

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

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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 D₄ receptor (*DRD4*), and the dopamine β-hydroxylase (*DBH*) genes and CSF concentrations of 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylglycol (MPHG) in healthy volunteers (n = 90).

Results: The *HTR3A* 178 C/T variant was associated with 5-HIAA levels (p = 0.02). The *DBH*-102I 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-hydroxyphe-nylglycol (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-HT₃ receptor is a ligand-gated ion channel [10]. In the brain 5-HT₃ 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-HT₃ 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 D₄ receptor has a predominantly cortical localisation in the human brain [16]. Among several effects, the dopamine D₄ receptor seems to modulate dopamine synthesis and turnover [17,18]. The dopamine D₄ 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.

HTR2C Allele/genotype	Sex	n	5-HIAA			HVA			MHPG		
			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.

HTR3A Genotype	Sex	n	5-HIAA			HVA			MHPG		
			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 D₄ receptor (DRD4) genotypes and relationships to monoamine metabolite concentrations in human lumbar cerebrospinal fluid.

DRD4 Genotype	Sex	n	5-HIAA			HVA			MHPG		
			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.

DBH Genotype	Sex	n	5-HIAA			HVA			MHPG		
			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 survive 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-HT₃ 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 norepinephrine 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.

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