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Tryptophan hydroxylase and catechol-O-methyltransferase gene polymorphisms: relationships to monoamine metabolite concentrations in CSF of healthy volunteers

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Abstract Concentrations of monoamine metabolites (MM) in lumbar cerebrospinal fluid (CSF) have been used extensively as indirect estimates of monoamine turnover in the brain. We investigated possible relationships between DNA polymorphisms in the tryptophan hydroxylase (TPH) and catechol-O-methyltransferase (COMT) genes and CSF concentrations of 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylglycol (MHPG) in healthy volunteers (n = 66). Lower CSF 5-HIAA levels were found in men with the TPH U allele (p = 0.005), but not in women. A similar but less significant pattern was observed for CSF HVA. No relationship was found between the TPH polymorphism and CSF MHPG. COMT genotypes did not relate significantly to MM concentrations. The results suggest that TPH genotypes participate differentially in the regulation of serotonin turnover rate under presumed steady state in the central nervous system of men. Due to the uncertain functional relevance of the DNA polymorphism investigated and the many calculations performed, the results should be interpreted with caution until replicated.

Key words Tryptophan hydroxylase gene · Catechol-O-methyltransferase gene · Monoamine metabolites (HVA, 5-HIAA, MHPG) · Cerebrospinal fluid

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Introduction

Concentrations of the major monoamine metabolites (MM) 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylglycol (MHPG) in lumbar cerebrospinal fluid (CSF) have been used extensively as indirect indices of monoamine turnover rate in the brain in humans. Studies of human twins indicate that CSF 5-HIAA and HVA levels are under familial influence of both genetic and environmental origin, whereas MHPG is under major genetic influence (Oxenstierna et al. 1986). In rhesus monkeys significant portions of CSF 5-HIAA, the major serotonin metabolite, HVA, the major dopamine metabolite, and MHPG, the major norepinephrine metabolite in the central nervous system, have been shown to be determined by genetic mechanisms (Higley et al. 1993). Tryptophan hydroxylase (TPH) catalyzes the first and rate-limiting reaction in the biosynthesis of serotonin (5-HT; Cooper and Melcer 1961). This makes the TPH gene, located on chromosome 11p15.3-p14 (Craig et al. 1991), a candidate for the regulation of 5-HT turnover and metabolism. The enzyme catechol-O-methyltransferase (COMT) catalyzes the O-methylation of catechol neurotransmitters, hormones, and drugs (Axelrod and Tomchick 1958; Guldberg and Marsden 1975). Together, monoamine oxidase and COMT contribute to the conversion of dopamine and noradrenaline into HVA and MHPG, respectively. COMT exists in a thermolabile low-activity form and a thermostable high-activity form, which is due to a DNA exonic polymorphism changing Met to Val at position 108 and 158 in the soluble and membrane-bound form of the enzyme, respectively (Boudikova et al. 1990; Grossman et al. 1992b; Klemetsdal et al. 1994; Lachman et al. 1996). Thus, the COMT gene, positioned on chromosome 22q11.2 (Brahe et al. 1986; Grossman et al. 1992a; Winqvist et al. 1992), is a candidate to influence the levels of HVA and MHPG in CSF.

In the present study a HaeIII marker which distinguishes a polymorphism in the TPH gene and a NlaIII restriction site associated with high and low COMT activity were examined for possible relationships to concentrations of 5-HIAA, HVA, and MHPG in lumbar CSF of healthy Swedish volunteers.

Materials and methods

Subjects

The characteristics and assessment of the subjects participating in the present study have previously been described (Jönsson et al. 1996). Briefly, Caucasian individuals (n = 66) were recruited predominantly among students or hospital staff. Lumbar puncture (LP) had been performed in all subjects in previous studies on MM in CSF. Height was also measured. Back length, defined as the distance between the external occipital protuberance and the insertion point of the lumbar needle with the subject in the lying position, was measured in 41 subjects. Eight to 19 years after these studies were accomplished, a structured interview was performed by a psychiatrist (E.J.) to assess psychiatric morbidity (according to DSM-III-R), somatic illness, and presence of mental and nervous system disorders among relatives. Hospital records were obtained and examined for diagnosis. Genealogical data for antecedents up to the third degree were obtained from parish registers to assess the origin of the individuals. Subjects previously excluded from the MM studies and those who admitted any lifetime psychiatric disorder were omitted.

Of the subjects 37 were men and 29 women. The age range at the time of the present study was 29–56 years (mean age 39 years). The mean age at LP was 27 years (range 19–43 years). Thirty subjects were university graduates. Twenty-one subjects had a family history of major mental illness defined as at least one first- or second-degree relative with schizophrenia, schizoaffective disorder, bipolar disorder, recurrent unipolar disorder, other non-organic psychosis, or who had committed suicide. Of the women, 10 used oral contraceptives at LP, 17 did not, and data were missing for two individuals. Except for oral contraceptives, all participants were drug free at LP. Genealogical data indicated that 88% of the chromosomes were of Swedish origin, 7% of Finnish, and the remaining 5% from seven other European countries.

CSF MM concentrations

The CSF was obtained by LP with the subject in the sitting (n = 36) or recumbent (n = 30) position. It was performed between 8 and 9 a. m. Subjects had at least 8 h of supervised bedrest in the hospital, abstaining from food and smoking. Samples of 12.5 ml CSF were drawn according to a standardized sampling procedure (Sedvall et al. 1980). 5-HIAA, HVA, and MHPG concentrations were measured with mass fragmentography using deuterium-labeled internal standards (Swahn et al. 1976).

Genotype analyses

Venous blood was taken from all individuals into EDTA-containing tubes. DNA isolation was performed according to Luthman and Datta (Geijer et al. 1994).

TPH genotype was determined by single-strand conformational polymorphism (SSCP) analysis essentially as previously described (Nielsen et al. 1995). Briefly, DNA was amplified using polymerase chain reaction (PCR) with the primers TPHI7-3: 5′-TTGTTTCTTTATTTGATTAGTGT-3′ and TPHI7-4a: 5′-AGTTCATGGCAGGTATCTCTGAA-3′. These primers hybridize within TPH intron seven on either side of the polymorphic nucleotide. Amplification was performed with 50 ng DNA, 0.2 μM of each primer, 250 μM each of dCTP, dGTP, dTTP, and dATP, 1.5 mM $MgCl_2$, 50 mM KCl, 0.001% gelatin, 10 mM Tris, pH 8.3, 3 μCi [α-^{32}P]dCTP, and 0.8 units of AmpliTaq (Perkin-Elmer Cetus, Foster City, CA 94404, USA) in a volume of 10 μl. Samples were amplified for ten cycles of 15 s at 94° C, 15 s at 52° C, and 30 s at 72° C, followed by 20 cycles of 15 s at 89° C, 15 s at 52° C, and 30 s at 72° C, followed by 5 min at 72° C. The resulting phosphorus 32-labeled fragment (2 μl) was diluted with 18 μl of 95% formamide, 10 mM NaOH, 0.05% bromophenol blue, and 0.05% xylene cyanole, and incubated at 100° C for 2 min. The denatured DNA was electrophoresced (2.5 μl/lane) through a nondenaturing 1X MDE gel (FMC BioProducts, Rockland, ME 04841, USA) and visualized on a PhosphorImager 400 (Molecular Dynamics, Sunnyvale, CA 94086, USA) and by autoradiography. The alleles, due to an adenine to cytosine transversion at position 779 of intron 7 (Nielsen et al. 1997), are designated according to their SSCP migration pattern with the faster migrating band designated "L" and the slower "U".

The COMT genotype was determined by RFLP analysis as previously described (Daniels et al. 1996). Briefly, genomic DNA was amplified using PCR with the primers 5′-ACTGTGGCTACTCAGCTGTG-3′ and 5′-CCTTTTTCCAGGTCTGACAA-3′. These primers are complementary to motifs in exon 4 and intron 4 of the human COMT gene so that the resulting fragment includes the polymorphic site in the 3′ part of exon 4. The 169 bp product was digested with the restriction enzyme Nla III (New England Biolabs (UK), Hitchin, Herts, England) and then run on a 6% non-denaturating polyacrylamide gel subsequently stained with ethidium bromide. The low-activity COMT polymorphism (methionine at amino acid 108/158) contains a Nla III restriction site resulting in 129- and 40-bp digest products, whereas the high-activity COMT polymorphism (valine at amino acid 108/158) contains no restriction site, leaving the 169-bp product undigested.

Data analysis

Analysis of variance (ANOVA) was used for the 30 primary calculations. Unpaired two-tailed t-test was used in post hoc analyses. Due to the exploratory nature of the investigation, the overall level of significance was set to 0.01 in the primary calculations and 0.005 in the post hoc analyses.

Results

Relationships between TPH genotypes and CSF MM concentrations

In the present sample the UL genotype was the most frequent (44%), followed by the LL (38%) and UU (18%) genotype. The allele frequencies were 0.60 (L) and 0.40 (U), respectively.

When the three tryptophan hydroxylase genotypes were compared across the total sample, there were no significant differences for CSF 5-HIAA, HVA, and MHPG levels (Table 1). However, when the sample was subdivided by gender it was observed that men with the LL genotype displayed higher 5-HIAA mean (114 nmol/l) than men with the UL (74 nmol/l) or UU (90 nmol/l) marker (F = 6.15, p = 0.005). In the post hoc analysis men with the LL genotype had higher 5-HIAA levels than men with UL and UU genotypes (means 114 and 78 nmol/l, respectively; t = 3.30, p = 0.002). Comparisons between men with homozygotic genotypes vs men with the UL genotype also revealed differences (t = 3.09, t = 0.004) with significantly higher 5-HIAA concentrations in the

Table 1 Comparisons (ANOVA) between tryptophan hydroxylase genotype constellations and homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), and 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations in lumbar cerebrospinal fluid in healthy volunteers

Genotype	Subgroup	*n*	HVA		5-HIAA		MHPG	
			Mean ± SD (nmol/L)	*F* *p*	Mean ± SD (nmol/L)	*F* *p*	Mean ± SD (nmol/L)	*F* *p*
LL	All	25	206 ± 83	$F = 2.94$	108 ± 33	$F = 1.73$	43 ± 6	$F = 0.85$
UL		29	157 ± 74	$p = 0.060$	89 ± 41	$p = 0.186$	42 ± 7	$p = 0.431$
UU		12	167 ± 57		95 ± 28		41 ± 7	
LL	Men	11	208 ± 99	$F = 3.89$	114 ± 38	$F = 6.15$	46 ± 7	$F = 1.37$
UL		20	138 ± 53	$p = 0.030$	74 ± 28	$p = 0.005$	41 ± 7	$p = 0.267$
UU		6	140 ± 50		90 ± 21		43 ± 8	
LL	Women	14	204 ± 72	$F = 0.04$	103 ± 29	$F = 1.16$	42 ± 4	$F = 1.10$
UL		9	202 ± 97	$p = 0.960$	124 ± 47	$p = 0.328$	43 ± 8	$p = 0.348$
UU		6	194 ± 52		99 ± 36		38 ± 6	
LL	No family history	13	214 ± 93	$F = 4.02$	109 ± 35	$F = 3.13$	44 ± 7	$F = 0.94$
UL		21	147 ± 58	$p = 0.025$	81 ± 33	$p = 0.054$	42 ± 7	$p = 0.400$
UU		11	159 ± 52		90 ± 25		40 ± 7	
LL	Family history[a]	12	197 ± 75	$F = 0.28$	106 ± 31	$F = 0.35$	43 ± 4	$F = 0.25$
UL		8	185 ± 105	$p = 0.756$	111 ± 55	$p = 0.710$	41 ± 9	$p = 0.778$
UU		1	254		142		43	

[a]At least one first- or second-degree relative with schizophrenia, schizoaffective disorder, bipolar disorder, recurrent unipolar disorder, other non-organic psychosis, or who had committed suicide

Table 2 Comparisons (ANOVA) between tryptophan hydroxylase genotype constellations and ratios between homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), and 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations in lumbar cerebrospinal fluid in healthy volunteers

Genotype	Gender	n	HVA/5-HIAA		5-HIAA/MHPG	
			Mean ± SD (nmol/L)	*F* *p*	Mean ± SD (nmol/L)	*F* *p*
LL	All	25	1.94 ± 0.47	$F = 0.55$	2.50 ± 0.75	$F = 0.83$
UL		29	1.82 ± 0.43	$p = 0.578$	2.19 ± 1.10	$p = 0.441$
UU		12	1.81 ± 0.51		2.33 ± 0.58	
LL	Men	11	1.86 ± 0.56	$F = 1.10$	2.52 ± 0.86	$F = 2.97$
UL		20	1.92 ± 0.43	$p = 0.345$	1.84 ± 0.77	$p = 0.065$
UU		6	1.58 ± 0.57		2.10 ± 0.29	
LL	Women	14	2.00 ± 0.39	$F = 3.89$	2.49 ± 0.68	$F = 0.72$
UL		6	1.59 ± 0.34	$p = 0.033$	2.96 ± 1.35	$p = 0.496$
UU		9	2.03 ± 0.37		2.57 ± 0.72	
LL	No family history	13	2.00 ± 0.54	$F = 0.51$	2.50 ± 0.79	$F = 2.33$
UL		21	1.89 ± 0.43	$p = 0.607$	1.95 ± 0.79	$p = 0.110$
UU		11	1.81 ± 0.54		2.24 ± 0.51	
LL	Family history[a]	12	1.87 ± 0.39	$F = 0.76$	2.51 ± 0.76	$F = 0.35$
UL		8	1.65 ± 0.39	$p = 0.480$	2.81 ± 1.55	$p = 0.713$
UU		1	1.79		3.30	

[a] At least one first- or second-degree relative with schizophrenia, schizoaffective disorder, bipolar disorder, recurrent unipolar disorder, other non-organic psychosis, or who had committed suicide

LL and UU (mean 105 nmol/l) than the UL (74 nmol/l) subjects. A similar but nonsignificant pattern was found in men when the TPH genotypes were compared for CSF HVA concentrations. Men with the LL genotype had higher HVA concentrations than men with UL or UU genotypes (mean 208, 138, and 140 nmol/l, respectively; $F = 3.89$, $p = 0.030$). In the post hoc analysis these differences were further attenuated but still nonsignificant when men with U-containing genotypes were compared with males with the LL genotype ($t = 2.83$, $p = 0.008$).

However, in women there were no significant differences when TPH genotypes or alleles were compared for CSF 5-HIAA or HVA levels. The MHPG levels did not relate to TPH genotypes or alleles either in men or women.

When subjects were divided into those with and without a family history of major mental illness, it was observed that family-history-negative subjects with the LL genotype had nonsignificantly higher HVA concentrations than subjects with UL or UU genotypes (mean 214, 147, and 159 nmol/l, respectively; $F = 3.89$, $p = 0.025$). There was also a trend for subjects without family history carrying the LL genotype to display higher 5-HIAA concentrations than subjects with the UL and UU genotype (Table 1).

When MM ratios were compared for TPH genotypes nonsignificantly higher HVA/5-HIAA ratios were found in homozygotic than heterozygotic women ($F = 3.89$, $p = 0.033$; Table 2). In a post hoc analysis comparing homo- vs heterozygotic female subjects this difference was not significant ($t = 2.84$, $p = 0.009$).

Relationships between COMT genotypes and CSF MM concentrations

The heterozygotic genotype was the most frequent (52%), followed by the Met/Met (32%) and Val/Val (17%) genotypes. The allele frequencies were 0.42 (Val) and 0.58 (Met), respectively.

There were no relationships between COMT genotypes or alleles and 5-HIAA, HVA, or MHPG concentrations or ratios between the different MM either in the total sample, or in the subsamples derived with regard to gender or family history (data not shown).

Discussion

The TPH L allele was found to be the most frequent (0.60). This is in accordance with previous studies of Caucasian subjects (Nielsen et al. 1992; Nielsen et al. 1994).

In the present study we found significantly different mean CSF 5-HIAA levels in men with different TPH genotypes. This finding is interesting, since TPH can be rate limiting in the biosynthesis of serotonin (Cooper and Melcer 1961). The data are compatible with the view that the TPH genotypes differentially regulate 5-HT synthesis. There was also a nonsignificant trend for different CSF HVA levels in relation to TPH genotypes in a similar way as for 5-HIAA. This finding may be expected in light of the well-established high correlation between HVA and 5-HIAA levels in CSF (Ågren et al. 1986; Hsiao et al. 1993).

The lack of relationship between TPH genotypes and MHPG levels is in accordance with the evidence suggesting TPH as an enzyme of direct importance for serotonin but not noradrenalin synthesis.

Interestingly, in women no significant relationship between TPH genotypes and CSF 5-HIAA was found. This may indicate gender differences in the relationship between TPH genotypes and 5-HIAA levels in CSF or major confounding variables among women. In a study of rhesus monkeys TPH mRNA expression was elevated by estrogen treatment (Pecins-Thompson et al. 1996). Prominent differences in translational efficiency for TPH mRNA has been documented (Dumas et al. 1989). Steroids also have rapid, nongenomic actions at the membrane level (Ke and Ramirez 1990; Majewska 1992). Hypothetically, such mechanisms may alter the serotonin turnover and hence the level of 5-HIAA in CSF; if so, for example, gender differences in steroid levels or distribution of steroid receptors may explain the present results rather than, or in concert with, DNA variations in the TPH gene. Changes in serotonin synthesis in relation to the menstrual cycle have been suggested on the basis of altered rat brain serotonin in relation to varying serum concentrations of sex steroids (Sietnieks et al. 1983). Reduction of premenstrual complaints demonstrated during treatment with 5-HT reuptake inhibitors may also possibly reflect changes in the serotonin synthesis (Eriksson et al. 1990; Rickels et al. 1990; Steiner et al. 1995). However, no significant differences were found when CSF MM levels were compared in the lutheal and follicular phases in women with or without premenstrual syndrome (Eriksson et al. 1994). In the present study the timing of the menstrual phases was not recorded. Some of the women used oral contraceptives, another confounder which could have reduced the possibility to find a relationship between TPH genotypes and CSF 5-HIAA levels also in women. However, when MM concentrations from each group of women (users or non-users of oral contraceptives at the time of lumbar puncture) were separately related to TPH genotypes, no significant differences emerged (data not shown).

Ratios of the three CSF MMs have been suggested to reflect interactions between the 5-HT, dopamine (DA) and norepinephrine (NE) systems (Ågren et al. 1986). In the present study no significant differences related to genotypes were found between MM ratios.

Despite a huge literature on confounding variables in CSF MM measurements controlling for factors such as height or back length, affecting acid metabolite transport seems to be controversial and is often not done, even in recent studies. Recently, 5-HT_3 receptors were reported to regulate development of the spinal column during fetal period (David Julius, 29th Winter Conference on Brain Research, 27 January to 3 February 1996, Snowmass, Colorado, USA). This implies that serotonin may influence growth and length of the spinal column and indirectly also influence height. Hence, assuming relationships between the serotonin turnover in fetus and the adult gives a rationale not to normalize for height or back length. However, in a previous report we found significant relationships between back length and CSF MMs in the present sample (Jönsson et al. 1996). Therefore, in the present study we also performed calculations with data corrected for back length. Overall, the results were very similar, but when TPH genotypes were compared with corrected MM levels the tendencies were somewhat

weaker. For example, in men comparisons between LL and the U-containing TPH genotypes decreased the probability for differences (5-HIAA, $p = 0.010$; HVA, $p = 0.039$).

The present findings differ from those of Nielsen et al. (1994). In the latter study no statistically significant difference of TPH genotype to CSF 5-HIAA concentration was detected when 20 healthy male controls were evaluated. However, in a series of 56 impulsive alcoholic male offenders with the UU genotype higher 5-HIAA levels were found. Interestingly, there was also an association between presence of suicide attempts within the sample of alcoholic Finnish offenders and the L allele. Analyzing another TPH polymorphism in a clinically heterogenous group of French suicide attempters, Abbar et al. (1995) could not find any association of TPH genotypes to suicidal behavior. In the present study, investigating 37 healthy male volunteers, men with the LL genotype displayed the highest CSF 5-HIAA concentrations, whereas men with the UL genotype had the lowest mean levels. In the study by Nielsen et al. (1994) the 20 controls had very similar 5-HIAA mean concentrations irrespective of TPH genotype. The cause of the difference between the studies is presently unclear. The control samples are different, with respect to number, sociodemographics, family history, selection, and ethnic profiles.

The present sample consists of an unusually high percentage of university graduates. There is also a high degree of mental illness among relatives in the present sample, whereas the Finnish controls were all assessed to be free of first-degree relatives with major mental illness or alcoholism. Although in the present study trends for relationships between TPH genotypes and CSF HVA and 5-HIAA levels emerged in subjects without, but not among those with, a family history of major mental illness, these relationships were far from significant after correction for back length making the interpretation difficult. If alcohol diagnoses in relatives up to the second degree also were included in the definition of family history, no relationships between TPH genotypes and CSF HVA and 5-HIAA emerged (data not shown). However, it could not be excluded that family history of some mental illness(es) may influence the relationship between TPH genotypes and MM in CSF. The control subjects in the present study had been clinically evaluated twice with about a decade in between. Approximately a third of the sample was excluded due to DSM-III-R psychopathology admitted at the second interview. In the Finnish study the control individuals were assessed only once. It is possible that a significant amount of the Finnish control subjects later in life will experience DSM-III-R disorders. If there are different relationships between TPH and CSF 5-HIAA levels in Swedes and Finns, the different origin may be a possible explanation for the differences in the studies. The smaller control sample size in the Finnish study may also have reduced the possibility of detecting a relationship.

The COMT polymorphism, directly associated with COMT activity, did not reveal any influence on lumbar MM concentrations in the present sample. This suggests that COMT activity does not significantly influence monoamine turnover or metabolite formation. This may be due to the high availability of COMT, which therefore, irrespective of high or low catecholaminergic activity, does not become rate limiting in the formation of HVA and MHPG. As COMT is not involved in the serotonin metabolism, the lack of relationship to CSF 5-HIAA levels was expected and may also further point to the significance of the significant TPH - 5-HIAA relationships found.

The significant CSF 5-HIAA differences with regard to TPH genotypes and alleles in the present study may in any case be chance findings, although the lack of or less significant associations to HVA and MHPG make such an interpretation less likely. The sample size is limited, especially when divided according to gender and family history. The functional relevance of the TPH polymorphism investigated is unclear. The TPH polymorphism is located in an intron and does not cause amino acid changes in the protein sequence. It is unlikely to convey a direct effect itself upon CSF 5-HIAA levels. Its relevance is based on an assumed linkage disequilibrium with other polymorphisms within the coding or regulatory sequence. Also considering the many variables tested, type-I errors can be expected to emerge. This means that until the present results are replicated in independent investigations, they must be regarded as preliminary and should be interpreted with caution.

If replicated, the present results suggest that TPH genotypes may participate differentially in the regulation of serotonin and dopamine turnover in the central nervous system of men and women. The results do not favour a differential influence of COMT gene activities on MM concentrations in lumbar CSF.

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References

Abbar M, Courtet P, Amadéo S, Caer Y, Mallet J, Baldy-Moulinier M, Castelnau D, Malafosse A (1995) Suicidal behaviors and the tryptophan hydroxylase gene. Arch Gen Psychiatry 52: 846–849

Ågren H, Meffords I, Rudorfer M, Linnoila M, Potter W (1986) Interacting neurotransmitter systems. A non-experimental approach to the 5HIAA-HVA correlation in human CSF. J Psychiatr Res 20: 175–193

American Psychiatric Association (1987) Diagnostic and statistical manual of mental disorders (DSM-III-R), 3rd edn, revised. American Psychiatric Press, Washington, DC

Axelrod J, Tomchick R (1958) Enzymic O-methylation of epinephrine and other catechols. J Biol Chem 233: 702–705

Boudikova B, Szumlanski C, Maidak B, Weinshilboum R (1990) Human liver catechol-O-methyltransferase pharmacogenetics. Clin Pharmacol Ther 48: 381–389

Brahe C, Bannetta P, Meera KP, Arwert F, Serra A (1986) Assignment of the catechol-O-methyltransferase gene to human chromosome 22 in somatic cell hybrids. Hum Genet 74: 230–234

Cooper JR, Melcer I (1961) The enzymic oxidation of tryptophan to 5-hydroxytryptophan in the biosyntehsis of serotonin. J Pharm Exp Ther 132: 265–268

Craig SP, Boularand S, Darmon MC, Mallet J, Craig IW (1991) Localization of human tryptophan hydroxylase (TPH) to chromosome 11p15.3-p14 by in situ hybridization. Cytogenet Cell Genet 56: 157–159

Daniels JK, Williams NM, Williams J, Jones LA, Cardno AG, Murphy KC, Spurlock G, Riley B, Scambler P, Asherson P, McGuffin P, Owen MJ (1996) No evidence for allelic association between schizophrenia and a polymorphism determining high or low catechol-O-methyltransferase activity. Am J Psychiatry 153: 268–270

Dumas S, Darmon MC, Delort J, Mallet J (1989) Differential control of tryptophan hydroxylase expression in raphe and in pineal gland: evidence for a role of translational efficiency. J Neurosci Res 24: 537–547

Eriksson E, Alling C, Andersch B, Andersson K, Berggren U (1994) Cerebrospinal fluid levels of monoamine metabolites. A preliminary study of their relation to menstrual cycle phase, sex steroids, and pituitary hormones in healthy women and in women with premenstrual syndrome. Neuropsychopharmacology 11: 201–213

Eriksson E, Lisjö P, Sundblad C, Andersson K, Andersch B, Modigh K (1990) Effects of clomipramine on premenstrual syndrome. Acta Psychiatr Scand 81: 87

Geijer T, Neiman J, Rydberg U, Gyllander A, Jönsson E, Sedvall G, Valverius P, Terenius L (1994) Dopamine D2 receptor gene polymorphisms in Scandinavian chronic alcoholics. Eur Arch Psychiatry Clin Neurosci 244: 26–32

Grossman MH, Emanuel BS, Budarf ML (1992a) Chromsomal mapping of the human catechol-O-methyltransferase to 22q11.1-q11.2. Genomics 12: 822–825

Grossman MH, Littrell JB, Weinstein R, Szumlanski CL, Weinshilboum RM (1992b) Identification of the possible molecular basis for inherited differences in human catechol-O-methyltransferase. Soc Neurosci 18: 70

Guldberg HC, Marsden CA (1975) Catechol-O-methyltransferase: pharmacological aspects and physiological role. Pharmacol Rev 27: 135–206

Higley JD, Thompson WW, Champoux M, Goldman D, Hasert MF, Kraemer GW, Scanlan JM, Suomi SJ, Linnoila M (1993) Paternal and maternal genetic and environmental contributions to cerebrospinal fluid monoamine metabolites in Rhesus monkeys (Macaca mulatta). Arch Gen Psychiatry 50: 615–623

Hsiao JK, Potter WZ, Ågren H, Owen RR, Pickar D (1993) Clinical investigation of monoamine neurotransmitter interactions. Psychopharmacology 112: S76–S84

Jönsson E, Sedvall G, Brené S, Gustavsson JP, Geijer T, Terenius L, Crocq M-A, Lannfelt L, Tylec A, Sokoloff P, Schwartz JC, Wiesel F-A (1996) Dopamine-related genes and their relationships to monoamine metabolites in CSF. Biol Psychiatry 40: 1032–1043

Ke F, Ramirez VD (1990) Binding of progesterone to nerve cell membranes of rat brain using progesterone conjugated to 125I-bovine serum albumin as a ligand. J Neurochem 54: 467–472

Klemetsdal B, Straume B, Giverhaug T, Aarbakke J (1994) Low catechol-O-methyltransferase activity in a Saami population. Eur J Clin Pharmacol 46: 231–235

Lachman HM, Papolos DF, Saito T, Yu Y-M, Szumlanski CL, Weinshilboum RM (1996) Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 6: 243–250

Majewska MD (1992) Neurosteroids: endogenous bimodal modulators of the GABA A receptor: mechanism of action and physiological significance. Prog Neurobiol 38: 379–395

Nielsen DA, Dean M, Goldman D (1992) Genetic mapping of the human tryptophan hydroxylase gene on chromosome II, using an intronic conformational polymorphism. Am J Hum Genet 51: 1366–1371

Nielsen DA, Goldman D, Virkkunen M, Tokola R, Rawlings R, Linnoila M (1994) Suicidality and 5-hydroxyindoleacetic acid concentration associated with a tryptophan hydroxylase polymorphism. Arch Gen Psychiatry 51: 34–38

Nielsen DA, Novoradovsky A, Goldman D (1995) SSCP primer design based on single-strand DNA structure predicted by a DNA folding program. Nucleic Acids Res 23: 2287–2291

Nielsen DA, Jenkins GL, Stefanisko KM, Jefferson KK, Goldman D (1997) Sequence, splice site and population frequency distribution analyses of the polymorphic human tryptophan hydroxylase intron 7. Mol Brain Res 45: 145–148

Oxenstierna G, Edman G, Iselius L, Oreland L, Ross SB, Sedvall G (1986) Concentrations of monoamine metabolites in the cerebrospinal fluid of twins and unrelated individuals: a genetic study. J Psychiatr Res 20: 19–29

Pecins-Thompson M, Brown NA, Kohama SG, Bethea CL (1996) Ovarian steroid regulation of tryptophan hydroxylase mRNA expression in Rhesus Macaques. J Neurosci 21: 7021–7029

Rickels K, Freedman EW, Sondheimer S, Albert J (1990) Fluoxetine in the treatment of premenstrual syndrome. Curr Ther Res 48: 161–166

Sedvall G, Fyrö B, Gullberg B, Nybäck H, Wiesel F-A, Wode-Helgodt B (1980) Relationships in healthy volunteers between concentrations of monoamine metabolites in cerebrospinal fluid and family history of psychiatric morbidity. Br J Psychiatry 136: 366–374

Sietnieks A, Andén N-E, Grabowska-Andén M (1983) Determination of serotonin in small brain structures by means of HPLC combined with electrochemical detection: the effect of ovarian steroids on serotonin turnover rate within several brain regions. Acta Pharmacol Toxicol 52: 398–400

Steiner M, Steinberg S, Stewart D, Carter D, Berger C, Reid R, Grover D, Streiner D (1995) Fluoxetine in the treatment of premenstrual dysphoria. N Engl J Med 332: 1529–1534

Swahn C-G, Sandgärde B, Wiesel F-A, Sedvall G (1976) Simultaneous determination of the three major monoamine metabolites in brain tissue and body fluids by a mass fragmentographic method. Psychopharmacology 48: 147–152

Winqvist R, Lundström K, Salminen M, Laatikainen M, Ulmanen I (1992) The human catechol-O-methyltransferase (COMT) gene maps to band q11.2 of chromosome 22 and shows a frequent RFLP with BglI. Cytogenet Cell Genet 59: 253–257