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Human 5-HT_{5A} Receptor Gene: Systematic Screening for DNA Sequence Variation and Linkage Mapping on Chromosome 7q34–q36 Using a Polymorphism in the 5′ Untranslated Region

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Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter that mediates a wide range of sensory, motor, and cortical functions by activating multiple 5-HT receptor subtypes. In the present study we performed a systematic mutation scan of the complete coding region of the 5-HT_{5A} receptor to explore its variability in the general population. Investigating 46 unrelated healthy subjects by single-strand conformation analysis no sequence changes of likely functional relevance were observed. The detection of a frequent G→C substitution at position −19 was used for fine scale linkage mapping of the 5-HT_{5A} gene. Employing a polymerase-chain-reaction based assay we genotyped 7 CEPH families (Centre d'Etude du Polymorphisme Humaine) and mapped the receptor to genetic markers on chromosome 7q34-q36.

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter that mediates a wide variety of sensory, motor, and cortical functions. Likewise, disturbances of serotonergic pathways have been implicated in many psychiatric disorders which include anxiety, affective disorder, schizophrenia, alcoholism, migraine, aggression, and suicidal behavior. The transduction of serotonergic signals across the neuronal membrane is mediated by multiple 5-HT receptor subtypes. Molecular biology techniques have revealed that many more heterogeneous subtypes exist than previously suspected from traditional pharmacological studies [Sadou & Hen, 1994; Shih et al., 1995]. Recently, the 5-HT_{5A} receptor was cloned in three mammalian species, namely rat [Erlander et al., 1993], mouse [Plassat et al., 1992; Matthes et al., 1993], and human [Rees et al., 1994]. The human 5-HT_{5A} receptor gene contains an open reading frame of 1,071 base pairs (bp) and encodes a protein of 357 amino acids (aa). On the genomic level the human $5HT_{5A}$ receptor gene consists of two exons separated by a large intron (> 5 kb) positioned in the third intracellular loop of the deduced aa sequence [Rees et al., 1994]. At present there is no information on the existence of genetic variation within the coding region of the $5HT_{5A}$ receptor gene.

Using the mouse 5-HT_{5A} cDNA as a probe the mouse 5-HT_{5A} gene was localized on chromosome 5, at position 5B, whereas its human counterpart was found on chromosome 7, at position 7q36 [Matthes et al., 1993]. Because no DNA sequence polymorphism has been reported so far for the human 5-HT_{5A} receptor gene locus, no information on genetic order and distance of the 5-HT_{5A} gene in the context of other markers and genes on chromosome 7 is available.

In the present study we report results from a systematic mutation scan of the human serotonin 5A receptor gene in 46 unrelated healthy control individuals. The identification of a frequent nucleotide substitution in the 5′ untranslated region (−19G/C) was utilized for fine-scale linkage mapping of the 5-HT_{5A} receptor gene.

MATERIAL AND METHODS

Proband samples. EDTA anticoagulated venous blood samples were collected from 46 unrelated healthy German individuals. Leukocyte DNA was isolated by salting out with saturated NaCl solution [Miller et al., 1988]. DNA from CEPH (Centre d'Etude du Polymorphisme Humaine, Paris) families K1329, K1331, K1333, K1340,

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K1345, K1349, and 884 comprising 120 individuals was used for linkage mapping.

Polymerase chain reaction (PCR) amplification and analysis. Seven sets of primers were chosen to produce 7 overlapping fragments encompassing the whole coding region and the exon-intron boundaries of the human 5-HT_{5A} gene (Table 1). Fragment sizes ranged from 177 bp - 245 bp. Standard PCR was carried out in a 25 μl volume containing 80 ng genomic DNA, 10 pmol of each primer, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μM of each dNTP, and 0.5 U Taq DNA polymerase (Life Technologies). For amplification of fragment 5HT5A.3 10% of glycerol and 5% of formamide were added to the reaction mixture. Samples were processed in a GeneAmp® PCR System 9600 (Perkin-Elmer Cetus). After an initial 5 min denaturation at 94°C, 35 temperature cycles were carried out consisting of 20 sec at 94°C, 20 sec at 57°C, and 20 sec at 72°C, followed by a final extension step of 10 min at 72°C. After amplification, the PCR products were screened by SSCA as described below. Four μl of the PCR product were mixed with 6 μl of formamide containing 0.0125% bromophenol blue and 0.75% Ficoll 400 in 1×TBE and denatured for 5min at 96°C. Samples were subsequently chilled on ice and then loaded on a 10% polyacrylamide (PAA) gel (acrylamide:bisacrylamide=49:1; 110mm × 120mm × 1.0mm, Multigel-Long/Biometra) containing 0.5×TBE. Gels were allowed to run for 16-18 h at 6 V/cm at room temperature and 7 V/cm at +4°C, respectively. Bands were visualized by silver-staining [Budowle et al., 1991].

PCR products from heterozygous individuals were cloned into pUC 18 SmaI/BAP vector (Pharmacia). Single colonies were lysated in 10 μl TE buffer by boiling for 10 min. The lysates were used as template for PCR with insert specific primer pairs. SSCA of PCR products allowed the identification of clones containing different alleles. From selected colonies a hemibiotinylated PCR product was generated using one biotinylated vector primer and one normal vector primer. The PCR product was incubated with streptavidine Dynabeads (Dynal) and magnetic beads were collected with a magnetic concentrator. After washing and denaturing both strands of DNA were sequenced by the dideoxy nucleotide chain termination method [Sanger et al., 1977] using Sequenase Version 2.0 Kit (U.S. Biochemicals).

To allow a rapid genotyping PCR-based RFLP-assays were developed. After amplification of genomic DNA with primer pairs 5HT5A.1MF (5′-CCCCTTCTGCAAGTACTCCA-3′) / 5HT5A.1MR (5′-GGAAGGTGCGTACACGGA-3′) and 5HT5A.6F / 5HT5A.6R (sequences see Table 1), respectively, 5 μl of the PCR product were digested with either 7.5 U of restriction enzyme *BsaJ*I, 5 U *Aci*I, 7.5 U *Hae*III, 10 U *Bsr*I, 10 U *Nla*III (New England BioLabs) according to the manufacturer's recommendations (Table 2, 3). The digested products were separated on a 15% PAA gel (acrylamide:bisacrylamide=49:1) containing 1×TBE at 15 V/cm. Restriction profiles were visualized by silver staining [Budowle et al., 1991].

Linkage analysis. Two-point linkage analysis was performed against all chromosome 7 markers from the CHLC (Cooperative Human Linkage Center) version 3 genotype resource [Buetow et al., 1994] using the LODSCORE module of the FASTLINK version of LINKAGE [Cottingham et al., 1993; Lathrop & Lalouel, 1984].

RESULTS AND DISCUSSION

We used SSCA to screen the entire coding region of the human 5-HT_{5A} receptor gene for possible sequence variation in 46 unrelated healthy controls (92 alleles). Although PCR products of each sample were run under two different temperature conditions, we detected no mutation of likely functional significance. This suggests that the 5-HT_{5A} receptor displays no common genetically determined structural variants.

Although the present mutation screening detected no functionally relevant mutation, two nucleotide substitutions were identified in the 5′ untranslated region (−19G/C and −18C/T) and two silent substitutions were found in the coding region (12A/T and 789C/T). To allow rapid and specific testing of these variants we

TABLE 1

PCR Primers for Amplification of Overlapping Fragments Covering the Coding Region and the Exon–Intron Boundaries of the Human 5-HT_{5A} Receptor Gene

Primer	Primer sequences	Nucleotide position (5′-3′)*	PCR-product (bp)
Exon 1 (nt 1–741)			
5HT5A.1F	5′-CTCTTGAACACCCCTTCTGC-3′	−51→−32	236 bp
5HT5A.1R	5′-AGCACCAGCAGGTTCCAG-3′	185→168	
5HT51.2F	5′-TCGGTCTTCGGAGTGCTTAT-3′	109→128	245 bp
5HT5A.2R	5′-ATCCAAAGCTGGCACAGC-3′	353→3336	
5HT5A.3F	5′-GAGCCTGGTGCATGAGCT-3′	288→305	228 bp
5HT5A.3R	5′-ATGACAGCGGAGAGTGCC-3′	515→498	
5HT5A.4F	5′-GGAATACACGCTCCGCAC-3′	441→458	222 bp
5HT5A.4R	5′-CTATGCCGTAACCGAAGTTTG-3′	662→642	
5HT5A.5F	5′-CTACGCCGTGTTCTCCACC-3′	597→615	177 bp
5HT5A.5R	5′-CGGGCATTTTTAAGGATTGC-3′	+32→+13	
Exon 2 (nt 742–1047)			
5HT5A.6F	5′-AGTGTCCAGGCTCAGCCTAA-3′	−69→−50	230 bp
5HT5A.6R	5′-AAGGGGATCCAGGCTCAGCCTAA-3′	902→885	
5HT5A.7F	5′-TCATGGTGGGCATCCTCA-3′	854→871	240 bp
5HT5A.7R	5′-TCAATCCTGGTCCTCTCCC-3′	1093→1076	

* Nucleotide numbering was in according to Rees *et al.* (1994).

TABLE 2

Characterization and Detection of DNA Sequence Variants in the Human 5-HT_{5A} Receptor Gene

Variant	Primer pair	PCR-product (bp)	Restriction enzyme	Allele	Fragment sizes (bp)	Allele frequency
−19G/C	5HT5A.1MF	255	*Bsa*JI	−19G	145 + 92 + 18	0.63
	5HT5A.1MR			−19C	145 + 110	0.37
−18C/T	5HT5A.1MF	255	see Table 3	−18C	see Table 3	0.92
	5HT5A.1MR			−18T		0.08
12A/T	5HT5A.1MF	255	*Bsr*I	12A	204 + 51	0.34
	5HT5A.1MR			12T	255	0.66
789C/T	5HT5A.6F	230	*Nla*III	789C	186 + 44	0.99
	5HT5A.6R			789T	118 + 68 + 44	0.01

developed PCR-based restriction assays (Table 2, 3). Since all enzymes recognizing the −18C/T variant also included the polymorphic −19 site in their recognition sequence we derived the −18 genotypes from a combination of three independent restriction assays (Table 3). This also allowed us to determine exact haplotypes for the −19G/C and −18C/T variants. Allele frequencies obtained in the 46 probands are given in Table 2. There was a highly significant linkage disequilibrium between −19G/C and −18C/T ($P<0.001$) and −19G/C and 12A/T ($P<0.00005$), respectively. Estimated frequencies of haplotypes for the three variants were −19G −18C 12T (0.29), −19G −18C 12A (0.34), −19C −18C 12T (0.29), and −19C −18T 12T (0.08).

We used the highly polymorphic nature of the −19G/C variant to perform fine scale linkage mapping of the 5-HT_{5A} gene. At a recombination fraction of 0.00, lod-scores >3.0 were obtained for markers D7S61 (3.01), D7S396 (4.51), and D7S1807 (4.21) located on chromosome 7q34-q36. The human locus for holoprosencephaly type III (HPE3) has been mapped to 7q36 [Muenke et al., 1994] and among the few genes mapped to this chromosomal region the 5-HT_{5A} receptor gene was previously considered a good candidate for this disorder [Matthes et al., 1993]. However, D7S396 is in >10 cM genetic distance to marker D7S22, which showed the strongest support for linkage with no recombination in a series of HPE3 pedigrees [Muenke et al., 1994].

Since the 5-HT_{5A} receptor belongs to the group of receptors mediating the central effects of the neurotransmitter 5-HT, it has to be considered a candidate gene for neuropsychiatric disorders where dysregulations of serotonergic pathways have been implicated, such as anxiety, affective disorder, schizophrenia, alcoholism, migraine, aggression, and suicidal behavior. The frequent variants identified in our study could therefore be valuable for studying genetic linkage of the 5-HT_{5A} receptor gene to these disorders. They may also be used for case-control association studies where linkage disequilibrium between the polymorphism and a nearby functionally relevant mutation is assumed.

TABLE 3

Detection of Haplotypes for −19G/C and −18C/T Variants in the Human 5-HT_{5A} Receptor Gene

Haplotype	*Bsa*JI	*Aci*I	*Hae*III	Observed (n=92)
−19G −18C	1	1	2	58
−19G −18T	1	2	2	0
−19C −18C	2	2	1	27
−19C −18T	2	2	2	7

Note. Fragment sizes (bp) for *Bsa*JI are 145 + 92 + 18 (allele 1) and 145 + 110 (allele 2), for *Aci*I 172 + 60 + 23 (allele 1) and 195 + 60 (allele 2), and for *Hae*III 231 + 24 (allele 1) and 255 (allele 2).

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