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Genetic Mapping of the Human Tryptophan Hydroxylase Gene on Chromosome 11, Using an Intronic Conformational Polymorphism

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Summary

The identification of polymorphic alleles at loci coding for functional genes is crucial for genetic association and linkage studies. Since the tryptophan hydroxylase (TPH) gene codes for the rate-limiting enzyme in the biosynthesis of the neurotransmitter serotonin, it would be advantageous to identify a polymorphism in this gene. By examining introns of the human TPH gene by PCR amplification and analysis by the single-strand conformational polymorphism (SSCP) technique, an SSCP was revealed with two alleles that occur with frequencies of .40 and .60 in unrelated Caucasians. DNAs from 24 informative CEPH families were typed for the TPH intron polymorphism and analyzed with respect to 10 linked markers on chromosome 11, between p13 and p15, with the result that TPH was placed between D11S151 and D11S134. This region contains loci for several important genes, including those for Beckwith-Wiedemann syndrome and tyrosine hydroxylase.

Introduction

Tryptophan hydroxylase (TPH) (E.C.1.14.16.4) catalyzes the biopterin-dependent monooxygenation of tryptophan to 5-hydroxytryptophan (Jequier et al. 1969; Kaufman 1987), which is subsequently decarboxylated to form the neurotransmitter serotonin. TPH expression is limited to a few specialized tissues in man, these being raphe neurons (Grahame-Smith 1964; Joh et al. 1975; Steinbusch 1981), pinealocytes (Lovenberg et al. 1967; Jequier et al. 1969), mast cells (Schindler 1958), mononuclear leukocytes (Finocchiaro et al. 1991), β-cells of the islets of Langerhans (Cetin 1992), and intestinal (Cooper and Melcer 1961; Gershon 1981) and pancreatic enterochromaffin cells (Cetin 1992). In the raphe neurons of the brain stem, TPH is the rate-limiting enzyme in the biosynthesis of the neurotransmitter serotonin (Cooper and Melcer 1961). Lower turnover of serotonin in brain, as indicated by 5-hydroxyindoleacetic acid in cerebrospinal fluid, has been associated with behaviors characterized by intolerance to delay. Therefore, a polymorphism of the TPH gene could serve, in linkage studies, as a marker for serotonergic behaviors.

Cloning and comparative mapping of the TPH gene has revealed interesting chromosome positional relationships between TPH and the two other members of the aromatic amino acid hydroxylase gene family (Ledley et al. 1985): tyrosine hydroxylase (TH) and phenylalanine hydroxylase (PAH). By means of somatic cell hybrids, the human TPH gene has been localized to the short arm of chromosome 11 (Ledley et al. 1987) and, by in situ hybridization, to the chromosome region 11p15.3→p14 (Craig et al. 1991). This places the TPH gene proximal to the TH gene, which maps to 11p15 (Craig et al. 1986). In addition, the TH gene is physically located 2,700 bases 5′ to the insulin gene (O'Malley and Rotwein 1988). However, in mice the TPH gene is not closely linked to TH, being found at the telomeric end of chromosome 7 near *Int-2* (Barton et al. 1988). The human PAH gene is not linked to the other aromatic amino acid hydroxylases

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but is located near the terminus of the long arm of chromosome 12 (Lidsky et al. 1985). Several other gene superfamilies are also split between chromosomes 11 and 12, apparently through a duplication and translocation of a region of chromosome 11 to chromosome 12 (Ledley et al. 1987). The TPH gene has been cloned from mouse (Stoll and Goldman 1991), and TPH cDNAs have been isolated from human (Boularand et al. 1990), mouse (Stoll et al. 1990), rat (Kim et al. 1991), and rabbit (Grenett et al. 1987). A 53% amino acid identity and a 48% amino acid sequence identity are shared with PAH and TH, respectively (Grenett et al. 1987; Ledley et al. 1987), and, as expected, the TPHs isolated from various mammalian species share a high degree of sequence identity.

In the present study, using information on the structure of the mouse TPH gene and the sequence of the human cDNA, we have identified a polymorphism in an intron of the human TPH gene. To confirm the location of this polymorphism within the TPH gene, we screened the CEPH Reference Family Panel (Dausset et al. 1990). A map was derived that genetically localizes the TPH gene.

Material and Methods

PCR

PCRs were performed on two regions of the human TPH gene that correspond to mouse TPH introns 5 and 6 and short stretches of adjacent exons. The "intron 5" region was amplified with the primers HTHSSCP1 and HTHSSCP3, which correspond to the human TPH sequence and amplify a region of approximately 240 bp. The "intron 7" region was amplified with the primers HTHSSCP4 and HTHSSCP5, which correspond to the human TPH sequence and amplify a region of approximately 885 bp. The primer sequences are HTHSSCP1, 5′-GCGGACTTGGCTATGAACTATAAAC-3′; HTHSSCP3, 5′-AATCTCCTCTTCAGTGAATTCAACC-3′; HTHSSCP4, 5′-TTCAGATCCCTTCTATACCCCAGAG-3′; and HTHSSCP5, 5′-GGACATGACCTAAGAGTTCATGGCA-3′. Amplification was performed with 100 ng DNA, 0.2 μM of each primer, 250 μM each of dCTP, dGTP, dTTP, and dATP, 250 μM spermidine, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 10 mM Tris pH 8.3, 7.5–15 μCi [a-^{32}P]dCTP, and 2 units of AmpliTaq (Perkin Elmer Cetus) in a volume of 25 μl (Saiki et al 1988). Samples were amplified for 30 cycles, each consisting of 1 min at 94°C, 2 min at 62°C (for intron 5 amplification) or 64°C (for intron 7 amplification), and 3 min at 72°C, followed by 7 min at 72°C.

Single-Strand Conformation Polymorphism (SSCP) Analysis

To generate smaller DNA fragments from the intron 7 amplification that would be more likely to show detectable variation by the SSCP procedure, 5 μl of this PCR mixture was digested with 10 units of *Hae*III (BRL) containing 1 × React Buffer 2 (BRL) in 30 μl for 2 h. In lanes 1 and 2 of figure 1*A*, 2 μl of digested DNA was diluted to 10 μl 5% glycerol, 0.05% bromophenol blue (BPB), and 0.05% xylene cylanol (XC), and 5 μl was used per lane. In lanes 3 and 4 of figure 1*A*, 2 μl of digested DNA was diluted with 8 μl 95% formamide, 10 mM NaOH, 0.05% BPB, and 0.05% XC and incubated at 100°C for 2 min, and 5 μl was used per lane. For the remaining SSCP analyses, 1 μl of digested DNA was diluted with 19 μl 95% formamide, 10 mM NaOH, 0.05% BPB, and 0.05% XC and incubated at 100°C for 2 min. Seven microliters of this denatured DNA was loaded per lane and electrophoresed on a 5% polyacrylamide gel by using a sequencing gel apparatus (Orita et al. 1989). Electrophoresis was carried out at room temperature for 17.5 h at 200 V. The gels were dried and autoradiographed at −70°C.

Linkage Analysis

Data were entered into the programs provided by CEPH, and files with chromosome 11 markers were prepared using SETPED. Two-point LOD score analyses were performed using LINKAGE (Lathrop et al. 1984) and MAPMAKER (Lander et al. 1987), and the two-point values were employed for multipoint analysis. A map of markers of known order from 11p was assembled (Junien and van Heyningen 1991), and TPH was located on the map by the TRY function of MAPMAKER. The odds of inverting the position of TPH and adjacent markers were also calculated. All primary data have been contributed to CEPH and are freely available (Dausset et al. 1990).

Results

To identify a polymorphism in the TPH gene, we performed SSCP analysis (Orita et al. 1989), this being a highly sensitive method for the detection of sequence variants (Dean et al. 1990). Since DNA sequences within introns are more likely to diverge than sequences in exons, we screened several TPH introns

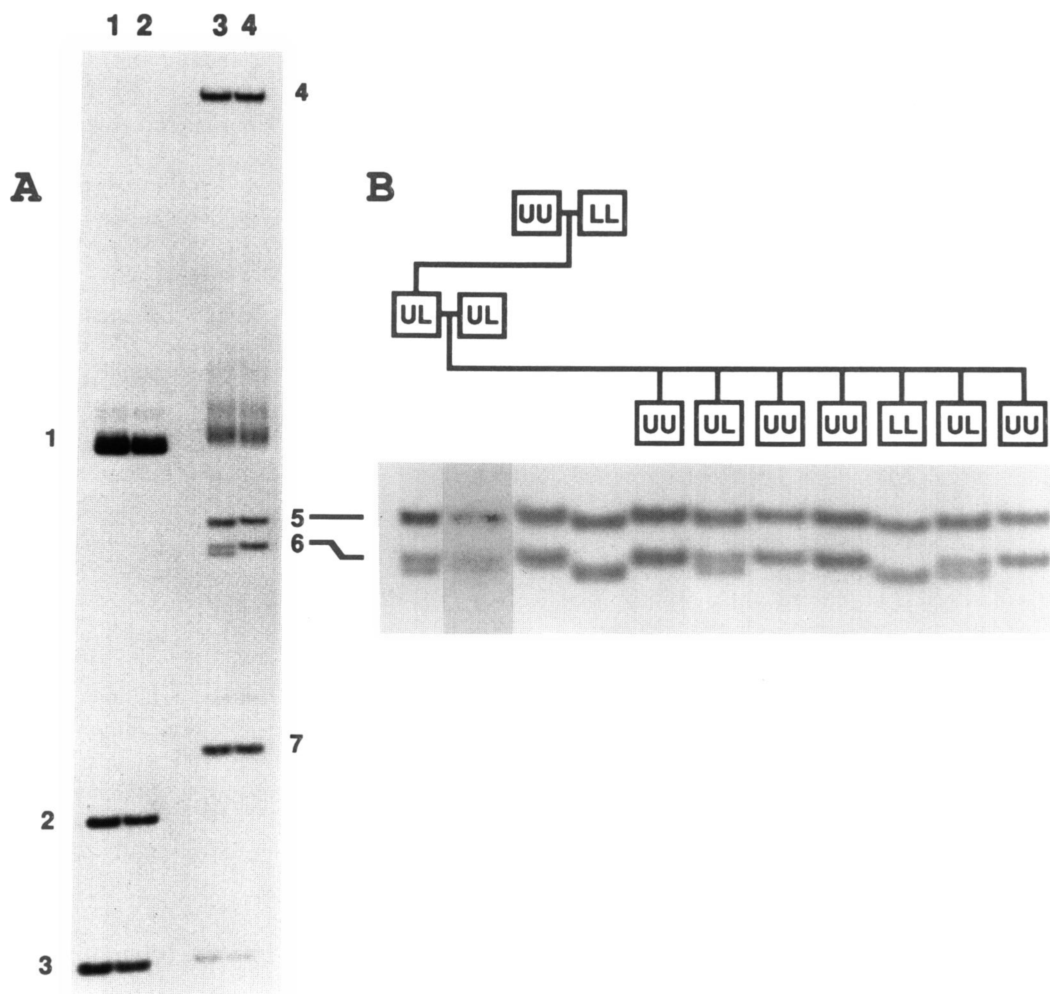

Figure 1 SSCP analysis of the human TPH intron 7. *A*, Autoradiogram of nondenatured DNA samples (lanes 1 and 2), versus denatured DNA samples (SSCP) (lanes 3 and 4), electrophoresed on a nondenaturing gel as described in Material and Methods. The bands labeled "1" and "4" correspond to the 510-bp fragment; the bands labeled "2," "5," and "6" correspond to the 220-bp fragment; and the bands labeled "3" and "7" correspond to the 155-bp fragment. Band 5 corresponds to the U allele, and band 6 corresponds to the L allele. *B*, Transmission of the TPH polymorphism in a CEPH family. SSCP analysis of the polymorphism was performed as described in Material and Methods. Shown is the SSCP autoradiogram derived from analysis of CEPH family 1423 (Dausset et al. 1990). The pedigree displayed at the top corresponds to the lanes below.

for polymorphisms. The human TPH cDNA sequence (Boularand et al. 1990) was aligned with the mouse genomic TPH sequence (Stoll and Goldman 1991) to identify putative intron locations in the human TPH genem, and oligonucleotides were synthesized to amplify human introns corresponding to mouse introns 5 and 7. These introns were chosen for analysis because they are the smallest introns in the mouse TPH gene. Although amplification of the intron 5 region identified an intron of approximately 170 bp, no polymorphism was detected (data not shown). When the region corresponding to intron 7 of the mouse TPH gene was amplified, a fragment of approximately 885 bp was amplified, demonstrating the presence of an intron of approximately 825 bp. This fragment also contained 27 bp of "exon 7" and 32 bp of "exon 8" sequences. When this relatively large, amplified DNA fragment was subjected to SSCP analysis, no polymorphism was detected in either denatured or native DNA (data not shown). To increase the probability of detecting a polymorphism, this amplified DNA fragment was digested with *Hae*III to yield fragments of 510,

Table 1

TPH: Linked Loci

Locus[a]	Probe	Enzyme[b]	Location[c]	Recombination Frequency with TPH		
				Male	Female	Sex Averaged (LOD Score)
INS	pINS-310	*Pvu*II	11p15.5-15.4	.15	.75	.37 (2.75)
HBB	JW102	*Sin*I	11p15.5-15.4	.00	.00	.00 (4.82)
PTH	pPTH-LF	*Pst*I	11pter-15.4	.04	.07	.05 (9.00)
CALCA	pCAL	*Taq*I	11p15.4	.01	.13	.05 (6.73)
D11S134 ...	CRI-L834	*Msp*I	11	.01	.26	.18 (1.54)
	CJ.5T1	*Pst*I	11	.09	.00	.05 (3.39)
TH	pTH-S8	*Hind*III	11p15.5	.12	.03	.09 (2.08)
D11S151 ...	p56H2.4	*Pst*I	11p13	.11	.09	.08 (6.01)
D11S324 ...	p60H1.4	*Taq*I	11p13	.13	.30	.19 (3.89)
D11S97	pMS51	*Taq*I	11q13	.27	.53	.40 (3.17)

[a] D11S151, D11S324, and D11S97 are anonymous DNA markers.
[b] Enzyme that detects the polymorphism.
[c] Known physical location of the marker on chromosome 11.

220, and 155 bp, in which conformational variation can be more sensitively detected. Although no polymorphism was detected with native DNA (fig. 1*A*, lanes 1 and 2), an SSCP polymorphism of the 220-bp fragment was observed by using the SSCP technique of running the denatured DNA on a native gel (fig. 1*A*, lanes 3 and 4). Two allelic variants were identified and labeled "U" and "L," as shown in figure 1. Allele frequencies were determined in 72 unrelated Caucasian individuals (CEPH parents), and the more common L allele had a frequency of .60.

The TPH alleles segregate in codominant Mendelian fashion, as can be seen in CEPH family 1423 in figure 1*B*. To genetically map the TPH gene to its chromosomal location, the polymorphism was typed in all 24 CEPH families that were informative. As cited above, TPH has been physically assigned by in situ hybridization to the chromosome region 11p15.3→p14 (Craig et al. 1991). To place the gene on the linkage map, two-point linkage analysis and multipoint linkage analysis were performed. Table 1 displays maximum LOD scores with respect to other chromosome 11 markers. As expected, TPH is tightly linked to several other markers in the telomeric region of the short arm of chromosome 11. Multipoint analyses with the closest markers place the TPH polymorphism centromeric to insulin (INS), hemoglobin-β

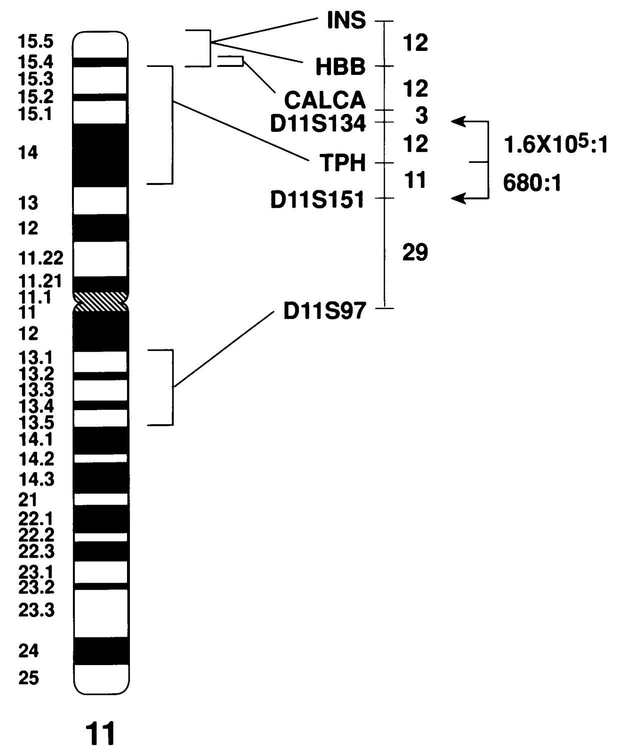

Figure 2 Genetic location of the human TPH gene. The location of the TPH gene is shown in relation to other mapped chromosome 11 loci. Odds against the placement of TPH in adjacent positions are shown.

(HBB), calcitonin α (CALCA), and D11S134 (fig. 2). Parathyroid hormone (PTH) and TH also lie within this region but could not be definitively ordered in relation to TPH. Although TPH and HBB show no recombination, multipoint analysis places TPH centromeric to HBB. This may reflect either a deficiency of informative meioses between the markers or errors in the data base. Taken together, these analyses place TPH between D11S134 and D11S151.

Discussion

The 11p15 region of human chromosome 11 is of interest because it contains three times as many known genes as the rest of 11p and has a higher recombination rate (Junien and van Heyningen 1991). Besides TPH, this region also contains the genes coding for insulin, TH, and β-globin (Moss et al. 1986). In the mouse genome, the TPH gene is localized to the proximal half of chromosome 7 (Stoll et al. 1990). Comparative mapping has shown that homology between the mouse chromosome 7 and human chromosome 11 includes at least 10 genes (Barton et al. 1988). Eight of these genes that are clustered in mouse chromosome 7 remain clustered in human chromosome 11, at region 11p15→p14.

In humans, several diseases of unknown etiology have been mapped to this region. Although a linkage of bipolar affective disorder to chromosomal region 11p15 (Egeland et al. 1987) has been greatly weakened by subsequent studies (reviewed in Ciaranello and Ciaranello 1991), a recent report (Pakstis et al. 1991) revealed that LOD scores at this region remain slightly positive, so that a linkage to a gene in this region cannot be ruled out. A low but positive LOD score may indicate genetic heterogeneity, partial penetrance, or the inclusion of nongenetic cases. The Beckwith-Wiedemann syndrome (BWS) has been mapped to 11p15.5 (Henry et al. 1989; Koufos et al. 1989; Ping et al. 1989). The BWS locus may contain, or be identical to, one of two genes believed to be involved in the development of Wilms tumor (Koufos et al. 1989; Ping et al. 1989).

This is the first demonstration of a polymorphism in the TPH gene. We have found that amplification and SSCP analysis of intronic DNA is an efficient technique for generating polymorphic markers at candidate genes. Relatively large sequences can be amplified and cut with restriction enzymes to provide fragments of shorter length that are suitable for SSCP analysis. By this technique, a moderately informative polymorphism can usually be discovered by analyzing only a few intronic regions and intron/exon boundaries. Furthermore, the intron locations and sequences, while useful, are not required in advance of using this technique, so that cDNA sequence data can be used directly, without the necessity of isolating genomic clones.

Because TPH is the rate-limiting enzyme in the biosynthesis of serotonin, and because serotonin is a major neurotransmitter involved in modifying behavior, this polymorphism should provide a valuable tool for studying the genetic linkage of TPH to various serotonergic behaviors and their disorders. The polymorphic fragment includes mostly intronic sequence; therefore it is unlikely that the variant itself would alter serotonin biosynthesis. However, it remains possible that a change in intronic sequence may alter pre-mRNA splicing of the intron, and it is, of course, highly probable that this variant will be tightly linked to mutations at the TPH locus that alter the activity, expression, or regulation of the TPH gene.

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