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Replication of functional serotonin receptor type 3A and B variants in bipolar affective disorder: a European multicenter study

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Serotonin type 3 receptors (5-HT_3) are involved in learning, cognition and emotion, and have been implicated in various psychiatric phenotypes. However, their contribution to the pathomechanism of these disorders remains elusive. Three single nucleotide polymorphisms (SNPs) in the *HTR3A* and *HTR3B* genes (rs1062613, rs1176744 and rs3831455) have been associated with bipolar affective disorder (BPAD) in pilot studies, and all of them are of functional relevance. We performed a European multicenter study to confirm previous results and provide further evidence for the relevance of these SNPs to the etiology of neuropsychiatric disorders. This involved analysis of the distribution of the three SNPs among 1804 BPAD cases and 2407 healthy controls. A meta-analysis revealed a pooled odds ratio of 0.881 ($P = 0.009$, 95% confidence intervals = 0.802–0.968) for the non-synonymous functional SNP *HTR3B* p.Y129S (rs1176744), thereby confirming previous findings. In line with this, the three genome-wide association study samples BOMA (Bonn-Mannheim)-BPAD, WTCCC (Wellcome Trust Case Control Consortium)-BPAD and GAIN (Genetic Association Information Network)-BPAD, including >3500 patients and 5200 controls in total, showed an overrepresentation of the p.Y129 in patients. Remarkably, the meta-analysis revealed a *P*-value of 0.048 (OR = 0.934, fixed effect model). We also performed expression analyses to gain further insights into the distribution of *HTR3A* and *HTR3B* mRNA in the human brain. *HTR3A* and *HTR3B* were detected in all investigated brain tissues with the exception of the cerebellum, and large differences in the A:B subunit ratio were observed. Interestingly, expression of the B subunit was most prominent in the brain stem, amygdalae and frontal cortex, regions of relevance to psychiatric disorders. In conclusion, the present study provides further evidence for the presence of impaired 5-HT_3 receptor function in BPAD.
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Introduction

Serotonin type 3 (5-HT_3) receptors are involved in neural processes related to cognition and emotion, and antagonists of these receptors have been shown to be beneficial in the treatment of various psychiatric disorders. In particular, improvement in depressive symptoms, memory and cognition have been described in psychiatric patients.[1] Furthermore, distinct 5-HT_3 receptor variants have been implicated in the pathophysiology of bipolar affective disorder (BPAD), schizophrenia and eating disorders.[2] Functional receptors contain five subunits of diverse composition. In humans, 5-HT_3 subunits are encoded by five genes: *HTR3A–E*. Of these subunits, the 5-HT3A and 5-HT3B have been characterized most extensively. *In vitro* studies have shown that only the 5-HT3A subunit is capable of forming functional homopentameric receptors whereas the other subunits require 5-HT3A to build heteropentameric complexes.[3] Comparative expression analyses of all five *HTR3* genes on the mRNA level, using conventional PCR have shown that expression of the 5-HT3E subunit is restricted to certain tissues of the gastrointestinal tract, whereas the other subunits are expressed ubiquitously.[4] Early studies in the 1990s using radioligand binding revealed 5-HT_3 selective binding in distinct brain regions. Different ligand-binding densities were observed in the forebrain regions analyzed, depending on the radioligand used; for example, zacopride, LY278584 or granisetron.[5–8] However, 5-HT3A was the only known subunit of this type of receptors at that time, the complexity of the human 5-HT_3 receptor system first became apparent during the last decade, and the composition of native receptor subtypes remains elusive.[2]

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Consequently, the difference in binding density depending on the applied ligand may reflect the existence of receptor subtypes of differing compositions and affinities.[3] Although expression of *HTR3A* has been reported in the cerebral cortex, amygdala, hippocampus, caudate and thalamus on the mRNA level in humans[9,10] contradictory data exist concerning the expression of *HTR3B* in the central nervous system.[11]

In two pilot case–control studies, the variants *HTR3A* c.−42C>T (rs1062613) and *HTR3B* p.Y129S (c.386A>C, rs1176744), as well as the small deletion *HTR3B* c.−104_−102delAGA (rs3831455) were found to be associated with BPAD.[12,13]

All of these variants have been shown to exhibit functional effects *in vitro*.[13–19] Furthermore, the two single nucleotide exchanges have also been implicated in anorexia nervosa and irritable bowel syndrome, both of which show high comorbidity with anxiety and depression,[14,20] whereas *HTR3B* p.Y129S appears to have a role in major depression.[21] In addition, *HTR3A* c.−42C>T has been correlated with altered amygdala activity and increased anxiety scores in paradigms testing emotional response *in vivo*.[22,23]

The identification of identical risk variants for different psychiatric phenotypes supports the hypothesis that in spite of discriminable clinical diagnoses, these phenotypes share some aspects of the underlying biology.[24] The aim of the present European multicenter study was to confirm the previous association findings and provide further evidence for the hypothesis that functional *HTR3* variants are implicated in the etiology of various psychiatric disorders. We successfully replicated the association between the *HTR3B* variant p.Y129S (rs1176744) and BPAD in a large European case–control cohort consisting of >1800 cases and 2400 controls. Furthermore, we were able to confirm co-expression of both the 5-HT3A and 5-HT3B subunits in brain structures of relevance to anxiety and depression.

Materials and methods

European patient–control samples. All protocols and procedures were approved by the respective local Ethics Committees. Written informed consent was obtained from all study participants before the study participation. All patients were assigned a lifetime diagnosis of BPAD type I or type II. This was based on Diagnostic and Statistical Manual of Mental Disorders-IV criteria and a consensus best-estimate procedure, including a structured interview-I, review of medical records, the family history method and the Operational Criteria Checklist for Psychotic Illness OPCRIT system. Patients were recruited from consecutive admissions to the psychiatric inpatient units of seven clinical centers: (A) the Central Institute of Mental Health, Mannheim, and the Department of Psychiatry and Psychotherapy of the University of Bonn ($n=378$ patients/768 controls); (B) Department of Psychiatry, Poznan University of Medical Sciences, Poznan, Poland ($n=446$ patients/558 controls); (C) Alexandru Obregia Clinical Psychiatric Hospital, Bucharest, Romania ($n=237$ patients/235 controls); (D) Civil Hospital Carlos Haya, Malaga, Spain ($n=297$ patients/401 controls); (E) Russian State Medical University, Moscow ($n=331$ patients/331 controls); (F) Psychiatric Clinic, Clinical Center of the University of Sarajevo, Sarajevo, Bosnia-Herzegovina ($n=124$ patients/115 controls). Ethnicity was assigned to patients and controls on the basis of self-reported ancestry at each of the seven recruitment sites. The given numbers reflect the sample sizes after quality control (sample call rate >90%). This sample was used before for replication purposes as part of a genome-wide association study study (replication I).[25]

Independent replication data sets. To further strengthen our association finding, rs1176744 was examined in three independent genome-wide data sets.[25–27] First, this study makes use of data generated for the BOMA (Bonn-Mannheim)-BPAD case–control cohort consisting of 682 cases and 1300 controls.[25] In addition, data for two independent, well-known BPAD studies were included. The Wellcome Trust Case–Control Consortium BPAD study[26] makes use of a cohort of 1868 patients and 2938 controls. As rs1176744 was not available in the set of genotyped markers for this study, the proxy rs4938058 ($r^2=0.898$) was analysed. Finally, a sample of 987 patients and 1000 controls was derived from the Database of Genotypes and Phenotypes data set[28] 'Whole Genome Association Study of Bipolar Disorder' (phs000017.v3.p1).[27] Here, another proxy rs1176743 ($r^2=0.919$) was used.

DNA extraction and genotyping. EDTA anti-coagulated venous blood samples were collected from all individuals. Lymphocyte DNA was isolated by salting-out with saturated sodium chloride solution 4, or by the use of a Chemagic Magnetic Separation Module I (Chemagen, Baesweiler, Germany) according to the manufacturer's recommendations.

Genotyping was performed using the Matrix-Assisted-Laser-Desorption/Ionization–Time-Of-Flight (MALDI-TOF)-based MassARRAY system and iPLEX Gold assays (Sequenom, San Diego, CA, USA). Primer sequences and assay conditions are obtainable upon request. Single nucleotide polymorphisms (SNPs) raw genotype data, that is, cluster plots, were visually assessed by two investigators. SNP and sample call rates were >90%.

Statistical analyses. PLINK (v1.07; http://pngu.mgh.harvard.edu/~purcell/plink/) was used for the analysis of statistical association between the single SNPs and affection status (trend test), and to test for deviations from Hardy-Weinberg equilibrium. The SNPassoc package (http://www.creal.cat/jrgonzalez/software.htm) for *R* was used to calculate *P*-values, odds ratios (OR) and 95% confidence intervals (CI), assuming a log-additive inheritance model adjusted for sex.[29] SPSS Statistics 17.0 (IBM Deutschland GmbH, Munich, Germany) was used in the performance of a Cochran-Maentel-Haentzel (CMH) meta-analysis to obtain pooled estimates of the ORs across nationalities, and Breslow-Day tests for homogeneity of the ORs. Significant *P*-values were corrected for three tests using the Bonferroni method, but are displayed uncorrected in text and tables. For the replication analysis in genome-wide association study data sets, a fixed effect model was used assuming an additive effect. To assess putative false-negative results, a power calculation

was carried out using Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/).[30]

Expression analyses on the mRNA level. The total RNA from human adult brain used in the present experiments was either obtained commercially or was isolated from brain tissue obtained from the German Brain Bank Brain-Net (http://www.brain-net.net). The human fetal material was provided by the Joint MRC Wellcome Trust Human Developmental Biology Resource (http://www.hdbr.org). Trizol reagent (Life Technologies, Darmstadt, Germany) was used for the extraction of RNA, as recommended by the manufacturer (Table 1). The experiments were approved by the ethics committee of the University of Heidelberg.

Total RNA (100 ng) served as input material in nCounter (Nanostring, Seattle, WA, USA) analysis using a customized codeset, as recommended by the manufacturer. In particular, the used codesets for *HTR3A* and *HTR3B* expression profiling among 18 other target genes are included in Table 2 (Supplementary data). Background correction and normalization of data was performed as follows. In brief, for each sample, the average + 2 SDS of background counts (negative controls) was calculated. For each gene for each sample, the average + 2 SDS was subtracted from the counts. Expression of *HTR3A* (NM_000869.5) and *HTR3B* (NM_006028.3) was normalized by taking the geometric mean of the expression of the three reference genes *ADAM9* (NM_003816.2), *PGK1* (NM_000291.3) and *SNX17* (NM_014748.2) into account.[31]

Results

In the current case–control study, >1800 cases and 2400 controls from six European countries were analyzed (Table 2). Genotype call rates were at least 97.6% for each SNP, and none of the tested variants deviated from Hardy-Weinberg equilibrium in patients or controls (data not shown).

For *HTR3B* p.Y129S (c.386A>C, rs1176744) a pooled odds ratio (OR) of 0.881 (95% CI = 0.802–0.968) was determined in a CMH analysis, yielding a significant *P*-value of 0.009 (Figure 1). This was because of an overrepresentation of the p.Y129 allele in BPAD patients. A Breslow-Day test for homogeneity of ORs revealed no significant deviation ($P = 0.076$). In the national subgroups, significant *P*-values were detected for the Polish and Spanish cohorts in a Cochran-Armitage trend test, under the assumption of a log-additive inheritance model (Table 3). The *P*-values

Table 2 Number of genotyped BPAD cases and controls, totals and according to nationality

Nationality	N *(cases)*	N *(controls)*	N *(Cases + controls)*
Bosnian-Herzegovinan	124	115	239
German	378	768	1146
Polish	446	558	1004
Romanian	237	233	471
Russian	331	333	663
Spanish	298	400	698
Total	**1814**	**2407**	**4221**

Abbreviation: BPAD, bipolar affective disorder.

Table 1 Brain tissue used for total RNA extraction followed by nCounter analysis

RNA	*Donor information*					
	Number	*Race*	*Gender*	*Age*	*COD*	*Company*
Brain	Pool of 3	Caucasian	Male	23	Cardiac arrest	FirstChoice Human Total RNA Survey Panel
		Caucasian	Male	81	Congestive heart failure	
		Caucasian	Female	78	Congestive heart failure	
Frontal cortex	Single	Caucasian	Male	23	Cardiac Arrest	FirstChoice Human Total RNA
Thalamus	Single	n.a.	Female	71	Chronic obstructive pulmonary disease	BioChain Total RNA
Right cerebellum	Single	n.a.	Male	82	Aortic stenosis	BioChain Total RNA
Hippocampus	Single	Asian	Male	27	Accident	BioChain Total RNA
Spinal cord	Single	Asian	Male	27	Accident	BioChain Total RNA
Striatum	Single	n.a.	Male	20	n.a.	Stratagene MVP Total RNA
Insula	Pool of 15	Caucasian	Male/ female	20–68	Sudden death	Clontech Total RNA
Amygdala	Pool of 2	Caucasian	Female	21	Pneumonia	BrainNet Germany
		Caucasian	Male	68	Cardiac arrest	BrainNet Germany
Brain stem	Pool of 2	Caucasian	Female	21	Pneumonia	BrainNet Germany
		Caucasian	Male	68	Cardiac arrest	BrainNet Germany
Fetal brain	Single	n.a.	n.a.	F2	Induced abortion	MRC Wellcome Trust Human Developmental Biology Resource
Fetal cerebellum	Single	n.a.	n.a.	F2	Induced abortion	MRC Wellcome Trust Human Developmental Biology Resource
Fetal thalamus	Single	n.a.	n.a.	F2	Induced abortion	MRC Wellcome Trust Human Developmental Biology Resource

Abbreviation: COD, cause of death.

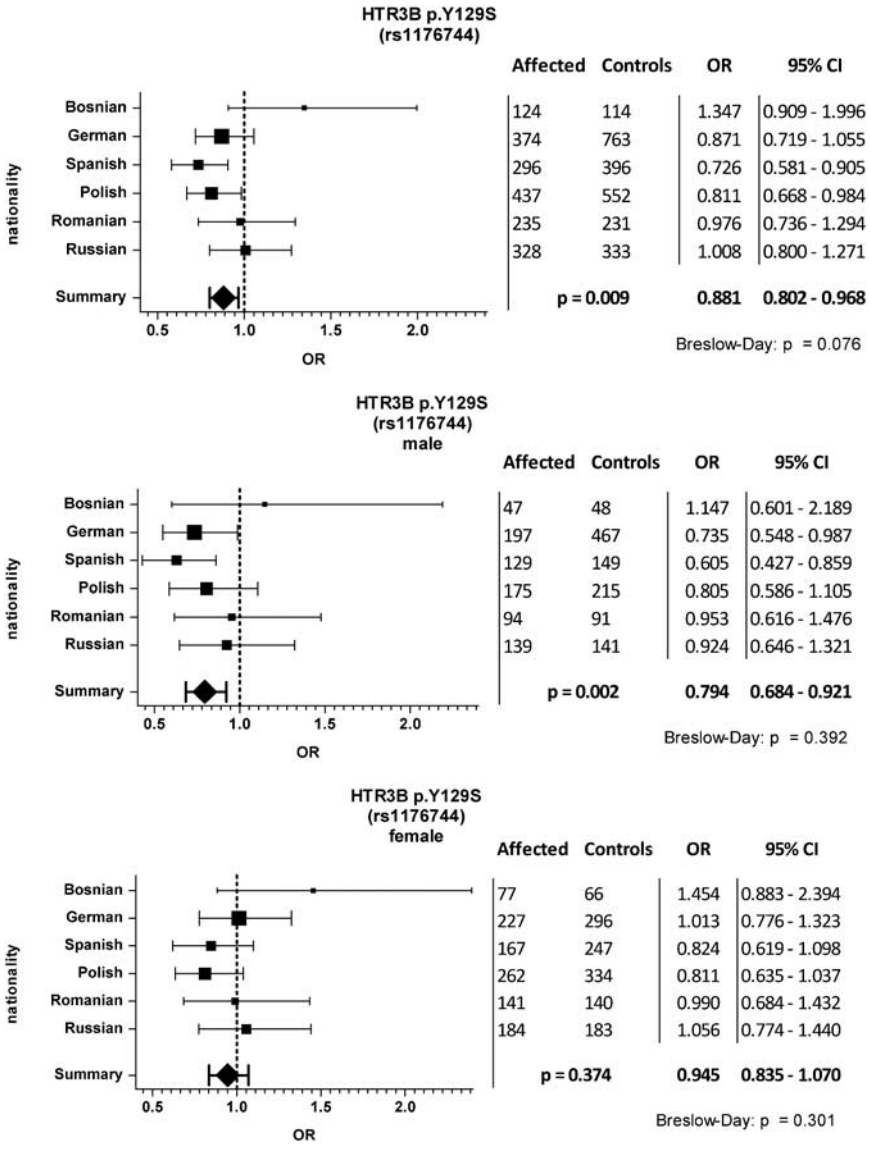

Figure 1 Odds ratio (OR) distribution across nationalities for *HTR3B* p.Y129S (rs1176744). The number of patients and healthy controls for each country is shown along with the respective OR and 95% confidence intervals (CI). The common *P*-value, OR and 95% CI (bold numbers) were determined using a Cochran-Maentel-Haenszel test. A Breslow-Day test was performed to test for homogeneity of ORs.

withstanding Bonferroni correction are highlighted in bold in Table 3. In view of statistical power issues, the national subgroups were not separated stratified according to sex. However, CMH analyses showed a clear sex-specific association. Although there was no association with the p.Y129S variant in females, the analysis of male patients vs controls resulted in a *P*-value of 0.0002 (OR = 0.794, CI = 0.684–0.921) (Figure 1).

In a further step to strengthen our association finding, rs1176744 (or its proxies) was examined in the three independent genome-wide data sets BOMA-BPAD, WTCCC (Wellcome Trust Case Control Consortium)-BPAD and Genetic Association Information Network-BPAD, including > 3500 patients and 5200 controls in total.[25–27] Remarkably, in all three samples, the p.Y129 allele was overrepresented in patients, and a final meta-analysis revealed a *P*-value of 0.048 (OR = 0.934, fixed effects model), thereby confirming the original association with BPAD.

No significant results were obtained for the *cis*-regulatory variants *HTR3A* c.−42C > T (rs1062613) and *HTR3B* c.−104 −102delAGA (rs3831455) in the CMH analyses (data not shown). The former was found to be associated in the Polish subgroup (*P*(log-additive) = 0.002, OR = 0.71, 95% CI = 0.57–0.89), and nominally associated in the Bosnian-Herzegovinian subgroup (*P*(log-additive) = 0.025, OR = 1.72, 95% CI = 1.05–2.80). However, the calculated ORs point to different risk alleles.

To address the possibility of false-negative findings, a power calculation was performed assuming the calculated OR of the *HTR3B* p.Y129S CMH analysis and a population risk of 2%. This yielded a statistical power of 66% for *HTR3A* c.−42C > T and of 47% for *HTR3B* c.−104_−102delAGA.

Table 3 Genotype counts and association statistics

SNP	*Study subgroup*	*Genotypes (and frequencies) cases* / *Genotypes (and frequencies) controls*			P-*value Trend test, d.f.* = *1*	P-*value (OR, (95% CI))* *Log additive, adjusted according to sex*
rs1062613		CC (%)	CT (%)	TT (%)		
	Bosnia-Herzegovina	70 (58)	44 (37)	6 (5)		
		81 (71)	30 (27)	2 (2)	0.073	*0.025* (1.72, (1.06–2.80))
	Germany	224 (62)	124 (34)	15 (4)		
		469 (62)	260 (34)	30 (4)	0.99	0.937 (0.99, (0.79–1.24))
	Poland	306 (70)	114 (26)	16 (4)		
		335 (61)	180 (33)	33 (6)	**0.01**	**0.002** (0.71, (0.57–0.89))
	Romania	144 (63)	70 (31)	13 (6)		
		142 (65)	69 (31)	8 (4)	0.579	0.524 (1.11, (0.8–1.53))
	Russia	205 (63)	109 (33)	11 (3)		
		205 (63)	105 (32)	14 (4)	0.808	0.865 (0.98, (0.74–1.28))
	Spain	167 (57)	107 (36)	20 (7)		
		207 (52)	157 (39)	34 (9)	0.416	0.190 (0.85, (0.67–1.08))
rs3831455		ins/ins (%)	ins/del (%)	del/del (%)		
	Bosnia-Herzegovina	97 (81)	21 (18)	2 (2)	0.312	0.195 (0.69, (0.40–1.21))
		82 (73)	29 (26)	2 (2)		
	Germany	278 (77)	80 (22)	5 (1)	0.702	0.477 (1.11, (0.84–1.47))
		594 (78)	158 (21)	7 (1)		
	Poland	328 (75)	99 (22)	9 (2)	0.221	0.099 (1.26, (0.96–1.65))
		430 (79)	107 (20)	6 (1)		
	Romania	174 (77)	46 (20)	6 (3)	0.591	0.812 (0.95, (0.65–1.40))
		162 (75)	52 (24)	3 (1)		
	Russia	252 (78)	63 (19)	9 (3)	0.204	0.239 (1.22, (0.87–1.70))
		261 (81)	59 (18)	4 (1)		
	Spain	245 (83)	47 (16)	3 (1)	0.597	0.295 (0.83, (0.58–1.18))
		318 (80)	75 (19)	5 (1)		
rs1176744		AA (%)	AC (%)	CC (%)		
	Bosnia-Herzegovina	54 (44)	57 (46)	13 (11)	0.3	0.152 (1.34, (0.90–1.99))
		61 (54)	44 (39)	9 (8)		
	Germany	191 (51)	152 (41)	31 (8)	0.321	0.178 (0.87, (0.72–1.06))
		355 (47)	335 (44)	73 (10)		
	Poland	235 (54)	157 (36)	45 (10)	**0.017**	*0.037* (0.82, (0.68–0.99))
		249 (45)	245 (44)	58 (11)		
	Romania	120 (51)	93 (40)	22 (9)	0.367	0.857 (0.97, (0.73–1.30))
		110 (48)	105 (46)	16 (7)		
	Russia	154 (47)	140 (43)	34 (10)	0.889	0.950 (1.01, (0.80–1.27))
		151 (45)	154 (46)	28 (8)		
	Spain	128 (43)	136 (46)	32 (11)	**0.013**	**0.005** (0.73, (0.59–0.91))
		142 (36)	181 (46)	73 (18)		

Abbreviations: CI, confidence interval; d.f., degrees of freedom; OR, odds ratio; SNP, single nucleotide polymorphism.
Frequencies (%) were rounded off to the nearest whole number. *P*-values are displayed in bold if withstanding Bonferroni correction, nominally significant *P*-values are shown in italics.

Using the nCounter technology, which measures mRNA expression levels directly without any further enzymatic modification of the sample,[32] we detected relative expression of both subunit genes on the transcript level, that is, *HTR3A* and *HTR3B* in total RNA samples from different human brain regions (Figure 2). In the tested adult brain tissues, relative code counts of the A and B subunits (code count B/code count A ratio) was found in the following descending order: brain stem (130), amygdala (30), frontal cortex (15), striatum (4.7), insula (4), thalamus (2.2) and hippocampus (1.6). The only tested tissue to show nearly equal code counts in adult tissue was the spinal cord (0.9). No expression was found in adult cerebellum. In the tested fetal tissues, the B:A code count ratio was 41 in the thalamus and 0.16 in hindbrain whereas in the cerebellum B was not detectable at all. Interestingly, the relative expression level of the B subunit was besides the brain stem highest in the amygdala and frontal cortex, that is, regions in which disturbances may lead to the manifestation of psychiatric disorders.

Discussion

In the present study, the *HTR3B* p. Y129S variant was successfully replicated overall, and showed significant associations in the CMH analysis and in the Polish and Spanish subgroups. The p.129Y allele was overrepresented in patients, and this finding is consistent with previous association data for BPAD, anorexia nervosa and major depression.[12,20,21] On a functional level, homozygous 5-HT$_3$AB p.129Y receptors displayed a decreased 5-HT maximum response, caused by a sevenfold decrease in single channel mean open time, compared with homozygous 5-HT$_3$AB p.129S receptors.[16,19] This striking difference may result in a disease-relevant change in 5-HT$_3$ receptor signaling.

The contribution of 5-HT$_3$ receptors to the etiology of psychiatric disorders is underlined by data from animal studies. Behavioral tests in primates and rodents revealed anxiolytic effects of 5-HT$_3$ antagonists secondary to blockage of the limbic hyperactivity response.[33] This is consistent with studies of 5-HT3A knockout mice, which show reduced

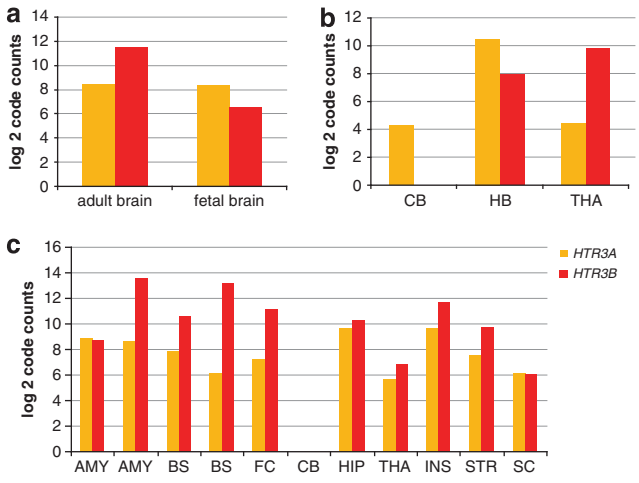

Figure 2 *HTR3A* and *HTR3B* mRNA expression profiling. mRNA levels were directly quantified using the nCounter technology. (**a**) Comparison of adult and fetal brain overall; (**b**) fetal brain areas and (**c**) adult brain regions. Abbreviations: CB, cerebellum; HB, hind brain; HIP, hippocampus; THA, thalamus; INS, insula; STRI, striatum; SC, spinal cord; AMY, amygdala; BS, brain stem; FC, frontal cortex.

anxiety-like behavior.[34] Further investigation of these knockout mice led to the conclusion that the regulation of depression- and anxiety-related behaviors by the 5-HT3A subunit differs between males and females.[35]

The effect of 5-HT3A on anxiety control has been reported to be mediated by regulation of hypothalamic-pituitary-adrenal responses to acute stress.[36] This suggests a potential interaction between 5-HT3A and corticotropin-releasing hormone in the amygdala. Sex differences in 5-HT3A- receptor-dependent hypothalamic-pituitary-adrenal responses in chronically stressed mice have also been reported.[36]

The observed association in males in the present study is in line with animal data.[35] A feasible hypothesis is that 5-HT$_3$ receptors are involved in the modulation of anxiety-related behaviors, which are important phenotypes in the symptomatic spectrum of BPAD.

Moreover, 5-HT$_3$ antagonist treatment in humans has been shown to be an effective treatment for anxiety and depression.[37,38]

A further aim of the present study was to gain insights into 5-HT$_3$ receptor subunit expression in the human brain. As outlined above, the expression of 5-HT$_3$ receptors has mainly been analyzed using 5-HT$_3$-binding site-selective compounds.[5–8] Our data are consistent with these early human brain ligand-binding data. Our results indicate that the expression of both subunits appears to switch during development. This switch in the 5-HT3A and 5-HT3B receptor ratio is interesting, since recent studies have described the relevance of 5-HT$_3$ receptors to neuronal plasticity in the cortex. Application of the 5-HT$_3$ receptor antagonist tropisetron led to a specific blockade of the 5-HT$_3$ response on Cajal-Retzius cells in mice, and 5-HT$_3$ receptors were shown to be involved in postnatal dendritic maturation and neuronal plasticity in the cortex.[39] In consequence, changes in the brain organization of variant carriers are conceivable. Disturbed column architecture of the cortex has indeed been reported in the brains of psychiatric patients.[40] Whether or not this is related to polymorphic changes in *HTR3* genes still awaits investigation.

Future studies are warranted to determine the functional and structural consequences of receptor polymorphisms *in vivo*. Recent studies by the present authors, using transiently transfected human cell lines, showed that the ratio between the subunits A and B determines receptor composition and consequently the formation of homomeric and heteromeric B subunits.[19,41]

We were able to confirm co-expression of both 5-HT3 subunits in brain regions involved in memory, cognition and anxiety control, that is, the frontal cortex, the hippocampus and the amygdala, respectively. Murine studies have shown that 70–80% of 5-HT$_3$ receptors are located presynaptically and are associated with axons and nerve terminals, with the exception of those in the hippocampus, which are mainly postsynaptic and located in somatodendritic regions.[42] The high presynaptic expression of 5-HT$_3$ receptors is consistent with their physiological role in neurotransmitter release (dopamine, cholecystokinin, glutamate, acetylcholine and gamma-aminobutyric acid (GABA)).[43] Accordingly, both the excitatory and the inhibitory effects of 5-HT$_3$R activation are mediated by activation of excitatory or inhibitory interneurons.[44] For example, 5-HT$_3$R activation of GABAergic interneurons innervating the amygdala is thought to exert an inhibitory influence on amygdala activity, whereas the activation of excitatory glutamatergic interneurons is hypothesized to have the opposite effect.[44] However, the situation in humans remains unknown. To date, systematic analysis has been hampered by the lack of specific antibodies.

Taken together, our results support the hypothesis that the *HTR3B* p.Y129S variant is implicated in BPAD, and the association could be replicated in a meta-analysis utilizing large published genome-wide association study data sets. Future studies are warranted to investigate a possible effect of this common genetic variant on further relevant neuropsychiatric phenotypes such as anxiety, stress response and depressive episodes, as well as memory and cognition. In doing so, possible gender-specific effects should also be taken into account.

In contrast to the *HTR3B* variant p.Y129S, the variants *HTR3A* c.−42C>T (rs1062613) and *HTR3B* c.−104 −102delAGA (rs3831455) were not replicated in our study overall. In fact, for the *HTR3A* variant, association analysis in the national subgroups generated contradictory results. Whereas pilot studies into BPAD, irritable bowel syndrome and eating disorders have implicated the less frequent T allele in the development of the disease phenotypes,[14,20,45] in the present study the C allele occurred more often in Polish patients. Although the nominally significant association in the subgroup from Bosnia-Herzegovina replicated earlier results (P(log-additive) = 0.025), the sample was small and probably lacked statistical power to detect true positives. Hence, our study does not support the hypothesis that *HTR3A* c.−42C>T SNP or the small deletion *HTR3B* c.−104_−102delAGA are involved in the etiology of BPAD. Nevertheless, the retrospective power calculation for the whole sample indicates that our study cohort may have been too small to detect minor effects.

In conclusion, the present study replicated the functional *HTR3B* variant p.Y129S as a predisposing factor for BPAD. Future studies should investigate the precise role of *HTR3B* in this disorder. The sex specificity of the association points to a putative function in the hypothalamic-pituitary-adrenal axis, resulting in anxiety-related phenotypes. Interestingly, differing code-count ratios of the A and B subunits were found in various adult and fetal brain regions, suggesting differences in receptor function and composition in the developing, compared with the adult brain.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)