BMC Psychiatry

BioMed Central

Research article

Monoamine related functional gene variants and relationships to monoamine metabolite concentrations in CSF of healthy volunteers

Erik G Jönsson*[1], Jessica Bah[2], Jonas Melke[2], Rami Abou Jamra[3], Johannes Schumacher[3], Lars Westberg[2], Roland Ivo[3], Sven Cichon[4], Peter Propping[3], Markus M Nöthen[4], Elias Eriksson[2] and Göran C Sedvall[1]

Address: [1]Department of Clinical Neuroscience, Psychiatry Section, HUBIN project, Karolinska Institutet, Stockholm, Sweden, [2]Department of Pharmacology, Göteborg University, Sweden, [3]Institute of Human Genetics, University of Bonn, Germany and [4]Department of Medical Genetics, University of Antwerp, Belgium

Email: Erik G Jönsson* - erikj@ks.se; Jessica Bah - jessica.bah@pharm.gu.se; Jonas Melke - jonas.melke@pharm.gu.se; Rami Abou Jamra - rami.aboujamra@uni-bonn.de; Johannes Schumacher - johannes.schumacher@uni-bonn.de; Lars Westberg - lars.westberg@pharm.gu.se; Roland Ivo - Roland.Ivo@gmx.de; Sven Cichon - Sven.Cichon@uia.ua.ac.be; Peter Propping - propping@uni-bonn.de; Markus M Nöthen - mnoethen@uia.ua.ac.be; Elias Eriksson - elias.eriksson@pharm.gu.se; Göran C Sedvall - goran.sedvall@ks.se

* Corresponding author

Published: 04 March 2004

BMC Psychiatry 2004, **4**:4

Received: 19 September 2003
Accepted: 04 March 2004

This article is available from: http://www.biomedcentral.com/1471-244X/4/4

Abstract

Background: Concentrations of monoamine metabolites in human cerebrospinal fluid (CSF) have been used extensively as indirect estimates of monoamine turnover in the brain. CSF monoamine metabolite concentrations are partly determined by genetic influences.

Methods: We investigated possible relationships between DNA polymorphisms in the serotonin 2C receptor (*HTR2C*), the serotonin 3A receptor (*HTR3A*), the dopamine D_4 receptor (*DRD4*), and the dopamine β-hydroxylase (*DBH*) genes and CSF concentrations of 5-hydroxyindolacetic acid (5-HIAA), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylglycol (MHPG) in healthy volunteers (n = 90).

Results: The *HTR3A* 178 C/T variant was associated with 5-HIAA levels (p = 0.02). The *DBH*-1021 heterozygote genotype was associated with 5-HIAA (p = 0.0005) and HVA (p = 0.009) concentrations. Neither the *HTR2C* Cys23Ser variant, nor the *DRD4* -521 C/T variant were significantly associated with any of the monoamine metabolites.

Conclusions: The present results suggest that the *HTR3A* and *DBH* genes may participate in the regulation of dopamine and serotonin turnover rates in the central nervous system.

Background

Concentrations of the major serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA), the major dopamine metabolite homovanillic acid (HVA), and the major

norepinephrine metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) in lumbar cerebrospinal fluid (CSF) have been used extensively as indirect measures of monoamine turnover in the brain of 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 [1]. In rhesus monkeys significant portions of CSF 5-HIAA, HVA, and MHPG in the central nervous system, have been shown to be determined by genetic mechanisms [2].

A number of serotonin receptors mediate the effects of serotonin. Among several functions, the serotonin receptor 5-HT_{2C}, which is densely expressed throughout the brain [3], seems to be directly involved in the regulation of serotonin and norepinephrine activities in the brain [4-6]. The 5-HT_{2C} gene (*HTR2C*) is localised to chromosome Xq24 [7]. An *HTR2C* variant giving rise to a Cystein to Serine substitution at position 23 of the protein has been identified [8]. This variant was shown to influence the CSF MHPG concentration in a Finnish sample of predominantly alcoholic offenders [9].

In contrast to all other serotonin receptors, which are G protein-coupled, the 5-HT_3 receptor is a ligand-gated ion channel [10]. In the brain 5-HT_3 receptors are localised in areas including the amygdala, hippocampus, and caudate nucleus. In addition to their effects on serotonin-regulated physiological processes, there are data suggesting that 5-HT_3 receptors influence the activity of several other neurotransmitters, including norepinephrine and dopamine [11-13]. The 5-HT_{3A} gene (*HTR3A*) is localised to chromosome 11q23.1-q23.2 [10]. An *HTR3A* single nucleotide polymorphism (178 C/T) in the upstream regulatory region was recently discovered to be of putative functional importance, because luciferase reporter assays in human embryonal kidney cells showed a two to three times higher activity of the rare allele compared to the wildtype [14]. This *HTR3A* variant was reported to be associated with bipolar disorder [14] and the personality trait harm avoidance in women [15].

The dopamine D_4 receptor has a predominantly cortical localisation in the human brain [16]. Among several effects, the dopamine D_4 receptor seems to modulate dopamine synthesis and turnover [17,18]. The dopamine D_4 receptor gene (*DRD4*) is located to chromosome 11p15.5 [19]. Recently, a putative functional *DRD4* upstream region variant (-521C/T) was discovered, where the -521C allele was reported to be 40% less active than -521T allele in a chloramphenicol acetyltransferase assay using human retinoblastoma cells [20]. This *DRD4* variant was associated with schizophrenia [20] as well as the personality trait novelty seeking in some [21-24] but not all studies [25-31]. However, meta-analyses suggested association with both conditions [32-34].

The enzyme dopamine β-hydroxylase (DβH) catalyses the conversion of dopamine to norepinephrine. DβH is localised in catecholamine-containing vesicles of noradrenergic and adrenergic cells [35,36]. DβH enzyme activity has been shown to be heritable to a great extent [1,37]. The DβH gene (*DBH*) is located on chromosome 9q34 [38]. Recently, a *DBH* promoter variant (-1021 C/T) was shown to strongly influence plasma DβH-activity [39-41], indicating a functional effect. In the present study we have examined the *HTR2C* Cys23Ser, *HTR3A* 178 C/T, *DRD4* -521 C/T and *DBH* -1021 C/T variants for possible relationships to concentrations of 5-HIAA, HVA, and MHPG in lumbar CSF from healthy Swedish volunteers.

Methods

Healthy human volunteers

The study was approved by the Ethics Committee of the Karolinska Hospital, Stockholm. Informed consent of the subjects was obtained after the nature of the procedures had been fully explained.

The characteristics and assessment of the subjects participating in the present study have been described previously [42,43]. Caucasian individuals (n = 90) were recruited predominantly among students or hospital staff. Lumbar puncture (LP) was performed in all subjects. Height was also recorded. 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 63 subjects. Eight to 19 years later a structured interview was performed by a psychiatrist (EJ) to assess psychiatric morbidity (DSM-III-R; [44]), somatic illness and presence of mental and nervous system disorders among relatives. Subjects completed a questionnaire regarding smoking habits. 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 who reported any lifetime psychiatric disorder were excluded.

Of the 90 subjects 52 were men and 38 women. The age range at the time of the structured interview was 29 to 56, with a mean ± standard deviation of 40.5 ± 6.4 years. The mean age ± standard deviation at LP was 27.4 ± 5.9 years, age range 18 – 43. Thirty-six 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 subjects 54 were or had been regular tobacco users, 25 were non-

smokers or had only used tobacco once or a few times in their life, while data were missing for 11 individuals. Of the women, 15 used oral contraceptives at LP, 21 did not, while data were missing for two individuals. Except for oral contraceptives all participants were drug free at LP. Genealogical data implicated that 89.6% and 4.7% of the genes originated in ancestors born in Sweden and Finland, respectively, and the remaining 5.7% were distributed on 8 European countries.

CSF monoamine metabolite concentrations

All subjects had at least 8 h of bed-rest in the hospital, abstaining from food and smoking. CSF samples were obtained by LP between 8 and 9 a.m. with the subjects in the sitting (n = 41) or recumbent (n = 47) position. Samples of 12.5 ml CSF were drawn according to a standardised sampling procedure [45]. Samples were stored at below -20°C and analysed within two months. 5-HIAA, HVA, and MHPG concentrations were measured by mass fragmentography with deuterium labelled internal standards [46].

Genotype analyses

Venous blood was taken from all individuals into EDTA-containing tubes. DNA was isolated as previously described [47]. The *HTR2C* Cys23Ser variant was genotyped in accordance with Lappalainen et al [8]. The *HTR3A* 178 C/T, *DRD4* -521 C/T, and *DBH* -1021 C/T variants were genotyped as previously described [15,26,48].

Data analyses

One way analysis of variance (ANOVA) was used for comparisons between genotypes and 5-HIAA, HVA, and MHPG concentrations, respectively. To correct monoamine metabolite levels for back length and use of oral contraceptives (among women), suggestive but discussed confounding variables for monoamine metabolite concentrations in lumbar CSF [42,43,49], analysis of covariance (ANCOVA) was used. For those subjects where back length was not available, estimated back length values, based on the relationship between back length and height, was used as previously described [42,43]. Significance level was defined as a p-value lower than 0.05. Power was estimated in accordance with published methods [50,51].

Results

Relationships between HTR2C genotypes and CSF monoamine metabolite concentrations

The *HTR2C* genotyping was successful in 86 individuals. Among men the allele frequencies were 0.88 (Cys23) and 0.12 (Ser23). In women the allele frequencies were 0.89 (Cys23) and 0.11 (Ser23), distributed on the following genotypes: Cys23Cys (81%), Cys23Ser (16%), and Ser23Ser (3%). As the *HTR2C* gene is localised on the X chromosome, each gender was analysed separately. Among women, the Ser23Ser and Cys23Ser genotypes were pooled and analysed versus the Cys23Cys genotype, because of the small number of Ser23Ser subjects. There were no significant relationship between genotypes and any of the CSF monoamine metabolite concentrations neither among men or women (table 1).

Relationships between HTR3A genotypes and CSF monoamine metabolite concentrations

The *HTR3A* 178 C/C genotype was the most frequent (67%), followed by the 178 C/T (30%) and the 178 T/T (3%) genotypes. The allele frequencies were 0.82 (178C) and 0.18 (178T). The 178 T/T and 178 C/T genotypes were pooled in the calculations, because of the small number of subjects carrying the 178 T/T genotype. In the total sample there were associations between the *HTR3A* variant and 5-HIAA (p = 0.002) and HVA (p = 0.006) concentrations, with higher concentrations of these monoamine metabolites in carriers of the T-containing genotypes (table 2). However, when corrected for back-length the association between the *HTR3A* variant and lumbar HVA concentrations was reduced to a trend (p = 0.08). In the male sub-sample, no significant relationships between *HTR3A* variation and 5-HIAA or HVA concentrations emerged. Among women the relationship between *HTR3A* variation and 5-HIAA concentrations, indicating higher 5-HIAA levels in subjects carrying the 178T allele, was significant both uncorrected (p = 0.004), corrected for back-length (p = 0.006), and corrected for use of oral contraceptives (p = 0.03; table 2). However, in the female sub-sample the association between *HTR3A* and HVA concentrations was of borderline significance (p = 0.05 uncorrected, p = 0.03 corrected for use of oral contraceptives), but was non-significant after correction for back-length (p = 0.12). Inspection of the CSF levels of the different genotype groups indicated a possible heterosis effect with regard to the CSF 5-HIAA and HVA concentrations [52]. We therefore also performed calculations pooling the homozygotic genotypes. However, the probability levels of significance did not exceed those obtained pooling the T/T and C/T genotypes (table 2). There were no significant relationships between the *HTR3A* genotype and MHPG concentrations (table 2).

Relationships between DRD4 genotypes and CSF monoamine metabolite concentrations

The *DRD4* -521 C/T genotype was the most frequent (58%), followed by the -521 T/T (28%) and the -521 C/C genotypes (14%). The allele frequencies were 0.57 (-521T) and 0.43 (-521C). When women were analysed separately, the -521 C/C and -521 C/T genotypes were pooled, because of the small number of -521 C/C subjects. There was no significant relationship between *DRD4* genotypes and any of the CSF monoamine metabolite

Table 1: Serotonin receptor 5-HT$_{2C}$ (*HTR2C*) genotypes and relationships to monoamine metabolite concentrations in human lumbar cerebrospinal fluid.

			5-HIAA			HVA			MHPG		
HTR2C Allele/ genotype	Sex	n	Mean ± SD (nmol/L)	F[a] p[a]	F[b] p[b]	Mean ± SD (nmol/L)	F[a] p[a]	F[b] p[b]	Mean ± SD (nmol/L)	F[a] p[a]	F[b] p[b]
Cys23	Men	43	92 ± 40	F = 0.27	F = 0.13	166 ± 75	F = 0.73	F = 0.50	43 ± 8	F = 1.75	F = 1.48
Ser23		6	86 ± 16	p = 0.61	p = 0.72	144 ± 39	p = 0.40	p = 0.48	39 ± 4	p = 0.19	p = 0.23
Cys23Cys	Women	30	105 ± 39	F = 0.66	F = 0.57	194 ± 77	F = 2.14	F = 1.34	40 ± 6	F = 1.03	F = 0.91
Cys23Ser[c]		6	118 ± 29	p = 0.42	p = 0.46[d]	231 ± 69	p = 0.15	p = 0.25[e]	44 ± 8	p = 0.32	p = 0.35[f]
Ser23Ser[c]		1	107			229			38		

5-HIAA = 5-hydroxyindoleacetic acid; HVA = homovanillic acid; MHPG = 3-methoxy-4-hydroxyphenylglycol. Statistical comparisons done on monoamine metabolite residuals corrected[a] and uncorrected[b] for back length. [c] Cys23Ser and Ser23Ser genotypes were combined in the analyses. [d] Correction for use of oral contraceptives, F = 0.21, p = 0.65. [e] Correction for use of oral contraceptives, F = 0.84, p = 0.37. [f] Correction for use of oral contraceptives, F = 0.22, p = 0.64.

Table 2: Serotonin receptor 5-HT$_{3A}$ (*HTR3A*) genotypes and relationships to monoamine metabolite concentrations in human lumbar cerebrospinal fluid.

			5-HIAA			HVA			MHPG		
HTR3A Genotype	Sex	n	Mean ± SD (nmol/L)	F[a] p[a]	F[b] p[b]	Mean ± SD (nmol/L)	F[a] p[a]	F[b] p[b]	Mean ± SD (nmol/L)	F[a] p[a]	F[b] p[b]
T/T[c]	All	3	105 ± 52	F = 5.75	F = 10.22	203 ± 124	F = 3.25	F = 7.95	44 ± 5	F = 0.45	F = 0.29
C/T[c]		27	117 ± 36	p = 0.02	p = 0.002	211 ± 67	p = 0.07	p = 0.006	43 ± 8	p = 0.50	p = 0.75
C/C		60	90 ± 35			166 ± 71			42 ± 7		
T/T[c]	Men	1	52	F = 0.31	F = 0.96	113	F = 0.75	F = 1.49	49	F = 0.20	F = 0.49
C/T[c]		11	106 ± 33	p = 0.58	p = 0.33	193 ± 50	p = 0.39	p = 0.23	44 ± 9	p = 0.66	p = 0.49
C/C		40	90 ± 37			159 ± 11			43 ± 7		
T/T[c]	Women	2	131 ± 34	F = 8.55	F = 9.75	248 ± 137	F = 2.49	F = 4.05	41 ± 3	F = 0.68	F = 1.00
C/T[c]		16	124 ± 37	p = 0.006	p = 0.004[d]	224 ± 76	p = 0.12	p = 0.05[e]	42 ± 7	p = 0.42	p = 0.32[f]
C/C		20	92 ± 30			179 ± 64			40 ± 6		

5-HIAA = 5-hydroxyindoleacetic acid; HVA = homovanillic acid; MHPG = 3-methoxy-4-hydroxyphenylglycol. Statistical comparisons done on monoamine metabolite residuals corrected[a] and uncorrected[b] for back length. [c] T/T and C/T genotypes were combined in the analyses. [d] Correction for use of oral contraceptives, F = 5.56, p = 0.02. [e] Correction for use of oral contraceptives, F = 1.76, p = 0.19. [f] Correction for use of oral contraceptives, F = 0.17, p = 0.68. Analysing heterosis, i.e. comparing homo- vs heterozygotes: All subjects 5-HIAA: F = 10.03, p = 0.002 (F = 5.71, p = 0.02 after correction for back length). All subjects HVA: F = 7.13, p = 0.009 (F = 2.28, p = 0.10). Men 5-HIAA: F = 1.96, p = 0.17 (F = 1.00, p = 0.32). Men HVA: 2.33, p = 0.13 (F = 1.40, p = 0.24). Women 5-HIAA: F = 6.73, p = 0.01 (F = 5.65, p = 0.02 and F = 3.76, p = 0.06 corrected for back length and use of oral contraceptives, respectively). Women HVA: F = 2.49, p = 0.12 (F = 1.03, p = 0.32 and F = 0.98, p = 0.33 corrected for back length and use of oral contraceptives, respectively).

concentrations neither in the total sample, nor among men or women (table 3).

Relationships between DBH genotypes and CSF monoamine metabolite concentrations

The *DBH* -1021 C/C genotype was the most frequent (71%), followed by the -1021 C/T (26%) and the -1021 T/T genotypes (3%). The allele frequencies were 0.84 (-1021C) and 0.16 (-1021T). The -1021 T/T and -1021 C/T genotypes were pooled in the calculations, because of the small number of subjects with the -1021 T/T genotype. In the total sample there was an association between *DBH* genotype and 5-HIAA concentrations (p = 0.01 uncorrected, p = 0.003 when corrected for back-length), with higher 5-HIAA levels in subjects with the -1021T containing genotypes (table 4). This relationship reached significance also among men (p = 0.06 uncorrected, p = 0.04 corrected for back length), but not among women (table 4). In the total sample there was a tendency for association (p = 0.09) between the *DBH* variant and HVA concentrations, with higher HVA levels in -1021T carriers (table 4). When corrected for back-length the strength of this difference was significant (p = 0.03). Neither in the

Table 3: Dopamine D_4 receptor (*DRD4*) genotypes and relationships to monoamine metabolite concentrations in human lumbar cerebrospinal fluid.

			5-HIAA			HVA			MHPG		
DRD4 Genotype	Sex	n	Mean ± SD (nmol/L)	F[a] p[a]	F[b] p[b]	Mean ± SD (nmol/L)	F[a] p[a]	F[b] p[b]	Mean ± SD (nmol/L)	F[a] p[a]	F[b] p[b]
C/C	All	13	104 ± 43	F = 0.76	F = 0.22	199 ± 94	F = 1.39	F = 0.52	42 ± 7	F = 2.62	F = 2.68
C/T		52	99 ± 34	p = 0.47	p = 0.80	176 ± 68	p = 0.25	p = 0.59	41 ± 7	p = 0.08	p = 0.07
T/T		25	96 ± 41			181 ± 77			45 ± 8		
C/C	Men	12	104 ± 44	F = 0.99	F = 0.98	197 ± 98	F = 1.49	F = 1.60	43 ± 7	F = 1.93	F = 2.06
C/T		26	91 ± 35	p = 0.38	p = 0.38	156 ± 63	p = 0.24	p = 0.21	41 ± 8	p = 0.16	p = 0.14
T/T		14	84 ± 32			155 ± 50			46 ± 8		
C/C[c]	Women	1	107	F = 0.004	F = 0.11	229	F = 0.003	F = 0.48	38	F = 0.78	F = 1.23
C/T[c]		26	106 ± 33	p = 0.95	p = 0.74[d]	195 ± 68	p = 0.96	p = 0.49[e]	40 ± 6	p = 0.38	p = 0.27[f]
T/T		11	111 ± 47			214 ± 93			43 ± 7		

5-HIAA = 5-hydroxyindoleacetic acid; HVA = homovanillic acid; MHPG = 3-methoxy-4-hydroxyphenylglycol. Statistical comparisons done on monoamine metabolite residuals corrected[a] and uncorrected[b] for back length. [c] C/C and C/T genotypes were combined in the analyses. [d] Correction for use of oral contraceptives, F = 0.38, p = 0.54. [e] Correction for use of oral contraceptives, F = 0.04, p = 0.84. [f] Correction for use of oral contraceptives, F = 1.09, p = 0.30.

Table 4: Dopamine β-hydroxylase (*DBH*) genotypes and relationships to monoamine metabolite concentrations in human lumbar cerebrospinal fluid.

			5-HIAA			HVA			MHPG		
DBH Genotype	Sex	n	Mean ± SD (nmol/L)	F[a] p[a]	F[b] p[b]	Mean ± SD (nmol/L)	F[a] p[a]	F[b] p[b]	Mean ± SD (nmol/L)	F[a] p[a]	F[b] p[b]
C/C	All	64	92 ± 35	F = 9.07	F = 6.42	172 ± 66	F = 5.21	F = 3.00	42 ± 7	F = 0.01	F = 0.01
C/T[c]		23	118 ± 39	p = 0.003	p = 0.01	208 ± 93	p = 0.02	p = 0.09	42 ± 9	p = 0.90	p = 0.93
T/T[c]		3	78 ± 25			151 ± 18			40 ± 5		
C/C	Men	36	86 ± 36	F = 4.68	F = 3.80	156 ± 56	F = 2.22	F = 1.81	43 ± 7	F = 0.08	F = 0.04
C/T[c]		15	107 ± 35	p = 0.04	p = 0.06	186 ± 98	p = 0.14	p = 0.18	43 ± 10	p = 0.78	p = 0.84
T/T[c]		1	95			160			45		
C/C	Women	28	101 ± 31	F = 4.08	F = 3.37	192 ± 73	F = 3.72	F = 1.79	41 ± 6	F = 0.03	F = 0.08
C/T[c]		8	139 ± 38	p = 0.05	p = 0.07[e]	249 ± 72	p = 0.06	p = 0.19[d]	41 ± 7	p = 0.87	p = 0.78[f]
T/T[c]		2	70 ± 30			147 ± 23			38 ± 4		

5-HIAA = 5-hydroxyindoleacetic acid; HVA = homovanillic acid; MHPG = 3-methoxy-4-hydroxyphenylglycol. Statistical comparisons done on monoamine metabolite residuals corrected[a] and uncorrected[b] for back length. [c] T/T and C/T genotypes were combined in the analyses. [d] Correction for use of oral contraceptives, F = 0.88, p = 0.36. [e] Correction for use of oral contraceptives, F = 0.46, p = 0.50. [f] Correction for use of oral contraceptives, F = 0.003, p = 0.96. Analysing heterosis, i.e. comparing homo- vs heterozygotes: All subjects 5-HIAA: F = 9.56, p = 0.003 (F = 13.07, p = 0.0005 after correction for back length). All subjects HVA: F = 4.43, p = 0.04 (F = 7.24, p = 0.009). Men 5-HIAA: F = 3.85, p = 0.06 (F = 4.6187, p = 0.04). Men HVA: 1.95, p = 0.17 (F = 2.31, p = 0.14). Women 5-HIAA: F = 9.14, p = 0.005 (F = 9.74, p = 0.004 and F = 4.11, p = 0.05 corrected for back length and use of oral contraceptives, respectively). Women HVA: F = 4.43, p = 0.04 (F = 6.42, p = 0.02 and F = 1.81, p = 0.19 corrected for back length and use of oral contraceptives, respectively).

smaller male or female sub-samples this difference reached significance (table 4).

We also performed calculations pooling the homozygotic genotypes, i.e. analysing possible heterosis [52]. In the total sample there was an association between *DBH* heterozygosity and 5-HIAA concentrations (p = 0.003 uncorrected, p = 0.0005 when corrected for back-length) (table 4). This relationship reached significance also among men (p = 0.06 uncorrected, p = 0.04 corrected for back length) and women (p = 0.005 uncorrected, p = 0.02 corrected for back length, p = 0.05 corrected for use of oral contraceptives; table 4). In the total sample there was an association (p = 0.04 uncorrected, p = 0.009 corrected for back-length) between *DBH* heterozygosity and HVA concentrations (table 4). This association failed to obtain sig-

nificance in the smaller male sub-sample. Among women there was an association (p = 0.04 uncorrected, p = 0.02 corrected for back-length), which however did not survived correction for use of oral contraceptives (p = 0.18; table 4).

There were no significant relationships between the *DBH* genotype and MHPG concentrations (table 4).

Given $\alpha = 0.05$ the present study had a power of 0.93 – 0.96 (total sample), 0.67 – 0.81 (men), or 0.53 – 0.67 (women) to detect differences of a large effect size (f = 0.40). For differences of a medium effect size (f = 0.25) the power was 0.54 – 0.65 (total sample) or less.

Discussion

The present study is, to our knowledge, the first to investigate three potentially functional candidate gene variants (*HTR3A* 178 C/T, *DRD4* -521 C/T, *DBH* -1021 C/T) in the context of monoamine metabolite concentrations in cerebrospinal fluid from human healthy volunteers. Association was detected between two of these gene variants (*HTR3A* 178 C/T, *DBH* -1021 C/T) and the indirect measures of monoamine activity in the brain.

However, we were not able to replicate the previously reported finding of higher CSF MHPG concentrations in *HTR2C* Ser23 compared to Cys23 carriers among men [9]. On the contrary, our male sub-sample showed a non-significant relationship in the opposite direction, i.e. higher MHPG concentrations in Cys23 subjects (table 1). When we added another 23 men, excluded from the main analysis because of reported life-time psychiatric disorder, mostly alcohol abuse, depressive or anxiety disorders, this difference reached nominal significance (F = 4.44, d.f. = 71, p = 0.04), a result robust for correction for back-length (p = 0.04) and presence of life-time psychiatric disorder (p = 0.04), respectively (data not shown). Reasons for the different results in the two studies may include the different selection of subjects. The previous study included 73% alcoholic violent offenders and 27% healthy controls of Finnish ethnicity, and the *HTR2C* genotype effect was most prominent among the offenders [9], whereas the present study only included healthy subjects. The present study may lack power to detect the previous relationship. Alternatively, assuming different relationships between alcoholic violent offenders and healthy controls, the Finnish study may be under-powered concerning control subjects. It is also possible that both results are valid, but the results reflect an association to a linked variant, and that the degree of linkage between the *HTR2C* Cys23Ser variant and the 'real' functional polymorphism differs between the two populations investigated. There may also be a difference between the two populations with regard to other genes interacting with the present to influence MHPG concentrations. It is also possible that the Finnish, the present or both results have emerged by chance.

Subjects carrying the rarer *HTR3A* 178T allele, which has been associated with higher protein expression than the wild-type variant [14], displayed higher lumbar CSF 5-HIAA concentrations. This suggests that a more efficient variant of the 5-HT_3 receptor, involved in the regulation of serotonin activities, enhances brain serotonin turnover, giving rise to higher levels of the serotonin degradation product in CSF.

We were not able to find any significant relationships between *DRD4* -521 C/T variation and CSF monoamine metabolite concentrations. This is in accordance with previous studies, analysing a *DRD4* exon 3 variable number of tandem repeat variant [42,53]. In the present report there was a trend for an association between the *DRD4* -521 C/T genotypes and CSF MHPG concentrations. This may mean that the present study does not have sufficient power to detect such a relationship, or that this trend reflects a tendency to a false positive finding. The results so far obtained suggest that the *DRD4* gene does not have a large impact on the monoamine turnover in the brain as reflected by the major degradation products of these compounds in healthy human subjects.

One would expect that functional variants of the gene encoding the dopamine β-hydroxylase would primarily affect the catecholamines, in particular norepinephrine. However, in the present study the strongest relationship emerged between the *DBH* -1021 C/T variant and CSF 5-HIAA levels. Complex interactions between the noradrenergic and serotonergic systems have been reported [54,55]. Altered noradrenergic activity may alter the firing activity of serotonergic neurons, leaving a possibility for a decreased or increased availability of norepinehprine to be involved in these interactions. One might speculate that a more effective *DBH* variant, giving rise to an enhanced norepinephrine formation, facilitates noradrenergic activity, which in its turn facilitates serotonergic activity, giving rise to larger amounts of the serotonin degradation product 5-HIAA. There was also an association between *DBH* -1021 C/T variation and the major dopamine degradation product, indicating higher HVA levels in subjects with a less effective enzyme variant. This is in accordance with the theory that a less effective conversion of dopamine to norepinephrine would lead to higher amounts of dopamine, and in turn to its degradation product HVA. However, the stronger associations between heterozygotic genotypes and 5-HIAA and HVA concentrations, examples of positive heterosis [52], rather indicate a more complex physiology including interactions based on hidden stratification of unknown factors or heterozygotic advantage [52]. In this context, an inter-

action with e.g. the monoamine oxidase A gene, where more effective variants have been reported to increase 5-HIAA concentrations [43,56], may be a possibility. However, these latter results further complicate the task, as they call into question the use of monoamine metabolites as straightforward, although indirect, measures of brain turnover. This may mean that high levels of 5-HIAA may reflect a more effective degradation process rather than an enhanced overall turnover giving a possibility for reduced serotonin transmission to be associated with high levels of 5-HIAA.

It is possible that the present associations may have emerged by chance. Applying Bonferroni's correction would give a p-value of < 0.0011 (0.05/45) to be considered significant. Only one of the reported relationships, i.e. the association between *DBH* heterozygosity and CSF 5-HIAA levels, would survive such a correction procedure. On the other hand, although relatively large to constitute a sample of healthy subjects investigated by a demanding procedure, i.e. lumbar puncture, the present sample is small from a statistical point of view. The power of the present study was adequate to detect differences of large, but not medium to small effect sizes. Thus, it cannot be excluded that relationships of smaller magnitudes may have escaped our analysis attempts. Applying strict corrections for multiple testing would make investigations like the present impossible to perform, because a sample big enough to withstand such a correction procedure would probably never be possible to obtain. This is especially true taking the detection of small effects into account.

Conclusions

If replicated, the present results suggests that the *HTR3A* and *DBH* variants participate differentially in the regulation of serotonin turnover in the central nervous system of human subjects. It is also suggested that the *DBH* variant differentially influence dopamine turnover in the brain. The results give some support for an influence of the *HTR2C* variant on norepinephrine turnover in men, but do not favour a major differential influence of *DRD4* gene activities on monoamine metabolite concentrations in lumbar CSF.

Competing interests

None declared.

Authors' contributions

EGJ performed the second clinical investigation, the statistical analyses, and drafted the manuscript. JB, JM, RAJ, JS, LW, and RI performed the genotyping. SC, PP, MMN and EE participated in the design and co-ordination of the study. GCS conceived of the study and participated in its design and co-ordination. All authors read and approved the final manuscript.

Acknowledgements

This study was supported by grants from the Swedish Research Council (8668, K2004-21X-15078-01A), Deutsche Forschungsgemeinschaft (SFB400, TPA3), the Fund for Scientific Research – Flanders (G.0425.02), the National Institute for Mental Health (44814), Söderström-Königska Foundation, the Lundberg Foundation, the Wallenberg Foundation, and the HUBIN project. We thank Kjerstin Lind and Alexandra Tylec for technical assistance.

References

1. Oxenstierna G, Edman G, Iselius L, Oreland L, Ross SB, Sedvall G: **Concentrations of monoamine metabolites in the cerebrospinal fluid of twins and unrelated individuals – a genetic study.** *J Psychiatr Res* 1986, **20:**19-29.
2. Higley JD, Thompson WW, Champoux M, Goldman D, Hasert MF, Kraemer GW, Scanlan JM, Suomi SJ, Linnoila M: **Paternal and maternal genetic and environmental contributions to cerebrospinal fluid monoamine metabolites in rhesus monkeys (Macaca mulatta).** *Arch Gen Psychiatry* 1993, **50:**615-623.
3. Pompeiano M, Palacios JM, Mengod G: **Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors.** *Brain Res Mol Brain Res* 1994, **23:**163-178.
4. Blandina P, Goldfarb J, Walcott J, Green JP: **Serotonergic modulation of the release of endogenous norepinephrine from rat hypothalamic slices.** *J Pharmacol Exp Ther* 1991, **256:**341-347.
5. Chiang G, Aston-Jones G: **A 5-hydroxytryptamine2 agonist augments gamma-aminobutyric acid and amino acid inputs to noradrenergic locus coeruleus neurons.** *Neuroscience* 1993, **54:**409-420.
6. Done CJ, Sharp T: **Biochemical evidence for the regulation of central noradrenergic activity by 5-HT1A and 5-HT2 receptors: microdialysis studies in the awake and anaesthetized rat.** *Neuropharmacology* 1994, **33:**411-421.
7. Milatovich A, Hsieh CL, Bonaminio G, Tecott L, Julius D, Francke U: **Serotonin receptor 1c gene assigned to X chromosome in human (band q24) and mouse (bands D-F4).** *Hum Mol Genet* 1992, **1:**681-684.
8. Lappalainen J, Zhang L, Dean M, Oz M, Ozaki N, Yu DH, Virkkunen M, Weight F, Linnoila M, Goldman D: **Identification, expression, and pharmacology of a Cys23-Ser23 substitution in the human 5-HT2c receptor gene (HTR2C).** *Genomics* 1995, **27:**274-279.
9. Lappalainen J, Long JC, Virkkunen M, Ozaki N, Goldman D, Linnoila M: **HTR2C Cys23Ser polymorphism in relation to CSF monoamine metabolite concentrations and DSM-III-R psychiatric diagnoses.** *Biol Psychiatry* 1999, **46:**821-826.
10. Miyake A, Mochizuki S, Takemoto Y, Akuzawa S: **Molecular cloning of human 5-hydroxytryptamine3 receptor: heterogeneity in distribution and functionamong species.** *Mol Pharmacol* 1995, **48:**407-416.
11. Matsumoto M, Yoshioka M, Togashi H, Tochihara M, Ikeda T, Saito H: **Modulation of norepinephrine release by serotonergic receptors in the rat hippocampus as measured by in vivo microdialysis.** *J Pharmacol Exp Ther* 1995, **272:**1044-1051.
12. Benloucif S, Keegan MJ, Galloway MP: **Serotonin-facilitated dopamine release in vivo: pharmacological characterization.** *J Pharmacol Exp Ther* 1993, **265:**373-377.
13. Tricklebank MD: **The functional importance of 5-HT3 receptors in the interactions between serotonergic and dopaminergic systems in the CNS.** In *Central and Peripheral 5-HT3 receptors* Edited by: *Hamen M. London: Academic Press*; 1992:189-205.
14. Niesler B, Flohr T, Nöthen MM, Fischer C, Rietschel M, Franzek E, Albus M, Propping P, Rappold GA: **Association between the 5' UTR variant C178T of the serotonin receptor gene HTR3A and bipolar affective disorder.** *Pharmacogenetics* 2001, **11:**471-475.
15. Melke J, Westberg L, Nilsson S, Landén M, Soderstrom H, Baghaei F, Rosmond R, Holm G, Björntorp P, Nilsson LG, Adolfsson R, Eriksson E: **A polymorphism in the serotonin receptor 3A gene (HTR3A) is associated with harm avoidance in women.** *Arch Gen Psychiatry* 2003, **60:**1017-1023.
16. Lahti RA, Roberts RC, Conley RR, Cochrane EV, Mutin A, Tamminga CA: **D2-type dopamine receptors in postmortem human**

brain sections from normal and schizophrenic subjects. *Neuroreport* 1996, **7:**1945-1948.
17. Rubinstein M, Phillips TJ, Bunzow JR, Falzone TL, Dziewczapolski G, Zhang G, Fang Y, Larson JL, McDougall JA, Chester JA, Saez C, Pugsley TA, Gershanik O, Low MJ, Grandy DK: **Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine.** *Cell* 1997, **90:**991-1001.
18. Tarazi FI, Baldessarini RJ: **Brain dopamine D(4) receptors: basic and clinical status.** *Int J Neuropsychopharmcol* 1999, **2:**41-58.
19. Gelernter J, Kennedy JL, Van Tol HHM, Civelli O, Kidd KK: **The D4 dopamine receptor (DRD4) maps to distal 11p close to HRAS.** *Genomics* 1992, **13:**208-210.
20. Okuyama Y, Ishiguro H, Toru M, Arinami T: **A genetic polymorphism in the promoter region of DRD4 associated with expression and schizophrenia.** *Biochem Biophys Res Commun* 1999, **258:**292-295.
21. Okuyama Y, Ishiguro H, Nankai M, Shibuya H, Watanabe A, Arinami T: **Identification of a polymorphism in the promoter region of DRD4 associated with the human novelty seeking personality trait.** *Mol Psychiatry* 2000, **5:**64-69.
22. Ronai Z, Szekely A, Nemoda Z, Lakatos K, Gervai J, Staub M, Sasvari-Szekely M: **Association between Novelty Seeking and the -521 C/Tpolymorphism in the promoter region of the DRD4 gene.** *Mol Psychiatry* 2001, **6:**35-38.
23. Bookman EB, Taylor RE, Adams-Campbell L, Kittles RA: **DRD4 promoter SNPs and gender effects on Extraversion in African Americans.** *Mol Psychiatry* 2002, **7:**786-789.
24. Lee HJ, Lee HS, Kim YK, Kim SH, Kim L, Lee MS, Joe SH, Jung IK, Suh KY, Kim S: **Allelic variants interaction of dopamine receptor D4 polymorphism correlate with personality traits in young Korean female population.** *Am J Med Genet* 2003, **118B:**76-80.
25. Mitsuyasu H, Hirata N, Sakai Y, Shibata H, Takeda K, Ninomiya H, Kawasaki H, Tashiro N, Fukumaki Y: **Association analysis of polymorphisms in the upstream region of the human dopamine D4 receptor gene (DRD4) with schizophrenia and personality traits.** *J Hum Genet* 2001, **46:**26-31.
26. Jönsson EG, Ivo R, Forslund K, Mattila-Evenden M, Rylander G, Cichon S, Propping P, Nöthen MM, Åsberg M, Sedvall GC: **No association between a promoter dopamine D4 receptor gene variant and schizophrenia.** *Am J Med Genet* 2001, **105:**525-528.
27. Jönsson EG, Ivo R, Gustavsson JP, Geijer T, Forslund K, Mattila-Evenden M, Rylander G, Cichon S, Propping P, Bergman H, Åsberg M, Nöthen MM: **No association between dopamine D4 receptor gene variants and Novelty Seeking.** *Mol Psychiatry* 2002, **7:**18-20.
28. Ekelund J, Suhonen J, Järvelin MR, Peltonen L, Lichtermann D: **No association of the -521 C/T polymorphism in the promoter of DRD4 with novelty seeking.** *Mol Psychiatry* 2001, **6:**618-619.
29. Strobel A, Lesch KP, Hohenberger K, Jatzke S, Gutzeit HO, Anacker K, Brocke B: **No association between dopamine D4 receptor gene exon III and-521C/T polymorphism and Novelty Seeking.** *Mol Psychiatry* 2002, **7:**537-538.
30. Strobel A, Spinath FM, Angleitner A, Riemann R, Lesch KP: **Lack of association between polymorphisms of the dopamine D4 receptor gene and personality.** *Neuropsychobiology* 2003, **47:**52-56.
31. Joyce PR, Rogers GR, Miller AL, Mulder RT, Luty SE, Kennedy MA: **Polymorphisms of DRD4 and DRD3 and risk of avoidant and obsessive personality traits and disorders.** *Psychiatry Res* 2003, **119:**1-10.
32. Schinka JA, Letsch EA, Crawford FC: **DRD4 and novelty seeking: results of meta-analyses.** *Am J Med Genet* 2002, **114:**643-648.
33. Munafò MR, Clark TG, Moore LR, Payne E, Walton R, Flint J: **Genetic polymorphisms and personality in healthy adults: a systematic review and meta-analysis.** *Mol Psychiatry* 2003, **8:**471-484.
34. Jönsson EG, Sedvall GC, Nöthen MM, Cichon S: **Dopamine D4 receptor gene (DRD4) variants and schizophrenia: meta-analyses.** *Schizophr Res* 2003, **61:**111-119.
35. Kemper CM, O'Connor DT, Westlund KN: **Immunocytochemical localization of dopamine-beta-hydroxylase in neurons of the human brain stem.** *Neuroscience* 1987, **23:**981-989.
36. Oka K, Kijikawa K, Ohuchi T, Yoshida H, Imaizumi R: **Distribution of dopamine-β-hydroxylase activity in subcellular fractions of adrenal medulla.** *Life Sci* 1967, **6:**461-465.
37. Weinshilboum RM: **Biochemical genetics of catecholamines in humans.** *Mayo Clin Proc* 1983, **58:**319-330.
38. Craig SP, Buckle VJ, Lamouroux A, Mallet J, Craig IW: **Localization of the human dopamine beta hydroxylase (DBH) gene to chromosome 9q34.** *Cytogenet Cell Genet* 1988, **48:**48-50.
39. Zabetian CP, Anderson GM, Buxbaum SG, Elston RC, Ichinose H, Nagatsu T, Kim KS, Kim CH, Malison RT, Gelernter J, Cubells JF: **A quantitative-trait analysis of human plasma-dopamine beta-hydroxylase activity: Evidence for a major functional polymorphism at the DBH locus.** *Am J Hum Genet* 2001, **68:**515-522.
40. Köhnke MD, Zabetian CP, Anderson GM, Kolb W, Gaertner I, Buchkremer G, Vonthein R, Schick S, Lutz U, Köhnke AM, Cubells JF: **A genotype-controlled analysis of plasma dopamine beta-hydroxylase in healthy and alcoholic subjects: evidence for alcohol-related differences in noradrenergic function.** *Biol Psychiatry* 2002, **52:**1151-1158.
41. Zabetian CP, Buxbaum SG, Elston RC, Köhnke MD, Anderson GM, Gelernter J, Cubells JF: **The structure of linkage disequilibrium at the DBH locus strongly influences the magnitude of association between diallelic markers and plasma dopamine beta-hydroxylase activity.** *Am J Hum Genet* 2003, **72:**1389-1400.
42. 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: **Dopamine-related genes and their relationships to monoamine metabolites in CSF.** *Biol Psychiatry* 1996, **40:**1032-1043.
43. Jönsson EG, Norton N, Gustavsson JP, Oreland L, Owen MJ, Sedvall GC: **A promoter polymorphism in the monoamine oxidase A gene and its relationships to monoamine metabolite concentrations in CSF of healthy volunteers.** *J Psychiatr Res* 2000, **34:**239-244.
44. American Psychiatric Association: *Diagnostic and Statistical Manual of Mental Disorders* Thirdth edition. *Washington DC: American Psychiatric Association*; 1987.
45. Sedvall G, Fyrö B, Gullberg B, Nybäck H, Wiesel F-A, Wode-Helgodt B: **Relationships in healthy volunteers between concentrations of monoamine metabolites in cerebrospinal fluid and family history of psychiatric morbidity.** *Br J Psychiatry* 1980, **136:**366-374.
46. Swahn C-G, Sandgärde B, Wiesel F-A, Sedvall G: **Simultaneous determination of the three major monoamine metabolites in brain tissue and body fluids by a mass fragmentographic method.** *Psychopharmacology* 1976, **48:**147-152.
47. Geijer T, Neiman J, Rydberg U, Gyllander A, Jönsson E, Sedvall G, Valverius P, Terenius L: **Dopamine D2 receptor gene polymorphisms in Scandinavian chronic alcoholics.** *Eur Arch Psychiatry Clin Neurosci* 1994, **244:**26-32.
48. Jönsson EG, Abou Jamra R, Schumacher J, Flyckt L, Edman G, Forslund K, Mattila-Evenden M, Rylander G, Åsberg M, Bjerkenstedt L, Wiesel F-A, Propping P, Cichon S, Nöthen MM, Sedvall GC: **No association between a putative functional promoter variant in the dopamine β-hydroxylase gene and schizophrenia.** *Psychiatr Genet* 2003, **13:**175-178.
49. Jönsson EG, Goldman D, Spurlock G, Gustavsson JP, Nielsen DA, Linnoila M, Owen MJ, Sedvall GC: **Tryptophan hydroxylase and catechol-O-methyltransferase gene polymorphisms. Relationships to monoamine metabolite concentrations in CSF of healthy volunteers.** *Eur Arch Psychiatry Clin Neurosci* 1997, **247:**297-302.
50. Cohen J: **Statistical power analysis for the behavioral sciences.** *Hillsdale, New Jersey: Lawrence Erlbaum Associates*; 1988.
51. Erdfelder E, Faul F, Buchner A: **GPower: a general power analysis program.** *Behav Res Methods Instruments Comput* 1996, **28:**1-11.
52. Comings DE, MacMurray JP: **Molecular heterosis: a review.** *Mol Genet Metab* 2000, **71:**19-31.
53. Adamson MD, Kennedy J, Petronis A, Dean M, Virkkunen M, Linnoila M, Goldman D: **DRD4 dopamine receptor genotype and CSF monoamine metabolites in Finnish alcoholics and controls.** *Am J Med Genet* 1995, **60:**199-205.
54. Svensson TH: **Brain noradrenaline and the mechanisms of action of antidepressant drugs.** *Acta Psychiatr Scand Volume 402. Suppl.*; 2000:18-27.
55. Blier P: **Crosstalk between the norepinephrine and serotonin systems and its role in the antidepressant response.** *J Psychiatry Neurosci* 2001, **26:**S3-S10.
56. Williams RB, Marchuk DA, Gadde KM, Barefoot JC, Grichnik K, Helms MJ, Kuhn CM, Lewis JG, Schanberg SM, Stafford-Smith M, Suarez EC, Clary GL, Svenson IK, Siegler IC: **Serotonin-related gene**

polymorphisms and central nervous system serotonin function. *Neuropsychopharmacology* 2003, **28:**533-541.

Pre-publication history

The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-244X/4/4/prepub