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Two Dinucleotide Repeat Polymorphisms (AC/TG and GT/CA) in the 5' Upstream Region of the Mouse Tryptophan Hydroxylase Gene

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Tryptophan hydroxylase (TPH), the rate-limiting enzyme in serotonin biosynthesis, is primarily expressed in serotonergic neurons of the raphe nuclei. Simple tandem repeat polymorphisms, typically one to four nucleotides long, are tandemly repeated several times and often characterized by many alleles. To identify the presence of polymorphic repeats, we sequenced the 5'-upstream region of the mouse TPH gene. For the detection of any allelic variants, polymerase chain reaction, nonisotopic single-strand conformation polymophism, and DNA sequencing analyses of the tandem repeat sequences were performed using genomic DNA extracted from 60 ICR mice. Two dinucleotide repeats, 5'-$(AC/TG)_{22}$-3' and 5'-$(GT/CA)_{17}$-3', were identified at approximately −5.7 kb and −3.4 kb upstream from the transcriptional initiation site of the mouse TPH gene, respectively. Minor allelic variants, 5'-$(AC/TG)_{21}$-3' and 5'-$(GT/CA)_{18}$-3', were observed in heterozygous pairs from 3 of 60 and 1 of 60 ICR mice, respectively. The identification of these microsatellites in the mouse TPH promoter raises the possibility that identical and/or other polymorphic sequences might exist in the upstream region of the human TPH gene.

Key Words: Mouse tryptophan hydroxylase (TPH), Microsatellite, Dinucleotide polymorphism, AC repeats, GT repeats

INTRODUCTION

Altered regulation of serotonin (5-HT) neurotransmission plays an important role in the pathophysiology of a variety of psychiatric disorders, such as affective disorders, schizophrenia, autism, anxiety disorders and sleep disorders (Comings, 1997). Tryptophan hydroxylase (TPH) catalyzes the first step of 5-HT biosynthesis in serotonergic neurons of the raphe nuclei and neuroendocrine cells of the pineal gland (Lovenberg et al, 1969; Joh et al, 1975).

Most psychiatric disorders have a genetic component, as suggested by twin, family, and adoption studies (Comings, 1997). Several groups have demonstrated the utility of simple repeated sequences as polymorphic markers (Hellevuo et al, 1997; Le et al, 1997; Nakayama et al, 1997). These microsatellites are more or less distributed throughout the human genome, and are commonly found within the noncoding parts of genes. Recently, microsatellite polymorphisms in candidate genes were studied for possible associations with psychiatric disorders. These genes include the tyrosine hydroxylase (TH) (Pérez de Castro et al, 1995), dopamine β-hydroxylase (DBH) (Wei et al, 1997), monoamine oxidase (MAO) A and B (Lim et al, 1995; Rubinsztein et al, 1996), tyrosinase (De Bruyn et al, 1994), and dopamine D2 receptor (DRD2) (Pérez de Castro et al, 1995). Polymorphism in the promoter region are also reported (Jongeneel et al, 1991; Braun et al, 1996; Heils et al, 1996; Ball et al, 1997). In addition, Lesch et al (1996)

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reported an association of anxiety-related traits with a polymorphism in the regulatory region of the 5-HT transporter gene. Huh et al (1994) demonstrated that a 6.1 kb flanking region of the mouse TPH gene directs the expression of the lacZ reporter gene in the raphe nuclei and the pineal gland. Recently, we also reported the transgenic mice carrying a construct consisting of a 6.1 kb upstream region of the mouse TPH promoter fused to the simian virus (SV) 40 T-antigen (Son et al, 1996).

We hypothesized that a microsatellite may exist in the mouse TPH upstream region. To identify a microsatellitic marker in the TPH promoter region, DNA sequence analysis, polymerase chain reaction (PCR) amplification, and nonisotopic single-strand conformation polymorphism (SSCP) for the mouse TPH gene were conducted. Here, we report two dinucleotide repeat polymorphisms (AC/TG and GT/CA) in the 5'-upstream region of the mouse TPH gene which is unknown.

METHODS

We performed sequencing analysis of the 5'-upstream region of the mouse TPH gene to detect the presence of repeat sequences with polymorphisms that have been implicated in genetic disorders. Genomic DNA was extracted from mice tails by a standard method. For establishment of a control for the number of dinucleotide repeats, DNA sequencing was done by the dideoxynucleotide chain termination procedure after subcloning the 6.1 kb fragment of the mouse TPH upstream region into a pBluescript SK vector. The sequences flanking the $(AC)_{22}$ and $(GT)_{17}$ repeat elements were used to design PCR primers. We used the primer sequences MAC-1 (sense 5'-GGAGATAGCCGTCACCATCCTTAGC-3') and MAC-2 (antisense 5'-TGATACTTAGAGAAGACATGGTTCT-3') for the AC repeats (Fig. 1A) and MGT-1 (sense 5'-GTCCTATCAGTTATTTGGCCTTCAT-3') and MGT-2 (antisense 5'-CTGGAGACCAAAGAATCCCCAAAGC-3') for the GT repeats (Fig. 1B). The PCR amplification was performed in a total volume of 40 μl containing 100 ng of genomic DNA, 10 pM of each primer, 250 μM of each dNTP, 1.5 mM $MgCl_2$, 40 mM KCl, 10 mM Tris-HCl (pH 8.3), 1 mM DTT, 0.5 μg/ml acetylated bovine serum albumin, and 2 U of *Taq* DNA polymerase. The conditions for the PCR included an initial denaturation at 94°C for 5 min, 30 cycles of 94°C for 30 sec, 53°C for 30 sec, and 72°C for 30 sec, and a final elongation at 72°C for 5 min.

To investigate whether these two tandem repeats show variations in length and how many polymorphic alleles exist in normal mouse population, we examined allelic variants of these repeats using PCR amplification and subsequent nonisotopic SSCP analyses of the genomic DNA from the 60 ICR mice (Chi et al, 1994). Twenty-thirty μl of the PCR products were directly mixed with 4 μl of alkaline solution (1 N NaOH and 10 mM EDTA) and 10 μl of denaturing loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol). The total volume was adjusted to 40 μl by adding double-distilled H_2O. After heating at 95°C for 5 min, each sample was rapidly chilled to 4°C and loaded onto a 10% nondenaturing polyacrylamide gel ($18 \times 22 \times 0.1$ cm) in $0.5 \times$TBE (45 mM Tris-borate, pH 8.3, and 2 mM EDTA) buffer. Each sample was run on a gel with 5% glycerol and a second gel without glycerol and each gel was run at 18°C and at 4°C using a buffer-jacketed gel apparatus (DGGE-II, Aladin Enterprises Inc., San Francisco, CA, U.S.A). Following a 3-to-5 hour run at 460 V, the gel was stained with ethidium bromide (1 mg/l in 0.5 x TBE) and photographed under UV light. All samples displaying different SSCP patterns were sequenced in both directions to confirm the findings.

A

GGAGATAGCC GTCACCATCC TTAGCACATA TTAATCAGAT GGGGGGAAAT
TCCATGTTCT TTTACATTTA AATGGGACAC CCATAAAACA GGTGACTCAG
ACTCTCTCCC CCCTACCCTG CACCGGAAAT CATATGTGCA TGTTTCCCAC
CTCCTATGCA ATAAAGCAGA GGAAGAA**ACA CACACACACA CACACACACA**
CACACACACA CACACACACA CTTGAAAAGA AATCAATTTT AAAACTTTAA
AAGAACCATG TCTTCTCTAA GTATCA

B

GTCCTATCAG TTATTTGGCC TTCATTTTAT GTATGTATTT ATGAGTATGT
ATAGGTATGT ATG**GTGTGTG TGTGTGTGTG TGTGTGTGTG TGTGTGT**AGG
AATGCACATA TCACAGAACA TATGAAGGGG ACAACCTGGG GTGCTCATGC
ACCCCCACTC CCCGTTTGAT GCAGAGTCTC TATGTGTTTG CTGCCAGGGT
AGCTGGCCAT GAGCTTTGGG GATTCTTTGG TCTCCAG

Fig. 1. Nucleotide sequences of AC (A) and GT (B) repeats and its flanking regions in the 5' upstream region of the mouse TPH gene. AC and GT repeats are indicated in *bold letters*, and the primers used for PCR amplification of the repeats are *underlined.* (GenBank accession numbers: U96811 and AF052201).

RESULTS

Two dinucleotide repeat sequences, 5'-$(AC/TG)_{22}$-3' (named MTPHAC) and 5'-$(GT/CA)_{17}$-3' (named MTPHGT), were identified to locate at approximately −5.7 kb and −3.4 kb upstream from the transcriptional initiation site of the TPH gene, respectively.

For SSCP analyses, the oligonucleotide primers were designed to generate optimal lengths of PCR products, 276 bp for 5'-$(AC/TG)_{22}$-3' (Fig. 1A) and 237 bp for 5'-$(GT/CA)_{17}$-3' (Fig. 1B). As shown in Fig. 2A, 3 out of 60 ICR mice were recognized to be heterozygous in length for the MTPHAC sequence. This minor polymorphic allele was determined as 5'-$(AC/TG)_{21}$-3' and designed as A2 to distinguish it from the predominant allele, A1. Based on this observation, the allelic frequencies in the ICR mice population are estimated as 97.50% for A1 and 2.50% for A2 in normal ICR mice population. The resulting heterozygosity (A1/A2) is calculated as 4.88%, as summarized in Table 1. A minor polymorphic allele of MTPHGT (B2) is also found in 1 of 60 ICR mice and characterized as 5'-$(GT/CA)_{18}$-3' (Fig. 2B). The allelic frequencies of B1 and B2 are estimated as 99.17% and 0.83%, respectively and the heterozygosity (B1/B2) is appeared as 1.65% (Table 1).

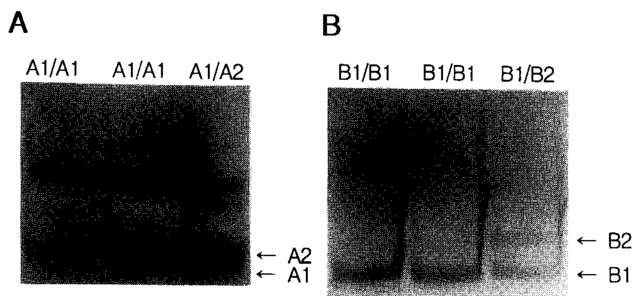

Fig. 2. Nonisotopic SSCP analysis of MTPHAC (A) and MTPHGT (B) polymorphisms in the 5'-upstream region of the mouse TPH gene. The two different genotypes of MTPHAC and MTPHGT polymorphisms are indicated at the top. Allele-specific bands are indicated by arrows.

DISCUSSION

In the present study, we found two dinucleotide repeat polymorphisms (AC/TG and GT/CA) located at approximately −5.7 kb and −3.4 kb upstream of the mouse TPH gene, which is not known yet.

Microsatellite polymorphisms have received considerable attention in mapping candidate genes for genetic diseases, since they are highly abundant, informative, and uniformly distributed genetic markers. Recently, psychiatric disorders such as schizophrenia and affective disorders have been studied with the aid of microsatellite polymorphisms used as genetic markers. Wei et al (1997) reported that a genotypic polymorphism containing (GT)n repeats in the human DBH gene is likely to be associated with biochemical variability of the catecholamine pathway in schizophrenia. A weak association for the TH and DRD2 genes with bipolar affective disorder in a Spanish sample were studied by Pérez de Castro et al

Table 1. Frequencies of alleles and genotypes for two microsatellites in 5-upstream region of the tryptophan hydroxylase gene in 60 ICR mice

Marker	Allele type	Repeated number (N)	Allelic frequency (%)	Genotype	Genotypic frequency (%)
MTPHAC					
(AC/TG)n	A1	22	97.50	A1/A1	95.06
	A2	21	2.50	A1/A2	4.88
				A2/A2	0.06
MTPHGT					
(GT/CA)n	B1	17	99.17	B1/B1	98.34
	B2	18	0.83	B1/B2	1.65
				B2/B2	0.01

MTPHAC: AC dinucleotide repeat microsatellite
MTPHGT: GT dinucleotide repeat microsatellite

(1995). Lim et al (1995) showed evidence for a genetic association of bipolar affective disorder with alleles on three MAO A markers, but not with alleles on MAO B. In addition, Nielsen et al (1992) reported two polymorphic sites, A218C and A779C, in the intron 7 of the human TPH gene. Association of suicidality and 5-hydroxyindoleacetic acid concentrations with a TPH polymorphism was also reported (Nielsen et al, 1994).

Huh et al (1994) demonstrated that a 5'-upstream region located at −6.1 kb in the mouse TPH gene directs the lacZ gene expression in the raphe nuclei and the pineal cells of transgenic mice. We also established the immortalized pineal cell line from the pineal tumors of transgenic mice by targeted tumorigenesis using 6.1 kb TPH promoter/SV-40 T-antigen (Son et al, 1996). Thus, our finding of two microsatellite markers containing the $(AC/TG)_{22}$ and $(GT/CA)_{17}$ repeat sequences in this region evokes the possibility that these two microsatellites may play a role in regulating the tissue-specific expression of the TPH gene.

Psychiatric disorders are thought to be polygenic inheritances. The dinucleotide, trinucleotide and other repeat polymorphisms may affect gene expression and thus maybe one source of polygenic effect (Comings, 1997). However, the mouse homologues of human genes containing an unstable repeat are notable for their shorter repeat length and less polymorphism (Sutherland & Richards, 1995). In the present study, we also found that both dinucleotide repeats only possessed two alleles in the ICR mouse strain. Abnormal microsatellite expansion of trinucleotide repeats appears so far to be a human-specific phenomenon. No mouse mutation has been linked with trinucleotide repeat expansion, and anticipation has not been reported for the mouse (Chambers & Abbott, 1996). Nevertheless, a transgenic animal model for some neuropsychiatric diseases would be valuable, particularly for processes by which simple tandem DNA repeats undergo abnormal expansion or contraction.

We are now characterizing the mouse TPH gene regulation by the dinucleotide microsatellites and screening microsatellites present in the human TPH upstream region.

In conclusion, the identification of two microsatellites in the upstream region of the mouse TPH gene raises the possibility that the identical and/or other microsatellite polymorphisms exist in the human TPH gene. With earlier reports of abnormal expansion of repeated sequences in the human genome and of corresponding genetic diseases, it is tantalizing to anticipate that these or other repeats could be highly polymorphic in humans and thus associated with serotonin-related disorders. Thus, it is possible that these polymorphic repeats of the TPH gene could be valuable markers for genetic analysis of neuropsychiatric disorders.

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