular level and the reported association of schizophrenia to a silent polymorphism within the coding region of the 5-HT 2a receptor gene needs substantiation in that a functional mutation in the regulatory region should be in close linkage disequilibrium with this variant (Williams et al. 1996). With regard to the serotonin transporter several post-mortem studies noted reduced serotonin reuptake sites in distinct brain areas of schizophrenics using radioligand binding (Joyce et al. 1994; Naylor et al. 1996). Hernandez and Sokolov (1997) recently reported on a significant increase of serotonin transporter mRNA in the frontal and temporal cortex of schizophrenic subjects. In order to test a possible contribution of the serotonin transporter gene to the development of schizophrenia, we determined frequencies of the 5-HTTLPR and the VNTR variant in a sample of unrelated schizophrenic patients and controls.

Subjects and methods

For the association study 180 schizophrenic patients and 233 blood donors as controls were investigated. The schizophrenic patients were collected from the Department of Psychiatry at the University of Würzburg and from the Psychiatric State Hospital of Lohr/Main. Subjects were diagnosed according to ICD-10. Among the ICD-10 schizophrenics (111 males and 69 females) the median age of onset was 24 years (mean 26.5 years, SD 9.7 years). The volunteer control subjects (137 males and 96 females) were recruited from the blood donor centre at the University of Würzburg. No assessment of their history of mental illness was recorded. The median age of the control subjects was 26 years (mean 29.9 years, SD 10.0 years). Even though the age of a significant number of control subjects was below the mean age of onset in schizophrenia, the population frequency of 0.6–1% for this disorder would mean that it is unlikely for more than 1 or 2 of the control subjects to develop the disease later in life. All subjects were unrelated and of German Caucasian descent. The study was approved by the Ethical Committee of Würzburg University and informed consent was obtained from all subjects.

EDTA anticoagulated venous blood samples were collected from all probands. Leucocyte DNA was isolated by salting out with saturated NaCl solution. Oligonucleotide primers were designed in the human 5-HTT regulatory region flanking the 5-HTT-linked polymorphic region (5-HTTLPR) and corresponded to nucleotide positions –1416 to –1396 (5′-GGCGTTGCCGCTCTGAATGCC) and from –1170 to –1149 (5′-CAGGGGAGATCCTGGGAGAGGT) according to the published sequence (Heils et al. 1996). The primers produced a 265 bp fragment (insertion variant) or 221 bp fragment (deletion variant). The VNTR in the intron 2 of the serotonin transporter gene was analysed using the intron-2 primer sequences 5′-GTCAGTATCACAGGCTGCGAG (nucleotide position +1 to +21) and 5′-TGTTCCTAGTCTTACGCCAGTG (+229 to +250) for PCR amplification as described by Ogilvie et al. (1996). Amplification products consisted of three alleles of 250 bp (9 repeats), 267 bp (10 repeats) and 300 bp (12 repeats).

Standard PCR was carried out in 25 µl volume containing 80 ng genomic DNA, 20 pmol of each primer, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl2, 0.01% gelatine, 5% DMSO, 200 µM of each dNTP and 0.5 U Taq DNA polymerase (Stratagene). The HTTLPR product was generated using 3.0 µM deoxyribonucleotides (dGTP/7-deaza-2′-dGTP). Samples were amplified in an UNO II Thermoblock (Biometra, Göttingen). After an initial 5 min denaturation at 95 °C, 35 temperature cycles were carried out consisting of 30 s at 95 °C, 45 s at 57 °C and 60 s at 72 °C, followed by a final extension step of 5 min at 72 °C. Alleles were resolved by agarose gel electrophoresis next to a DNA size standard.

Statistical analysis consisted of χ^2 tests for Hardy-Weinberg proportions and χ^2 tests for homogeneity of genotypic and allelic frequencies in the patient and control groups. Odds ratios (OR) with 95% confidence intervals (CI) were estimated for the effects of high-risk genotypes and alleles.

Results

Both the schizophrenic and the control group did not significantly differ between genotype frequencies ($\chi^2 = 0.168$;

Table 1 Genotype and allele frequencies of the HTTLPR of the promoter region and the VNTR of the intron 2 of the human serotonin transporter gene in the schizophrenic ($n = 180$) and control groups ($n = 233$)

5-HTTLPR	Genotype			Allele	
	221/221	221/265	265/265	221	265
Schizophrenics	32 (18%)	90 (50%)	58 (32%)	154 (43%)	206 (57%)
Controls	42 (18%)	112 (48%)	79 (34%)	196 (42%)	270 (58%)

VNTR intron 2	Genotype				Allele	
	9/12	10/10	10/12	12/12	9/10	12
Schizophrenics	2 (1%)	29 (16%)	83 (46%)	66 (37%)	143 (40%)	217 (60%)
Controls	8 (4%)	54 (23%)	96 (41%)	75 (32%)	212 (45%)	254 (55%)

NOTE: In schizophrenics vs controls χ^2 for genotype frequencies of the HTTLPR was 0.168, $p = 0.92$ (ns), and of the VNTR 3.267, $p = 0.195$ (ns). χ^2 for allele frequencies of the HTTLPR was 0.043, $p = 0.836$ (ns), and of the VNTR 2.760, $p = 0.097$ (ns)

Table 2 Genotype and allele frequencies of the HTTLPR of the promoter region of the serotonin transporter gene in schizophrenic subtypes according to ICD 10

Schizophrenic subtype	Genotype			Allele	
	221/221	221/265	265/265	221	265
Paranoid (n = 68)	10 (15%)	32 (47%)	26 (38%)	52 (38%)	84 (62%)
Non-paranoid (n = 112)	22 (20%)	58 (52%)	32 (28%)	102 (46%)	122 (54%)

NOTE: In the group of paranoid schizophrenics vs non-paranoid schizophrenics (combined hebephrenic, catatonic, undifferentiated and residual subtypes) χ^2 = for genotype frequencies of the HTTLPR was 2.00, p = 0.369 (ns), and χ^2 for allele frequencies was 1.84, p = 0.175 (ns)

Table 3 Haplotype analysis of the 5-HTTLPR in the promotor region and the VNTR of intron 2 in schizophrenics and controls

Allele	Schizophrenics	Controls	All combined
221/10	0.131	0.135	0.132
221/12	0.297	0.285	0.291
265/10	0.266	0.320	0.297
265/12	0.306	0.260	0.279

Schizophrenics vs controls: χ^2 = 3.22; df = 3, p = 0.36
Overall linkage disequilibrium: χ^2 = 17.09; p = 0.00004

p = 0.920) and allele frequencies (χ^2 = 0.043; p = 0.836) with regard to the 5-HTTLPR (Table 1). We found no excess of the short, low-activity allele among the schizophrenic patients with a frequency of 43% compared with controls with 42%. The estimated odds ratio (OR) for the schizophrenic subjects with the short allele compared with those with the long allele was was 1.03 (95% CI 0.78–1.36). By genotype the OR for the homozygous short variant (221/221) compared with the homozygous long variant (265/265) was 1.04 (95% CI 0.59–1.84), and for the heterozygous genotype compared with the homozygous long variant 1.10 (95% CI 0.71–1.70). Genotypes were in Hardy-Weinberg equilibrium among schizophrenics (χ^2 = 0.08; p = 0.775) and controls (χ^2 = 0.04; p = 0.834). Enhanced allelic or genotypic frequencies did not appear to characterize any specific subtype in the schizophrenic sample if classified in paranoid (n = 68), hebephrenic, catatonic, undifferentiated or residual schizophrenia (non-paranoid group: n = 112) according to ICD-10 (Table 2).

The 5-HTT-associated VNTR showed a frequency of allele 9 of 1.7% among controls and of 0.6% among schizophrenics (Table 1). Since allele 9 was rare and only the heterozygous genotype with allele 12 was observed, genotypes were pooled into three groups for further analyses: homozygous for allele 12, heterozygous for allele 12 and homozygous for allele 10. Allele 12 was the most common repeat element in controls (54%) as well as schizophrenics (60%). No significant differences between either the allelic distributions (χ^2 = 2.760; p = 0.097) or the genotypic distributions (χ^2 = 3.267; p = 0.195) were obvious in both samples. Regarding genotypes Hardy-Weinberg equilibrium was apparent in the control (χ^2 = 2.33; p = 0.127) as well as in the schizophrenic sample (χ^2 = 0.03; p = 0.852). Haplotype analysis showed a significant overall linkage disequilibrium (Table 3; χ^2 = 17.09; p = 0.00004). No differences were observed between schizophrenics and controls (χ^2 = 3.22; p = 0.36).

Discussion

Our findings do not suggest a significant association of schizophrenia with either the genotype or the allele frequency of both the 5-HTT-linked polymorphic region (5-HTTLPR) and the 5-HTT-associated VNTR relative to population control subjects.

Recently, we reported that the low-activity genotype of the 5-HTTLPR and anxiety-related traits significantly cosegregated within families and accounted for 7–9% of inherited variance. Furthermore, patients with affective disorder exhibited a significant association with the short, low-activity allele. Estimated OR for the short allele reached 1.23 (95% CI 1.02–1.49) and for the homozygous low-activity genotype 1.53 (95% CI 1.04–2.23). Equal frequencies of the insertion/deletion variant (5-HTTLPR) in schizophrenic patients and controls gave evidence that this genetic variation is of no major aetiological role in schizophrenia. Furthermore, in the schizophrenic sample OR were low at 1.03 (95% CI 0.78–1.36) for the short allele and 1.04 (95% CI 0.59–1.84) for the homozygous low-activity genotype and indicated that the variant determing low activity of the 5-HTT gene expresssion does not substantially contribute to susceptibility to develop schizophrenia. Weak potential linkage of markers on chromosome 17q12 (near the locus of the 5-HTT gene) to schizophrenia has been reported by Moises et al. (1995). On the basis of our results, we cannot exclude that the 5-HTTLPR or other yet-unknown genetic variants near the 5-HTT locus may play a pathogenic role in some high-density schizophrenia families. However, it seems more likely that susceptibility to affective symptoms or anxiety-related personality traits may cosegregate within some pedigrees with familial schizophrenia. Furthermore, there remains the possibility that the effect of the functional variant might not be present in the investigated subtypes of the disease. The failure to find a link between schizophrenia and occurrence of the low-activity variant might additionally result from a type-II error, i.e. that our sample size might have been too small so that we incorrectly accepted the null hypothesis of no association. However, on the basis of our sample size we had a power of 90.2% to detect a significant association of schizophrenia and homozygosity for the low-activity variant under the assump-

tion of a relative risk of 1.5 for heterozygous and of 2.5 for homozygous subjects ($\alpha = 0.05$).

Preliminary data (Collier et al. 1996a; Ogilvie et al. 1996) suggested an association of different alleles of the 5-HTT-associated VNTR in intron 2 with unipolar (allele 9) or bipolar affective disorder (allele 12), but independent samples failed to replicate these findings (Stöber et al. 1996). Interestingly, allelic frequencies of this VNTR seem to vary widely among geographical populations. The observed frequency of allele 12 in Caucasians with 55% (German controls) and 54% (English controls) contrasts to 88% in a Chinese population (Collier et al. 1996a). In our sample of schizophrenic patients the most abundant repetitive element was allele 12 with a frequency of 60% corresponding to a frequency of 63% in an English sample (Collier et al. 1996a). None of the three alleles of the VNTR was significantly associated with susceptibility to schizophrenia. The report of a non-significant over-representation of allele 12 seems merely a chance false positive. Collier et al. (1996b) had found only modest linkage disequilibrium at a level of $p = 0.005$ of both polymorphisms in a subsample of patients with affective disorders and controls and suggested a functional variant in or near the 5-HTT-associated VNTR which acts independently. In our sample of combined schizophrenics and controls we found a highly significant overall linkage disequilibrium of $p = 0.00004$ between the 5-HTTLPR and the 5-HTT-associated VNTR which are only separated by ~15 kb. Analysis of the haplotype frequencies in our published sample of affective psychosis likewise indicated a highly allelic association of the 5-HTT-associated VNTR and the 5-HTTLPR ($p = 0.0006$). Our data argue against a direct role for the VNTR in the pathophysiology of both schizophrenia and affective disorders and against an additional causative mutation in close linkage disequilibrium to the VNTR.

However, our results do not contradict the observation of post-mortem studies that discrete brain regions of schizophrenics showed reduced serotonin reuptake sites or abnormal expressions of 5-HTT mRNA (Naylor et al. 1996; Hernandez and Sokolov 1997). Expression of 5-HTT may be dysregulated in a tissue-specific manner and there are several lines of evidence that 5-HTT expression is promoted selectively during early brain development and aberrant expression may contribute to alterations of synaptic function and to physiological abnormalities of serotonergic pathways (Lebrand et al. 1996; Slotkin et al. 1996). Furthermore, the promoter activity of the 5-HTT gene is regulated by an interplay of different positive and negative acting regulatory elements (Heils et al. 1995). Systematic screening for additional functional polymorphisms in the promoter region followed by case-control association studies would be the next step in dissecting the pathophysiological relevance of the 5-HTT gene for neuropsychiatric disorders.

In conclusion, the present study investigated allelic and genotypic variations of a recently described functional polymorphic region in the promoter of the human serotonin transporter gene (5-HTTLPR) and a variable tandem repeat (VNTR) in intron 2 of the 5-HTT gene. The low-activity genotype of the 5-HTTLPR which exhibited strong association with both anxiety-related personality traits and affective disorders seemed not to be associated with susceptibility to schizophrenic psychoses. The 5-HTT-associated VNTR that appeared to be in close overall linkage disequilibrium with the 5-HTTLPR is also unlikely to play a direct role in the genetic predisposition to schizophrenia. However, there remains the possibility that the effect of the functional variant might not be present in the investigated subtypes of the disorder. Furthermore, the observed altered levels of 5-HTT expression in brains of schizophrenics may be due to interactions in serotonergic neuronal function.

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References

Collier DA, Arranz MJ, Sham P, Battersby S, Vallada H, Gill P, Aitchison KJ, Sodhi M, Li T, Roberts GW, Smith B, Morton J, Murray RM, Smith D, Kirov G (1996a) The serotonin transporter is a potential susceptibility factor for bipolar affective disorder. NeuroReport 7: 1675–1679

Collier DA, Stöber G, Li T, Heils A, Catalano M, DiBella D, Arranz MJ, Murray RM, Vallada HP, Bengel D, Müller CR, Roberts GW, Smeraldi E, Kirov G, Sham P, Lesch KP (1996b) A novel functional polymorphism within the promoter of the serotonin transporter gene: possible role in susceptibility to affective disorders. Mol Psychiatry 1: 453–460

DiBella D, Catalano M, Balling U, Smeraldi E, Lesch KP (1996) Systematic screening for mutations in the coding region of the human serotonin transporter (5-HTT) gene using PCR and DGGE. Am J Med Genet 67: 541–545

Gelernter J, Pakstis AJ, Kidd KK (1995) Linkage mapping of serotonin transporter gene SLC6A4 on chromosome 17. Hum Genet 95: 677–680

Heils A, Teufel A, Petri S, Seeman M, Bengel D, Balling U, Riederer P, Lesch KP (1995) Functional promoter and polyadenylation site mapping of the human serotonin (5-HT) transporter gene. J Neural Transm 102: 247–254

Heils A, Teufel A, Petri S, Stöber G, Riederer P, Bengel D, Lesch KP (1996) Allelic variation of the human serotonin transporter gene expression. J Neurochem 6: 2612–2624

Hernandez I, Sokolov BP (1997) Abnormal expression of serotonin transporter mRNA in the frontal and temporal cortex of schizophrenics. Mol Psychiatry 2: 57–64

Joyce J, Shane A, Lexow N, Winokur A, Casanova MF, Kleinman JE (1994) Serotonin uptake sites and serotonin receptors are altered in limbic system of schizophrenics. Neuropsychopharmacology 8: 315–336

Lebrand C, Cases O, Adelbrecht C, Doye A, Alvarez C, Elmestikawy S, Seif I, Gaspar P (1996) Transient uptake and storage of serotonin in developing thalamic neurons. Neuron 17: 823–835

Lesch KP, Balling U, Gross J, Strauss K, Wolozin BL, Murphy DL, Riederer P (1994) Organization of the human serotonin transporter gene. J Neural Transm 95: 157–162

Lesch KP, Gross J, Franzek E, Wolozin BL, Riederer P, Murphy DL (1995) Primary structure of the serotonin transporter in unipolar depression and bipolar disorder. Biol Psychiatry 37: 215–223

Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Müller CR, Hamer DH, Murphy DL (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274: 1527–1531

Moises HW, Yang L, Kristbjarnson H, Wiese C, Byerley W, Macciardi F, Arolt V, Blackwood D, Liu X, Sjögren B, Aschauer HN, Hwu HG, Jang K, Livesley WJ, Kennedy JL, Zoega T, Ivarsson O, Bui MT, Yu MH, Havsteen B, Commenges D, Weissenbach J, Schwinger E, Gottesman II, Pakstis AJ, Wetterberg L, Kidd KK, Helgason T (1995) An international two-stage genome-wide search for schizophrenia susceptibility genes. Nat Genet 11: 321–324

Naylor L, Dean B, Opeskin K, Pavey G, Hill C, Keks N, Copolov D (1996) Changes in the serotonin transporter in the hippocampus of subjects with schizophrenia identified using [3H] paroxetine. J Neural Transm 103: 749–757

Ogilvie AD, Battersby S, Bubb VJ, Fink G, Harmar AJ, Goodwin GM, Smith CAD (1996) Polymorphism in serotonin transporter gene associated with susceptibility to major depression. Lancet 347: 731–733

Roth BL, Meltzer HY (1995) The role of serotonin in schizophrenia. In: Bloom FE, Kupfer DJ (eds) Psychopharmacology: the fourth generation of progress. Raven Press, New York, pp 1215–1227

Routledge C, Middlemiss DN (1996) The 5-HT hypothesis of depression revisited. Mol Psychiatry 1: 437

Slotkin TA, Barnes GA, McCook EC, Seidler FJ (1996) Programming of brainstem serotonin transporter development by prenatal glucocorticoids. Dev Brain Res 93: 155–161

Stöber G, Heils A, Lesch KP (1996) Serotonin transporter polymorphism and affective disorder. Lancet 347: 1340–1341

Williams J, Spurlock G, McGuffin P, Mallet J, Nöthen MM, Gill M, Aschauer H, Nylander PO, Macciardi F, Owen MJ (1996) Association between schizophrenia and T102C polymorphism of the 5-hydroxytryptamine type 2a-receptor gene. Lancet 347: 1294–1296