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Localization of the hydroxyindole-O-methyltransferase gene to the pseudoautosomal region: implications for mapping of psychiatric disorders

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ABSTRACT

The human hydroxyindole-O-methyltransferase (HIOMT) gene has been mapped to the X chromosome using cDNA probes from the 3′ and 5′ regions of a human cDNA clone. Southern blot analysis of a panel of human–rodent somatic cell hybrid DNAs revealed that the gene was localized to the short arm of chromosome X, and most probably the pseudoautosomal region of the human X (Xp22.3) and Y (Yp11.3) chromosomes. Several multiallelic restriction fragment length polymorphisms were detected at this locus allowing further localization of the gene by two-point and multipoint linkage analysis in the 40 CEPH families. These results confirmed the pseudoautosomal localization of the HIOMT gene and allowed ordering of the gene in close proximity to DXYS17 at a position about 600–800 kb from the pseudoautosomal boundary and about 1800 to 2000 kb from the telomere. It will be possible to readily identify or exclude the involvement of this gene in genetic diseases by linkage analysis of the disease locus with the high frequency multiallelic polymorphisms at this locus.

INTRODUCTION

The hormone melatonin, which is secreted from the pineal gland, exhibits a dramatic circadian rhythm in both synthesis and secretion (1, 2, 3). Hydroxyindole-O-methyltransferase (S-adenosyl-L-methionine:N-acetylserotonin O-methyltransferase, EC 2.1.1.4) catalyzes the final reaction in the synthesis of melatonin (4) and is exclusively localized to the pineal gland and retina (5, 6, 7). Although HIOMT activity increases only marginally at night, nerve activity plays a role in controlling this enzyme (8).

Human HIOMT is of interest due to its limited distribution and neural control of enzyme activity as well as the potential involvement of this enzyme in psychiatric disorders. Although there is no current evidence to support this hypothesis, it is interesting that individuals with bipolar illness or seasonal affective disorder (SAD) exhibit an altered sensitivity to light and/or a phase shift in circadian rhythms when compared to control individuals (9, 10, 11, 12). It should be noted that our laboratory (Donahue *et al.*, unpublished) and another group (13) found no difference in post-mortem HIOMT levels in the pineal glands from controls and schizophrenics.

Bovine HIOMT has been cloned and enzyme activity expressed (14, 15). More recently, a clone with sequence homology to bovine HIOMT has been isolated from a human pineal cDNA library (16), and this cDNA has been used as a probe to determine the chromosomal localization of the human gene. Polymorphisms have been found at the HIOMT locus due to the presence of multiallelic VNTRs (i.e. variable number of tandem repeats). Genetic linkage analysis in CEPH families using these polymorphic markers is reported herein, and the results confirm the chromosomal localization of HIOMT and permit ordering the gene with respect to other loci within the pseudoautosomal region (PAR) of the sex chromosomes. The presence of these polymorphisms should permit the evaluation of this gene as a possible candidate locus in various psychiatric disorders.

RESULTS

HIOMT mapping

The HIOMT gene was chromosomally localized by Southern analysis of a panel of human–rodent hybrid cell DNAs (17, 18) with a ^{32}P-labeled HIOMT 3′cDNA probe. This probe detected a 6.6 kb hybridizing band in *Eco*RI digests of human DNA as well as a smaller polymorphic band. No cross-hybridization with rodent sequences was detected at high stringency (i.e. allowing less than 10% divergence). Analysis of a group of 93 independent hybrids and subclones allowed the gene to be localized unambiguously to the human X chromosome, and it segregated discordantly ($\geq$34%) with all other human chromosomes. The gene was further regionally localized by examination of hybrids containing a well-characterized X;14 (q13;q32) translocation (18). Twelve independent hybrids retained only one of the two translocation chromosomes, and the HIOMT gene always segregated with Xpter-q13. The HIOMT gene could be further assigned to Xp since it was not detected in a hybrid containing only the human X chromosome long arm translocated to a mouse chromosome (not shown). Finally, the human HIOMT gene is absent (not shown) in a human–mouse hybrid line A9/HRBC2 (19) which contains only human X and a portion of chromosome 2p (20) but the X chromosome in this hybrid has been shown (21) to lack the pseudoautosomal region (Xp22.3-pter). These combined results strongly suggest that the HIOMT gene is present in the pseudoautosomal region of the human X (Xp22.3) and Y

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(Yp11.3) chromosomes, and all results were confirmed by hybridization of the same blots with a 5′ cDNA probe.

Analysis for RFLPs

DNAs isolated from peripheral leukocytes of 39 unrelated individuals were digested separately with *Eco*RI, *Hin*dIII, *Bam*HI, *Pst*I, *Sst*I, *Msp*I, *Taq*I, *Bgl*II, *Xba*I, *Pvu*II, *Kpn*I, and *Eco*RV. Southern blots of these restriction digests were hybridized with the 3′ cDNA probe. A polymorphism resulting from the presence of a variable number of tandem repeats (VNTR) was detected vith this probe in most of the restriction digests, and *Sst*I, *Pst*I, and *Bgl*II digests were especially useful for this analysis (Figure 1). A VNTR vas also detected in *Msp*I digests, but additional complexity was generated by the probable presence of a frequent single-site polymorphism at one of the flanking *Msp*I sites (Figure 1). Most males and females were heterozygous for these VNTR alleles. The fact that the HIOMT gene can be mapped to chromosome Xp and that males contain two alleles of HIOMT indicates that the gene is present on a DNA sequence shared by both sex chromosomes, and this is consistent with its location on the pseudoautosomal region of both the X and Y chromosomes.

The same DNAs were examined with a 5′ cDNA probe and two additional VNTRs were found (not shown). The VNTR with the smaller (i.e. 1.8−2.2 kb) alleles could be used for linkage analysis. All RFLPs are summarized in Table 1.

The results of hybridization with 5′ and 3′ cDNA probes were used to estimate the size of the human HIOMT gene. Based upon the total sizes of overlapping and unique bands specifically detected in *Eco*RI, *Pvu*II, *Bam*HI, etc. digests with both the 5′ and 3′ cDNA probes, the length of the HIOMT gene does not exceed about 30 kb. It also was estimated that the intragenic distance between the 5′ and 3′ cDNA probes does not exceed 6.1−6.4 kb since bands of this size hybridized with both probes in *Pvu*II and *Eco*RI digests (Table 1). Efforts to amplify this region by PCR (22) using genomic DNA as template and flanking oligomeric primers from coding sequences were unsuccessful.

Genetic linkage analysis

At least eight different alleles could be distinguished in both *Pst*I and *Msp*I digests with the human HIOMT 3′ cDNA probe. The pattern of inheritance in one family is shown (Figure 2), and seven different alleles were observed in these four grandparents. Among nine offspring in this family, one X/Y recombinant was found between HIOMT and the sex-determining region (SRY) which is located within 35 kb of the pseudoautosomal boundary (23). Similar examination of all CEPH families for recombinants between HIOMT and sex determination for meioses in males disclosed $\theta = 0.106$ and $Z = 42.0$. This indicates that the HIOMT locus is considerably closer to the pseudoautosomal boundary than to the telomere where a recombination fraction of about 0.5 would be expected.

All parents in the CEPH pedigrees were typed with the HIOMT 3′ cDNA probe and 38 of the 40 families were informative with each enzyme. There were no recombinants between these two probe−enzyme combinations in 382 informative meioses (i.e. $Z = 115.1$ at $\theta = 0.00$). Hence, these two sets of results were combined as a haplotype, and the resulting heterozygosity and PIC (polymorphic information content) were each 0.900. This locus was tested pairwise for linkage to markers from other laboratories in the CEPH database version 5 (Table 2). Linkage was demonstrated to all markers in the pseudoautosomal region, and particularly close linkage was found to DXYS17. As expected, the recombination fractions between HIOMT and each locus was about ten-fold greater in male meioses than in females (24, 25, 26) since a single obligatory recombination event occurs within the PAR at meiosis in males.

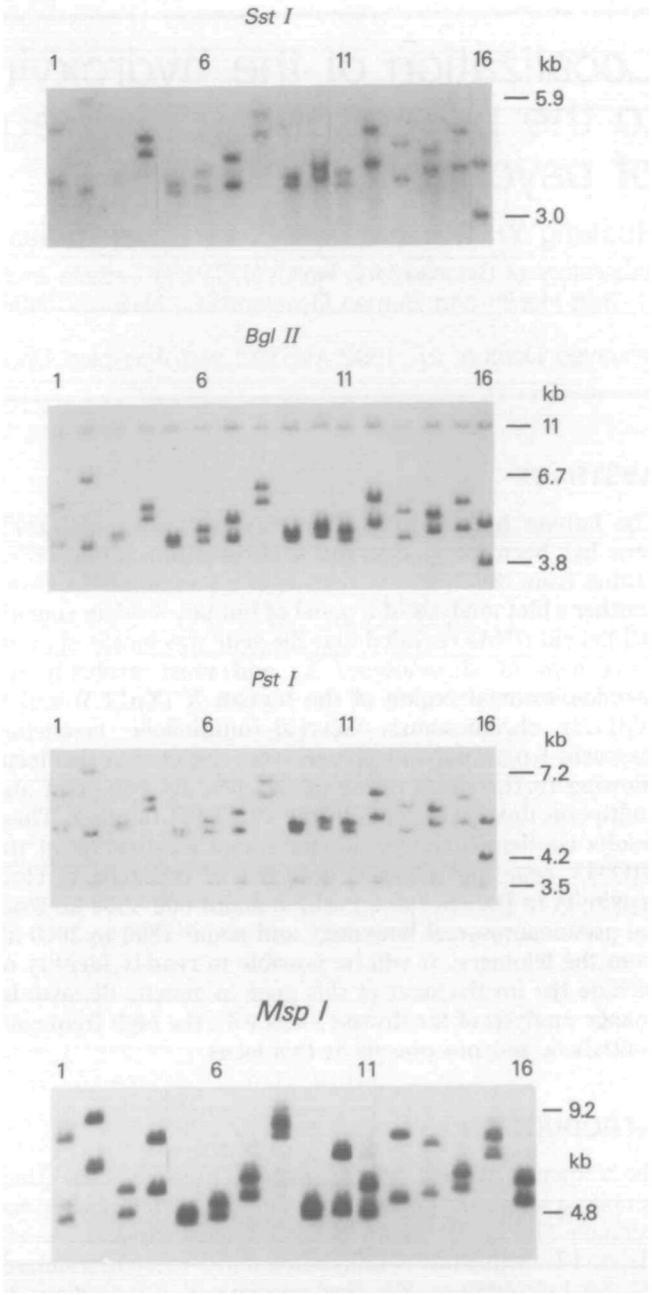

Figure 1. Detection of RFLPs in the 3′ flank of the HIOMT gene using a 3′ cDNA probe. Each lane contains DNA isolated from an unrelated individual (i.e. both males and females are present), and the order of these DNAs is identical in all four digests. A constant band of 11 kb and 3.5 kb was observed in *Bgl*II and *Pst*I digests, respectively. Allelic bands of about 3.0 to 5.9 kb, 3.8 to 6.7 kb, and 4.2 to 7.2 kb were found in the *Sst*I, *Bgl*II, and *Pst*I digests, respectively. The correspondence in size between allelic bands in all three digests can be seen, representing the presence of restriction sites flanking a variable number of tandem repeats (VNTR) in this region. The *Msp*I alleles vary in size from about 4.8 kb to 9.2 kb. Some of these alleles correspond closely in size to those observed in the other three digests (i.e. lanes 5 and 6), whereas one or both of the alleles are considerably larger in *Msp*I digests in other cases (i.e. lanes 2, 3, and 4). This probably reflects the presence of a high frequency single site polymorphism at one of the *Msp*I sites flanking the VNTR (i.e. two superimposed polymorphisms).

Table 1. RFLPs detected by HIOMT cDNA probes[a]

Enzyme	Pattern	Constant Bands (kb)	Alleles Size	Freq
A. 3′ cDNA Probe[a]				
KpnI	2 allele	–	A1 34	0.45
			A2 23	0.55
PstI	VNTR[b]	3.5	4.2–7.2	
BglII	VNTR[b]	11	3.8–6.7	
SacI	VNTR[b]	1.1	3.0–5.9	
PvuII	VNTR[b]	6.1[f]	5.6–8.8	
		4.8 weak		
EcoRI	VNTR[b]	6.4[f]	3.7–6.7	
HindIII	VNTR[b]	–	10–12.6	
TaqI	VNTR[b]	0.9	2.2–5.5	
		2.1		
BamHI	VNTR[b]		21–24[f]	
MspI	VNTR[c]	0.9	4.8–9.2	
B. 5′ cDNA Probe[a]				
PstI	VNTR[d]	3.9	1.8–2.2	
		5.8		
PstI	VNTR[e]	–	7.0–7.8	

[a]Probes described in Methods.
[b]Same VNTR detected in all these restriction digests and at least 8 different alleles were present in each digest. In each case, both restriction sites appear to flank the VNTR, and the differences in band sizes reflect a differing length of DNA flanking the VNTR. Allele frequencies were not determined but in 80 CEPH parents heterozygosity = 0.70 and PIC = 0.70
[c]MspI also detects a VNTR but the pattern of alleles differs from that observed with the other enzymes. Heterozygosity = 0.863 and PIC = 0.850 in 80 CEPH parents. When the results from b and c were combined as haplotypes, the heterozygosity and PIC were each increased to 0.900.
[d]Different VNTR with relatively small differences in the sizes of alleles. Heterozygosity = 0.725 and PIC = 0.608 in 51 CEPH parents.
[e]Third VNTR which is not very useful due to the small difference in size of the large allelic fragments.
[f]Same band also detected with 5′ cDNA probe.

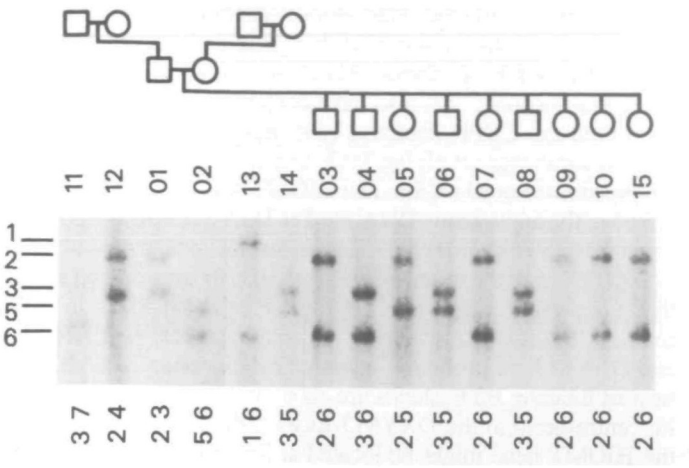

Figure 2. Pseudoautosomal inheritance of *Msp*I RFLP at the HIOMT locus in three-generation CEPH family 1416. The pedigree of the family is shown (squares, males; circles, females) above the autoradiogram, and the alleles in each lane are indicated below the lanes. The father (individual 01) inherits allele 3 on his Y chromosome from his father (individual 11) and allele 2 on his X chromosome from his mother (individual 12). Three of his sons (individuals 04, 06, and 08) inherit the Y chromosome with allele 3 whereas the fourth son (individual 03) inherits allele 2 from his father, and this represents an X;Y pseudoautosomal recombination event. All five daughters inherit nonrecombinant allele 2 from the father's X chromosome.

Table 2. Two-point LOD scores for HIOMT with other pseudoautosomal loci

Locus[a]	θm,θf[b]	Z(θf,θf)[b]	θm+f[c]	Z(θm+f)[c]
DXYS17	0.017,0.016	27.4	0.017	27.4
DXYS15	0.204,0.018	17.0	0.117	14.4
DXYS28	0.315,0.026	22.1	0.189	15.8
D17S2	0.357,0.020	13.6	0.197	8.6
DXYS60	0.280,0.000	5.4	0.164	3.9
DXYS20	0.389,0.062	33.4	0.217	22.3
SRy[d]	0.106	42.0		

[a]See Methods for description of loci and probe–enzyme combinations involved.
[b]The most likely recombination fractions and LOD scores under the assumption of independent rates of recombination in males (θm) and females (θf).
[c]Most likely recombination fractions and LOD scores assuming no difference in recombination rates in the two sexes.
[d]Segregation of paternal HIOMT alleles with sex phenotype in offspring.

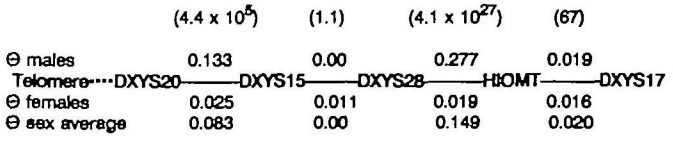

Figure 3. Multipoint linkage analysis of loci in the pseudoautosomal region of the X and Y chromosomes. The most likely order is shown. The most likely sex-specific recombination fraction between each locus is shown above (males) and below (females) the lines separating loci, and sex average values represent most likely recombination fractions assuming no sex difference. The odds against reversing the order of adjacent loci are shown in parentheses above the figure. DXYS15 and DXYS28 cannot be ordered and the odds for ordering HIOMT and DXYS17 are not significant. All other possible orders are excluded.

Multipoint linkage analysis was also performed with four loci from this region to better order HIOMT in this cluster of markers using the Linkage program CILink. All possible orders were considered and the most likely order is shown in Figure 3. The loci DXYS15 and DXYS28 could not be ordered since there were no recombinants between these two loci in 35 informative meioses. The results indicate that HIOMT is located in close proximity to DXYS17 but there are only two recombinants between these loci in about 100 informative meioses. Hence, the odds for ordering HIOMT and DXYS17 (i.e. 67 to 1) are not significant. Excluding the ambiguity in the orders of DXYS15 and DXYS28 as well as HIOMT and DXYS17, all other possible orders could be excluded with odds of at least 4.4×10^5. The order and spacing of loci in Figure 5 is generally consistent with previously published linkage maps (24, 25, 26, 27) and physical maps (28, 29) of the PAR. This is expected since the same database has been used. It is known from physical mapping (28) that DXYS15 is located 200–250 kb proximal to DXYS28 with respect to the telomere. The loci in Figure 5 span about 2000 kb including the distal 80 percent of the pseudoautosomal region. DXYS20 contains a VNTR and the locus spans 40–60 kb, and it is located only 20–25 kb from the telomere (28).

DISCUSSION

A cloned human HIOMT cDNA has been used as a probe to localize this gene to the pseudoautosomal region (PAR) of human chromosomes X and Y by Southern analysis of human–rodent somatic cell hybrids and by genetic linkage analysis in the 40 CEPH families. Both genetic linkage maps (24, 25, 26, 27) and physical maps (28, 29) of the entire pseudoautosomal region have

been reported from other laboratories. A complete physical map of the human Y chromosome involving sequence tagged sites (30) and contiguous YAC clones (31) as well as an updated map of the X chromosome (32) also have been published recently. We have used the CEPH database containing published data from other laboratories involving PAR loci to order HIOMT within the pseudoautosomal region, and HIOMT was found to be closely linked to the anonymous DNA marker DXYS17 which is located about 600–800 kb from the pseudoautosomal boundary and 1800 to 2000 kb from the telomere. Petit *et al.* (28) have demonstrated that a one centimorgan interval represents about 55 kb in male meiosis. Based on this value, we would estimate that HIOMT and DXYS17 are probably separated by about only 100 kb. The nearest flanking HTF islands are about 350 kb telomeric and 300 kb centromeric to the DXYS17 locus (28). The possibility that the HIOMT gene might be located at the more distal site about 1550 kb from the telomere is attractive but a somewhat higher recombination frequency between HIOMT and DXYS17 would be anticipated under these circumstances.

Only two other functional genes have previously been assigned to the PAR including MIC2 which encodes a cell surface antigen of unknown function (27, 33) and the granulocyte–macrophage colony stimulating factor receptor alpha subunit (CSF2RA) (34). The former is located near the PAR boundary (i.e. 2600 kb from the telomere) whereas CSF2RA is located in a region between 1100 and 1300 kb from the telomere (35).

Although HIOMT has not been implicated in any human disease, it probably can be considered a candidate gene in neuropsychiatric diseases based upon its chromosomal location and its tissue specific expression in brain. It has been proposed that a gene for bipolar affective illness and schizophrenia (36) and a cerebral dominance gene (37) may be located in the PAR. Evidence for this proposal was the demonstration of concordance by sex for psychosis in sibling pairs or dizygotic twins when there was paternal inheritance (36, 37). This would require that the locus be linked to the sex determining region (i.e. not too distant from the pseudoautosomal boundary). In a study of 35 sib pairs with schizophrenia or schizo-affective disorder, significant evidence was found for nonrandom assortment of alleles at the DXYS14 locus with the disorder (37, 38). This contrasts with the first observation since DXYS14 is located within 10–20 kb of the telomere in the PAR (39) and accordingly is unlinked to sex. Based upon the location of HIOMT within the PAR, it can be predicted that a reasonable concordance by sex in paternal inheritance would be expected for a disorder involving this gene. Most importantly, the highly informative polymorphisms at this locus should allow direct testing of HIOMT as a candidate gene in neuropsychiatric diseases by genetic linkage analysis.

It is interesting that hypervariable minisatellite sequences are found in both the 3′ and 5′ regions of the HIOMT gene. There is a remarkably high incidence of very polymorphic VNTR loci among probes previously isolated from the PAR. These include DXYS14, DXYS15, and DXYS17 (40), DXYS20 and DXYS28 (26), and CSF2RA (34). It is not clear whether the pseudoautosomal region is uniquely enriched in these hypervariable minisatellite sequences or whether their presence simply reflects the general clustering of these sequences in proterminal (i.e. near telomeric) regions (41). Instability of cloned sequences from the PAR in YAC vectors has been reported (31). The high frequency of minisatellite sequences and other tandemly repeated sequences within the PAR may be responsible for this instability. Of particular interest is the fact that the region containing the HIOMT gene is apparently unstable in YAC vectors and the gene has not been isolated from a YAC library.

MATERIALS AND METHODS

Preparation of probes

Probes were prepared from restriction digests of a 1250 bp full-length human HIOMT cDNA cloned in pBluescript vector (16) (the nucleotide sequence has been submitted to the GenBank™/EMBL Data Bank with accession number M83779). A 494 bp *Bam*HI/*Hin*cII fragment from the 5′ end and a 482 bp *Mae*II/*Dde*I 3′ cDNA fragment were isolated from 1% NuSieve GTG gel, melted, and labeled with α-^{32}P dCTP by random hexanucleotide primed DNA synthesis (42) to specific activity of greater than 10^9 cpm/μg. Alternatively, 570 bp 5′ cDNA and 520 bp 3′ cDNA probes were prepared by PCR amplification (22) using specific internal primers and universal primers in the flanking vector sequences, and these fragments were also labeled by the random primer method. The internal oligonucleotide primers for cDNA PCR amplification of 5′ and 3′ cDNA sequences were 5′ CTAAGGAATGCATGTCTCTG and 5′ GTTGACGC-TCCAGACCTCCT, respectively. Identical results were obtained using probes prepared from restriction digests or from PCR amplification of the corresponding cDNA fragments.

DNA restriction fragment length polymorphisms (RFLPS)

DNAs were isolated from the peripheral leukocytes of 39 unrelated normal individuals, digested with restriction endonucleases, size-fractionated by gel electrophoresis, transferred to nylon membranes, and hybridized with the 5′- and 3′-cDNA probes as described (43).

Linkage analysis

Both *Pst*I and *Msp*I digests of DNAs from all 40 CEPH families (44) were typed with a ^{32}P-labeled HIOMT 3′ cDNA probe. Filters were washed under conditions of high stringency (43) and autoradiographed for 1–14 days with intensifying screens. Two-point and multipoint linkage analyses were performed using LINKAGE v 5.10 for PC compatibles (45). All relevant markers in the CEPH database v. 5 were used in these analyses. The probe–enzyme combinations for loci were: DXY17 uses probe 601 (24) with data for enzymes *Eco*RI and *Taq*I combined as haplotype; DXYS15 uses probe 113D (21) with enzyme *Taq*I; DXYS 28 uses probe pDP411a (26) with data for enzymes *Eco*RI and *Taq*I combined as haplotypes; D17S2 uses probes L1.31 (46) with *Bgl*II; DXYS60 uses probe U7A (47) with *Eco*RI; DXYS20 uses probe pDP230 (26) with enzymes *Hin*dIII, *Msp*I, and *Taq*I and the data was combined as haplotypes. Similar data for DXYS15 was obtained in two different laboratories and discrepancies between labs were found for two individuals (133206 and 133212); these two individuals were omitted from the analyses.

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Note added in proof

Since submission of this manuscript, the official name acetylserotonin methyltransferase (ASMT) has been assigned to HIOMT by Dr Phyllis McAlpine of the HGM Nomenclature Committee.