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Pilot study on HTR2A promoter polymorphism, −1438G/A (rs6311) and a nearby copy number variation showed association with onset and severity in early onset obsessive–compulsive disorder

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Abstract A previous study showed that a single nucleotide polymorphism (SNP), −1438G/A (rs6311), found in the transcriptional control region of the gene that encodes the serotonin-receptor 2A (HTR2A) was associated with obsessive–compulsive disorder (OCD) in a sample of children and adolescents. In this study, we reanalyzed the association of this SNP with OCD in an enlarged population of 136 cases (55 previous + 81 new cases) and compared them to 106 newly recruited, healthy, age-matched controls. We also investigated whether this SNP or its copy number variations (CNV) was associated with OCD severity and age of onset. The CNV was analyzed in a DNA region located near rs6311. The results confirmed the association between the A-allele and early onset OCD in children and adolescents, with an odds ratio (OR) of 1.69 [95% CI (1.17, 2.46); $p = 0.005$]. Strikingly, we found that carriers of one copy (deletion) of the CNV were associated with a very early onset OCD (2.5 years earlier than the typical onset), and they had increased CY-BOCS scores (8.7 points higher compared to "normal" CNV and duplications); which is related to increased severity of OCD symptoms ($p = 0.031$; $p = 0.004$, respectively). Compared to the normal CNV and duplications, the association between the deletion and OCD showed an OR of 7.56 [95% CI (1.32, 142.84); $p = 0.020$]. These results pointed to the functional importance of this promoter region of HTR2A; it influenced the occurrence, the onset, and the severity of OCD.

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Introduction

Obsessive–compulsive disorder (OCD) is a heterogeneous psychiatric disorder characterized by clinically significant, recurrent, intrusive, and disturbing thoughts (obsessions), accompanied by repetitive, stereotypic behaviors, usually associated with anxiety or dread (compulsions) (American Psychiatric Association 1996). According to the National Comorbidity Survey Replication, the median age of OCD onset is 19 years. However, symptoms of OCD often begin during childhood or adolescence; 21% of all cases start experiencing symptoms by age 10 years old (Kessler et al. 2005). The early onset OCD often develops a chronic course and has a poor long-term outcome. According to a review of twin studies (van Grootheest et al. 2005), the authors concluded that the heritability for obsessive compulsive symptoms ranges from 0.45 to 0.65 in children and from 0.27 to 0.47 in adults. Grados and colleagues (2007) summarized that family studies showed OCD five- to sevenfold more frequent in relatives of patients than in relatives of controls. In accord to the results of twin studies, in family studies, the earlier age of onset of OCD in the index patients was correlated with a higher prevalence of OCD in first-degree relatives (Walitza et al. 2010). However, there is a wide range of prevalence rates of OCD in first-degree relatives across different studies, which merits discussion. The definition of early onset OCD differs among studies at the cut-off for age of onset; under 7 years of age was the lowest and under 18 years of age was the highest age as definition criteria. Furthermore, e.g. including different comorbidities as tic-disorders could have some effects on formal and molecular genetic results. Heterogeneity of the phenotype, the young age of siblings in studies of childhood OCD patients may result in an over/underestimation of the prevalence of OCD in relatives.

Dysfunction of the serotonin (5-HT) system has been implicated in OCD and is thought to contribute to behavioral traits, like perfectionism, obsessiveness, anxiety, and depression (Serretti et al. 2007; Zohar et al. 2000). Selective serotonin re-uptake inhibitors (SSRI; e.g. fluoxetine) that block the serotonin transporter represent the most effective pharmacological treatment of OCD, early onset OCD, and anxiety disorders (Geller et al. 2003; Pediatric OCD Treatment Study (POTS) Team 2004). Therefore, most genetic association studies in OCD have investigated genes related to serotonergic signaling. Although there is extensive evidence that the heritability of early onset OCD is higher than the heritability of adult onset OCD association studies in early onset OCD are comparably rare.

Our group previously conducted an association study within a case–control design to determine whether early onset OCD was associated with the single nucleotide polymorphism (SNP), −1438A/G (rs6311), in the serotonin receptor 2A (HTR2A) promoter (Walitza et al. 2002). We detected a significant association between OCD and the A-allele of 1438A/G (Walitza et al. 2002). Our results were in accordance with the results from a study conducted by Enoch and colleagues in adult patients with OCD as well as in early onset OCD determined retrospectively (Enoch et al. 1998, 2001). However, several other case–control association studies on adult OCD patients (Denys et al. 2006; Saiz et al. 2008a; Tot et al. 2003) reported no association with SNP rs6311 as well as negative transmission-disequilibrium tests (Dickel et al. 2007). Similarly, several association studies for the synonymous T102C (rs6313) variant, which was reported to be in complete linkage disequilibrium with rs6311 in different populations (Spurlock et al. 1998; Meira-Lima et al. 2004; Martinez-Barrondo et al. 2005; Saiz et al. 2008b), reported conflicting results in adult OCD patients (Tot et al. 2003; Meira-Lima et al. 2004; Hemmings et al. 2003; Frisch et al. 2000; Saiz et al. 2008a; Hemmings et al. 2006). Since Kato and colleagues (1996) presented evidence for genomic imprinting (complete inactivation of one allele) of the HTR2A, several studies investigate the effects of the 5HTR2A imprinting on expression, but up to now conflicting results have been reported. Bunzel and colleagues (1998) showed in 4 out of 18 post-mortem brain tissues monoallelic expression of the HTR2A due to polymorphic imprinting in the brain. Other authors suggested that HTR2A is unlikely to have any polymorphisms that alter gene expression in the brain in an allele-dependent pattern (Bray et al. 2004). But CpG sites, which are apparently potential target regions for methylation have been detected within the rs6311 polymorphism are relevant for expression pattern (Bray et al. 2004; Polesskaya et al. 2006). In contrast to the findings of Bunzel et al. (1998), de Luca et al. (2007) found no evidence of genomic imprinting in a sample of 29 heterozygous psychotic patients and 16 heterozygous controls. The authors concluded that complete polymorphic imprinting of the HTR2A gene may be a rare event. Although there are conflicting perspectives regarding imprinting of the HTR2A we decided to enlarge our previous sample with early onset OCD to reinvestigate the association with the 5HT2A promoter polymorphism. In parallel, we focused on a copy number variation (CNV) near the rs6311promoter region. Recent investigations have implicated de novo and/or rare CNVs as potentially pathogenic factors in attention-deficit/hyperactivity disorder (ADHD) (Williams et al. 2010; Lesch et al. 2011), autism

(Stankiewicz and Lupski 2010; Pinto et al. 2010), and schizophrenia (Lee et al. 2010). Rare CNV is defined having lower frequency than 1% in the population, while common CNV also referred as polymorphism has higher frequency (Beckmann et al. 2007). The duplication or deletion process of CNVs can disrupt a variable number of genes, which can result in alternate gene products or changes in gene dosage. Moreover, the disruption of regulatory regions in the genome can lead to altered gene expression, and this may contribute to disease predisposition. Given the highly heritable and variable nature of OCD, we hypothesized that individual, CNVs near the SNP rs6311 might contribute to OCD risk and severity. Therefore, after searching for CNVs reported in the Database of Genomic Variants (DGV; http://projects.tcag.ca/variation/) site as well as other CNVs in the HTR2A gene or in its vicinity, that resulted in no known CNV, we aimed to detect new putative CNV using the pre-designed TaqMan CNV-assay (Applied Biosystems Co., Germany) that correspond to the nearest CNV to the SNP (68-bp upstream of SNP rs6311, while amplicon size was 100 bp) and still on the promoter region (Fig. 1), assuming that this region is of functional relevance.

In the present study, we extended the number of patients included in our previous study (Walitza et al. 2002) and also included new independent controls. We aimed to confirm our previous results of the association between the rs6311 polymorphism and early onset OCD. In addition, we aimed to investigate the association between early onset OCD and the not yet described CNV located near SNP rs6311 on the HTR2A promoter.

Materials and methods

Subjects

The study sample comprised patients that had received inpatient treatment at the Department of Child and Adolescent Psychiatry of the Universities of Würzburg, Marburg, Aachen, and Freiburg. All patients and healthy controls were of German ethnicity. All participants and the parents of minors gave written informed consent. The ethics committees of all participating universities approved the study.

Patients fulfilled the diagnostic criteria for current OCD according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) (1996) and the International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10) (Dilling et al. 1996). To describe the broad range of clinical features, we ascertained systematically initial symptoms from baseline with psychopathology schemes as CASCAP-D (Döpfner et al. 1999). To assess the criteria for OCD and

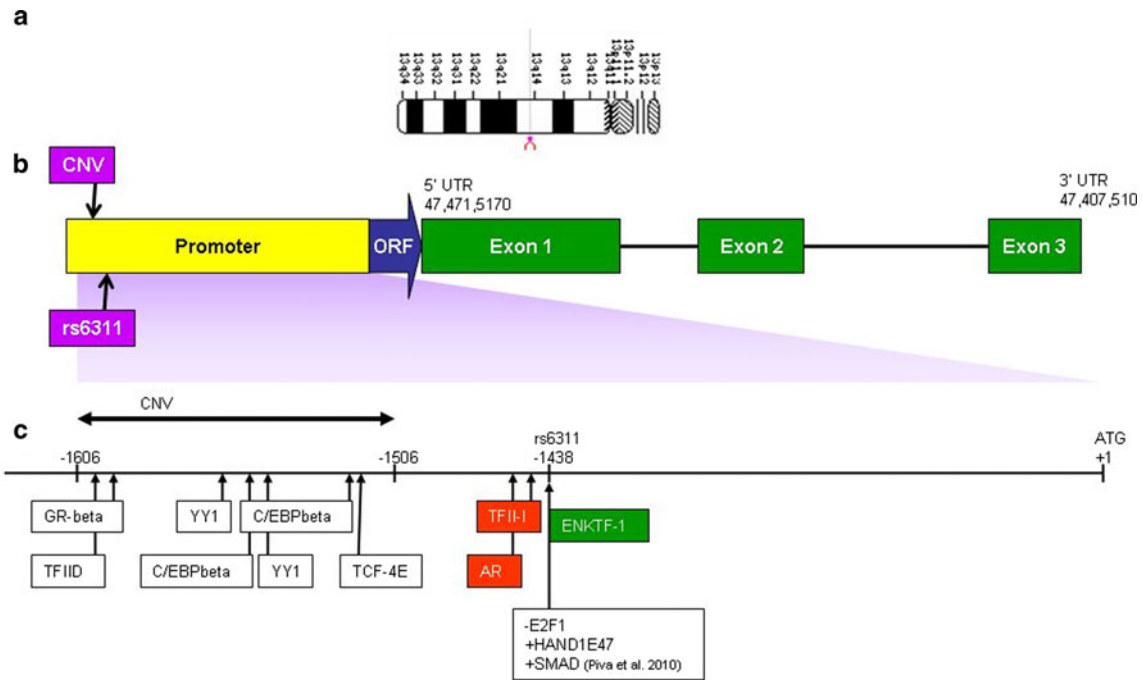

Fig. 1 Alignment of CNV and SNP rs6311 on the HTR2A promoter sequence and their effects on transcription factors. **a** The alignment of HTR2A on chromosome 13. **b** The HTR2A gene alignment with its promoter region (not to scale). **c** The promoter region with the CNV and rs6311 alignment (not to scale); the indicated transcription factors may be affected according to PROMO version3 (http://algenn-lsi.ups.es). *Red* G-allele, *green* A-allele, + gain, − loss, of transcription factors (according to Piva et al. 2010)

comorbid psychiatric disorders, both patients and parents were interviewed separately with the semi-structured diagnostic interview of psychiatric disorders in children and adolescent (Kinder-DIPS; children and parents version) (Unnewehr et al. 1995). The Kinder-DIPS is used to assess a wide range of psychiatric disorders in childhood and adolescence, including ADHD, and conduct and oppositional-, anxiety-, affective-, eating- and tic-disorders, diagnosed according to the criteria of the ICD-10 (Dilling et al. 1996). The Kinder-DIPS further includes a general clinical screening component to assess substance use, abuse, psychosis and somatic diseases. Autistic spectrum disorders were screened within the ascertainment of psychopathology (CASCAP-D) (Döpfner et al. 1999).

The Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS) (Scahill et al. 1997) was used to assess further characteristics and severity of OCD. A summary score above 16 points was determined to be the cut-off for clinical impairment caused by OCD symptomatology; a score of 38 points was the maximum for describing severity according to the CY-BOCS. Of note, the DSM-IV does not require children to fulfill the criterion of insight regarding the irrationality of the symptoms. This may result in low summary scores; therefore, it is possible that the CY-BOCS evaluations underestimated the true severity of symptomatology. For diagnostic assessments of present and lifetime Tourette's Syndrome and tic-disorders, we used the adapted German version (Hebebrand et al. 1997) of the Child and Adult Schedule for Tourette and Other Behavioral Syndromes (STOBS), according to Pauls et al. (1991).

All interviews were performed by senior clinicians of the university clinics for child and adolescent psychiatry. Subjects with comorbid disorders were only included in the study when the OCD symptoms were most prominent, based on two criteria: (1) OCD had to predate the onset of the comorbid disorders (except ADHD), and (2) two senior clinicians independently diagnosed the predominance of the OCD symptoms.

Exclusion criteria for patients were: lifetime history of psychotic disorders, Gilles-de-la-Tourette's syndrome and chronic tic-disorder, autistic spectrum disorder (e.g. infantile autism, atypical autism, Asperger's syndrome), alcohol dependence, or mental retardation (IQ < 70).

The patient sample consisted of 136 patients (children and adolescents). The patient sample was a superset that comprised 55 children with OCD from a previous study (Walitza et al. 2002), plus 81 newly recruited children with OCD.

In a previous case–control study regarding the −1438G/A polymorphism in early onset OCD (Walitza et al. 2002), the control group consisted of students that were older than the patients. For this study, we recruited 106 healthy German children from the Department of Child and Adolescent Psychiatry of the University of Würzburg to use as new independent controls. They were screened with the Child Behavior Checklist (CBCL) (Döpfner et al. 1991) and a validated German depression self-rating inventory (DIKJ) (Stiensmeier-Pelster et al. 2000). All controls and their mothers were interviewed with the Schedule for Affective Disorders and Schizophrenia for school-aged children (Kiddie-Sads) (Kaufman et al. 1997). The intelligence level was assessed with the Culture Fair Test (CFT 1/20) (Cattell et al. 1997). Exclusion criteria for participation of controls were: any severe somatic or neurological disease; any psychiatric diagnosis according to the Kiddie-Sads; a DIKJ score that exceeded the threshold of 18 for clinically relevant depressive symptoms; any T-score above 63 on the internalizing, externalizing, or total score of the CBCL; or an IQ below 80.

Genotyping for the HTR2A −1438 A/G polymorphism

Genomic DNA was extracted from whole blood with the desalting Proteinase K methodology (Miller et al. 1988). Genotyping for the −1438 A/G polymorphism (rs6311) (location 47,471,478 on the GRCh37; 13q14 q21) was conducted with the TaqMan® SNP genotyping assay (Assay no. C-8695278-10; Applied Biosystems Co.) on the iCycler iQ Real-Time thermocycler (BioRad Laboratories Inc., Germany), according to manufacturer instructions. Genotypes were assessed according to the Allelic discrimination method with the iCycler IQ software (version 3.1.7050; BioRad Laboratories).

CNV analysis

The CNV located 68 bp upstream of SNP rs6311 (location 47,471,310-47,471,410 on GRCh37; 13q14.2a; amplicon size 100 bp; Fig. 1) was analyzed with the TaqMan® Copy Number Assay (Assay no. Hs06366812_cn; Applied Biosystems Co.) on the iCycler iQ Real-Time thermocycler (BioRad Laboratories Inc.), according to manufacturer instructions. Each sample was adjusted to 20 ng genomic DNA per well. The TaqMan® Copy Number Reference assay for RNase P was used for normalization. Four replicates were run per sample. The CNV per sample were evaluated by the Cycles threshold (C_T) values assigned with the CopyCaller® software (version 1.0; Applied Biosystems Co.). A "normal"CNV was defined as two copies; a "low" CNV was defined as ≤ 1 copy (deletion); and a "high" CNV was defined as ≥ 3 copies (duplication). For each sample, an independent replication of the assay was conducted in order to eliminate false determinations of CNV.

Statistical analysis

All statistical tests were two-sided with an alpha-level of 0.05. The results were unadjusted for multiple testing, because the analysis was exploratory and the number of tests was comparably small. The association study of the SNP was a replication study. Assuming an OCD prevalence of 2% and a disease allele frequency of 50%, we have a power of 91.1% for detecting an odds ratio (OR) of at least 2 using Armitage's trend test and only the 81 new patients (Slager and Schaid 2001). This was analyzed primarily by omitting the cases analyzed in the previous study that gave similar significant results (data not shown). Secondly, we analyzed the complete sample. All confidence intervals were 95% profile likelihood intervals (Pawitan 2001).

Gender and age distributions were compared with Fisher's exact test and the Student's t test, respectively. Differences in average OCD onset and severity (measured by the CY-BOCS score) were assessed with one- and two-way ANOVAs for separate and simultaneous comparisons, respectively. In the case–control analysis, discrepancies in SNP and CNV distributions were separately tested with Armitage's trend test and Fisher's exact test, respectively (Sasieni 1997). Logistic regression for a simultaneous association analysis of SNP and CNV with OCD assumed an additive model for SNP and an unrestricted model for CNV. An additional interaction of SNP with gender was tested with the Student's t test.

The computations were performed with the statistical computing software R (R Development Core Team 2010), version 2.11.0.

Table 1 Demographic data

	N	Frequency (%)
136 patients		
Gender		
Male	77	56.6
Female	59	43.4
Age at investigation		13 ± 2.9 years (range 4.7–17.9 years)
Age at onset		11.1 ± 3.2 years (range 3–17.4 years)
Comorbid diagnosis		
None	74	54.4
ADHD	15	11.0
Anxiety disorders	10	7.4
Depression disorders	8	5.9
Eating disorders	5	3.7
Conduct disorders	4	2.9
Others*	7	5.1
Tic-disorder**	13	9.6
106 controls		
Gender		
Male	65	61.3
Female	41	38.7
Age		11.6 ± 2.6 years (range 5.1–17.8 years)

ADHD attention-deficit hyperactivity disorder

* Comorbidities as dyslexia, enuresis, histrionic personality disorder and behavioral and emotional disorders (as separation anxiety disorder of childhood, social anxiety disorder of childhood, depressive conduct disorder, elective mustism), which occur, respectively, only in one of the patients

** Lifetime-diagnosis of tic-disorder according to STOBS, without Tourette's syndrome

Results

Demographic data

The study population included slightly more males than females in both the OCD and the healthy control groups ($p = 0.51$; Table 1). The healthy controls were significantly younger than the patients with OCD ($p < 0.0001$; Table 1); however, the average age of the controls was not significantly different from the average age of the patients at OCD onset ($p = 0.21$; Table 1). For the comorbidities and gender data, see Table 1.

Case–control analysis of the HTR2A −1438 A/G polymorphism and CNV

The association between SNP rs6311 and OCD was first analyzed in only the 81 newly recruited patients with OCD (those not included in the previous study (Walitza et al. 2002). There was a significant difference between the genotype distributions of controls and these OCD cases ($p = 0.030$). Note that the previous study, with a different control group and a smaller OCD group, also reported a significant association between genotype and OCD ($p = 0.046$). When all 135 available cases with SNP information were included, the association was highly significant ($p = 0.007$). We also found that patients with early onset OCD showed a higher frequency of the AA genotype (19.3%) compared to the healthy controls (16%) (Table 2). The estimated OR for the A allele was 1.69 [95% CI (1.17, 2.46); $p = 0.005$], adjusted for CNV. Assuming an additive effect of the A allele, this indicated that the risk of OCD was increased by 69% in patients with the heterozygous GA genotype and by 286% for the homozygous AA genotype.

The association (adjusted for SNP) between the HTR2A CNV ("low" vs. "normal/high") and OCD was also significant ($p = 0.020$). The SNP-adjusted OR estimated for a "low" CNV relative to a "normal/high" CNV was 7.56

Table 2 Frequencies of SNP rs6311 genotypes and CNV sizes in patients with early onset OCD and healthy controls

	Controls *N* (frequency %)	OCD *N* (frequency %)
Genotype (rs6311)		
GG	54 (50.9)	39 (28.9)
GA	35 (33.0)	70 (51.9)
AA	17 (16.0)	26 (19.3)
Fisher's exact test		$p = 0.002$
Armitage's test for trend		$p = 0.007$
Two-sided asymptotic (adjusted for CNV-pooled)		$p = 0.005$
CNV		
"Normal/high"	105 (99.1)	127 (94.1)
"Low"	1 (0.9)	8 (5.9)
Fisher's exact test		$p = 0.082$
Two-sided asymptotic (adjusted for SNP)		$p = 0.020$

One sample missing for the genotyping and one for CNV from technical reasons

[95% CI (1.32, 142.84)]. We did not find a significant gender effect in our case–control analysis ($p = 0.72$).

HTR2A −1438 A/G polymorphism and CNV associations with age of onset and severity of OCD

The HTR2A genotype was not significantly associated with age of onset or severity of OCD (Table 3a). In addition, there was no significant difference between "normal" and "high" CNV with respect to age of onset or severity (*t* test $p = 0.47$ and $p = 0.30$, respectively). Because we hypothesized that the deletion ("low" CNV) would affect the phenotype by impairing the function of the promoter activity, we pooled the "normal" and "high" data into a joint "normal/high" CNV group for comparisons to the "low" CNV group (Table 3b). This analysis method revealed a significant association between "low" CNV and the age of onset ($p = 0.031$); the estimated average age of onset was 2.5 years earlier than that of the "normal/high" group [95% confidence interval (CI): (0.2 years, 4.8 years)]. Similarly, we observed a significant association between "low" CNV and the OCD severity measured with CY-BOCS scores ($p = 0.004$); the estimated average severity score was 8.7 points higher than that of the "normal/high" group [95% CI: (2.9 points, 14.5 points)].

Discussion

In this study, we confirmed the previously described association between the A allele of SNP rs6311 and early onset OCD (Walitza et al. 2002; Enoch et al. 2001). We estimated that the OR per A allele was 1.69 [95% CI (1.17, 2.46)] for early onset OCD, but we found no significant association between the A allele of SNP rs6311 and age of onset or OCD severity. This might be due to the fact that the OCD population in this study contained only patients

Table 3 Age at onset and severity of OCD, based on the SNP rs6311 genotype (A) and the CNV size (B)

A Genotype (rs6311)	Age at onset (years) (mean ± SD)	*N*	Missing	CY-BOCS (score) (mean ± SD)	*N*	Missing
GG	11.0 ± 3.3	38	1	23.9 ± 8.4	34	5
GA	10.9 ± 3.3	65	5	21.7 ± 7.0	66	4
AA	11.7 ± 2.6	25	1	21.0 ± 8.4	26	0
One-way ANOVA	$p = 0.55$			$p = 0.28$		
Two-way ANOVA (adjusted for CNV)	$p = 0.48$			$p = 0.29$		
Two-way ANOVA (adjusted for CNV-pooled)	$p = 0.44$			$p = 0.33$		

B CNV	Age at onset (years) (mean ± SD)	Estimate (95% CI) (years)*	*N*	Missing	CY-BOCS (score) (mean ± SD)	Estimate (95% CI) (score)*	*N*	Missing
"Normal/high"	11.2 ± 3.0		120	7	21.7 ± 7.6		119	8
"Low"	8.8 ± 4.8	−2.5 (−0.2; −4.8)	8	0	30.6 ± 3.7	8.7 (2.9; 14.5)	7	1
One-way ANOVA	$p = 0.036$				$p = 0.003$			
Two-way ANOVA*	$p = 0.031$				$p = 0.004$			

One sample missing for the genotyping from technical reasons

One sample missing for the CNV from technical reasons

* Estimates and confidence intervals (CIs) are adjusted for SNP

with very early onset of the disease (average age of onset was 11.1 ± 3.2 years, range 3–17.4 years). Thus, we could not rule out an age dependent association for the-rs6311 A allele. The association found in this study, without age dependence, could be consequence of polymorphism influencing HTR2A gene expression through the promoter activity. An extensive reporter gene assay suggested that the rs6311 SNP modulated HTR2A promoter activity (Parsons et al. 2004); however, another study on the effect of the rs6311 SNP on promoter activity revealed no significant difference in expression of HTR2A mRNA (Spurlock et al. 1998).

Several studies on the polymorphic imprinting have presented some conflicting results regarding monoallelic expression of HTR2A (Bunzel et al. 1998; Kato et al. 1996; Fukuda et al. 2006; Myers et al. 2007). Nevertheless, we investigated whether the rs6311 polymorphism on the promoter confirm association of previous findings in very early onset OCD, since current findings do show that specifically this particular SNP has transcriptional effects on the gene. A recent study reported an in silico selection of phenotypes that reflected different polymorphisms in the HTR2A gene (Piva et al. 2010). The SNP rs6311 was found to cause loss of the E2F1 transcription factor site and the gain of two other transcription factor sites (HAND1E47 and SMAD; see Fig. 1). Another investigation of epigenetic factors affected by rs6311 revealed that a newly created E47-binding site (A-allele), a glucocorticoid receptor-binding site at position −1420, and an Sp1-binding site at CpG methylation site +1224 caused alterations in methlylation rates at both −1420 and −1439 sites (Falkenberg et al. 2011). Therefore, modifiers should be taken into account in functional studies.

One problem with genetic analyses is the inability to completely match a control group or minimize ethnic differences between patients and controls. In the present study, we included 136 German children and adolescents with early onset OCD, of which 107 patients were children from Würzburg, Germany. All patients and controls were of German origin. Furthermore, all 106 healthy German children were also recruited at the Department of Child and Adolescent Psychiatry of the University of Würzburg; therefore, we assumed that we were able to minimize ethnic stratification.

We also found that one copy (deletion) of a CNV located near the rs6311 was associated with very early onset OCD and an increased CY-BOCS score, which was related to enhanced symptom severity. Of note, the frequency of this deletion in our OCD group ($n = 8$) was increased compared to that in the healthy controls ($n = 1$). Since this CNV is not yet reported in any platform such as DGV or any publication, little is known about its frequency or size. From our findings one could assume that according to its frequency in control group that it is a rare CNV, but concerning the size further analysis should be conducted up/downstream the amplicon location. Characterizations of CNVs and their associated polymorphisms are important, because they may provide key links to understanding of the genetic basis of quantitative traits and the different susceptibilities to psychiatric diseases. An initial study that explored the effects of CNVs on gene expression in lymphoblastoid cell lines from human populations reported that changes in copy number explained nearly 20% of the detected gene expression variation (Stranger et al. 2007). This was suggested to result from altered dosages of genes that mapped within CNVs; however, it may also potentially result from the impact of CNVs on neighboring genes (Stranger et al. 2007). Recent studies have shown that CNVs can influence the expression of genes both within the CNV and outside the CNV, in the vicinity of up to half a megabase (Henrichsen et al. 2009). It was suggested that, for disorders like Williams–Beuren (WBS; MIM 194050), Prader–Willi (MIM 176270), Angelman (MIM 105830), and DiGeorge/velocardio-facial syndromes (DG/VCFS; MIM 188400), patients with duplications might have different clinical syndromes and milder phenotypic features than those with deletions. This might be explained by the fact that excess information tends to be less detrimental to the organism than a deficiency (Zhang et al. 2009). This effect might explain our observations of the effect that the "low" CNV had on the HTR2A promoter region; this effect might also influence promoter activity. A potential mechanism might be the loss of transcription factor sites within the CNV deletion (Fig. 1). However, this theory requires further investigation.

Limitations of the present study: the small sample size is one of the main limitations of the study. Therefore, we believe that the relatively narrow phenotype (e.g. early onset OCD, without Tourette's syndrome) as one of the strengths of the study. A limitation could be also that we have not used a special interview to exclude all autistic spectrum disorders. For future studies, we recommend to use interviews as the Kiddie-SADS in a recent version with a separate interview of autistic symptoms according to DSM-IV. As the HTR2A is imprinted, the results of the association studies (patients compared to controls) cannot be interpreted without caution. Nevertheless, these replicated findings of association of the 5HT2A promoter polymorphism with early onset OCD and with other psychiatric disorders lead us to the CNV analysis in the 5HT2A promoter region, which showed interesting and promising findings. Further independent and extended studies of the CNV as well as the SNP analysis in early onset OCD, but also in related disorders need to be performed. These findings may suggest additional applications for investigating the genomic effects associated with OCD in large-scale populations.

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Conflict of interest The authors declare no conflict of interest.

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