Polymorphisms in the glutamate transporter gene *SLC1A1* and obsessive–compulsive symptoms induced by second-generation antipsychotic agents

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Background **A large subgroup of schizophrenic patients develops obsessive–compulsive symptoms (OCS) during treatment with second-generation antipsychotics (SGA). A genetic risk factor for these secondary OCS was recently described in the gene *SLC1A1* encoding the neuronal glutamate transporter excitatory amino acid carrier 1. The aim of this study was to replicate these findings in a European sample.**

Methods **A total of 103 schizophrenic patients treated with SGAs were included. Three single nucleotide polymorphisms in *SLC1A1* (rs2228622, rs3780412 and rs3780413), which had been associated with SGA-induced OCS, were investigated. Single marker and haplotype analyses were tested with logistic regressions using age, sex and medication type as covariates.**

Results **Treatment with markedly antiserotonergic SGAs such as clozapine was more prevalent in the subgroup of patients with comorbid OCS ($P<0.001$). The dosage and duration of clozapine treatment correlated significantly with the severity of OCS. In contrast to the Asian sample, no genetic associations were found with OCS.**

Conclusion **Larger samples are necessary to unravel the interplay of pharmacological and genetic risk factors for OCS in schizophrenia. *Psychiatr Genet* 00:000–000 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Epidemiologic studies have shown that schizophrenic patients have an increased lifetime risk of up to 30% for comorbid obsessive–compulsive symptoms (OCS) (Buckley *et al.*, 2009; Mukhopadhaya *et al.*, 2009; Achim *et al.*, 2011). These obsessive, distressing, intrusive thoughts and related compulsions (e.g. repeated hand washing, checking behaviour, counting or cleaning) are associated with a high subjective burden of disease, a negative impact on general prognosis and high treatment costs. Conversely, the risk of patients with obsessive–compulsive disorder of suffering from comorbid schizophrenic symptoms does not exceed the common risk for schizophrenia in the general population (de Haan *et al.*, 2009).

Within current pathogenetic theories of obsessions and compulsions, a dysregulation of serotonergic neurotransmission (Charnay and Leger, 2010) in a network comprising cortical, striatal and thalamic centres has been proposed, but close interactions between serotonergic, dopaminergic and glutamatergic neurotransmission must be considered (Pogarell *et al.*, 2003; El Mansari and Blier, 2006).

Several theories have attempted to explain the high prevalence of OCS in schizophrenia: a large subgroup of comorbid patients experiences OCS manifestation after the initiation of antipsychotic treatment (Schönfelder *et al.*, 2011). This type of second-onset OCS was considered an unfavourable effect of antiserotonergic, pro-obsessive second-generation antipsychotics (SGA), most importantly clozapine (Schirmbeck and Zink, 2011b). Baker *et al.* (1992) and de Haan *et al.* (1999) were the first to assume an aggravation or an induction of OCS by SGAs due to their antiserotonergic properties. We recently carried out a study stratifying 70 patients with schizophrenia by their mode of atypical antipsychotic treatment and compared a group of patients treated with predominantly antiserotonergic SGAs (clozapine and olanzapine) with a population medicated with SGAs predominantly affecting the dopaminergic system (aripiprazole and amisulpride) (Schirmbeck *et al.*, 2011a). OCS was found to be significantly more prevalent and severe in group I, in which 25 (64%) patients reported the de-novo occurrence of OCS under their antipsychotic treatment. These estimations are in agreement with independent studies reporting even higher proportions of SGA-induced OCS (70–88%) in

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schizophrenic patients suffering from comorbid OCS (Poyurovsky *et al.*, 2004; Lin *et al.*, 2006; Lim *et al.*, 2007). Positive correlations between the duration and the dose of antipsychotic treatment with the severity of OCS (de Haan *et al.*, 2002; Reznik *et al.*, 2004; Lin *et al.*, 2006; Mukhopadhaya *et al.*, 2009; Schönfelder *et al.*, 2011) substantiate the assumption that SGAs with pronounced antiserotonergic effects possess pro-obsessive properties. Nevertheless, further prospective clinical studies are needed to confirm causal interactions.

Genetic contributions in the aetiology of obsessions and compulsions in general are supported by family and twin studies showing strong evidence of heritability (Nicolini *et al.*, 2009; Pauls, 2010). The results from genetic association studies are rather ambiguous. On the basis of the pathophysiology and pharmacology of obsessive–compulsive disorder, the focus is primarily on candidate genes of serotonergic and dopaminergic neurotransmission. The only associations that have consistently been replicated refer to the gene *SLC1A1* on chromosome 9p24, encoding the neuronal glutamate transporter excitatory amino acid carrier 1 (EAAC1) (Veenstra-VanderWeele *et al.*, 2001; Arnold *et al.*, 2006; Dickel *et al.*, 2006; Stewart *et al.*, 2007; Shugart *et al.*, 2009; Wendland *et al.*, 2009). All these studies have found an association between one or more single nucleotide polymorphisms (SNPs) or haplotypes within SLC1A1 and OCS. Furthermore, independent lines of evidence support the glutamatergic theory in the pathophysiology of OCS, most importantly animal models (Joel, 2006; Albelda *et al.*, 2010; Yang and Lu, 2011), human magnetic resonance spectroscopy (Rosenberg and Hanna, 2000; Rosenberg *et al.*, 2004; Whiteside *et al.*, 2006; Starck *et al.*, 2008; Wu *et al.*, 2011), treatment approaches addressing the glutamatergic system (Coric *et al.*, 2005; Poyurovsky *et al.*, 2005; Lafleur *et al.*, 2006; Pittenger *et al.*, 2006; Poyurovsky *et al.*, 2010) and finally genetic studies (Bienvenu *et al.*, 2009; Nicolini *et al.*, 2009; Pauls, 2010).

To investigate whether genetic variations in the candidate gene *SLC1A1* modulate the susceptibility to SGA-induced OCS, Kwon *et al.* (2009) evaluated the associations between specific SNPs of the candidate gene *SLC1A1* and the prevalence of OCS in an Asian sample of 94 SGA-treated schizophrenic patients. The results show strong associations between OCS and the A/C/G haplotype rs2228622/rs3780413/rs37801412 of *SLC1A1*. According to an odds ratio (OR) of 3.96, patients carrying this A/C/G haplotype are almost four times more likely to suffer from SGA-induced OCS. Neither the gene *SLC1A1* nor its chromosomal region has been associated with vulnerability to schizophrenia spectrum disorders so far (Deng *et al.*, 2007).

To replicate these findings, we carried out a study with 103 schizophrenic patients of European descent. We carefully characterized the clinical phenotype and analysed the allele frequencies of the rs2228622–rs3780413–rs3780412 haplotype in an OC and a non-OC group of SGA-treated schizophrenic patients.

Methods

Participants

Participants were recruited from among former or current patients at the Central Institute of Mental Health. The study was approved by the local ethical committee of the University of Heidelberg (reference number 2008-235N-MA) and was carried out in accordance with the guidelines of good clinical practice. The general inclusion criteria were as follows: (a) diagnosis of schizophrenia spectrum disorder according to the *Diagnostic and Statistical Manual version IV* research (DSM IVR). (b) SGA treatment in established dose ranges. (c) Stable psychopathological states over a period of at least 2 weeks. Recruitment was performed irrespective of the presence or absence of OCS, until sufficient sample sizes according to the power analysis (see below) were attained. This naturalistic mode of recruitment justifies the assumption of representative samples. After providing the participants with a complete description of the study, written informed consent was obtained from all participants. To determine ethnicity, patients were asked about their nationality, place of birth and the ancestry of their parents. The majority of the participants were of German descent (85%). With the exception of one German citizen of Pakistani origin, all other parents were born in central European countries [Turkish ($N = 8$), Greek ($N = 2$), Romanian ($N = 2$), Polish ($N = 2$), Georgian ($N = 1$)]. Therefore, with one exception, our participants represent the central European gene pool (103 out of 104).

Clinical assessments

Sociodemographic data on age, sex, level of education, age of onset, duration of illness and comorbidity were collected. Schizophrenia was diagnosed according to the DSM IVR. OCS was assessed by trained and certified raters (F.S., M.Z.) by Yale-Brown Obsessive–Compulsive Scale (YBOCS) interviews. Compulsions and obsessions were rated separately on five five-point (0–4) scale items (time, handicap, frequency, controllability, discomfort), yielding subtotal scores (range 0–20) for compulsions and for obsessions, respectively, summing up to a total score range of 0–40 (de Haan *et al.*, 2006). In each case, the criterion of insight was explored with caution to distinguish between obsessions and delusions. The chronological order of the events 'first manifestation of psychosis', 'initiation of atypical antipsychotic treatment' and 'onset of OCS' was carefully explored both from patients and from available relatives. Additional information was obtained from hospital chart reviews. Further, we documented previous and current pharmacological treatment including the dosage and serum levels of antipsychotic agents.

Power analysis

On the basis of previously published data obtained by Kwon *et al.* (2009), power calculations were performed using the Genetic Power Calculator (*http://pngu.mgh.harvard.edu/~purcell/gpc/*). The following assumptions were made: (a) SGA-induced OCS prevalence of 124 (Lim *et al.*, 2007), (b) high-risk allele frequencies of 4 and (c) genotypic relative risk of 3.955. As a consequence, a sample size of 44 patients in the OCS group and 59 in the control group was estimated to gain 85% power at a significance level of P less than or equal to 0.05.

DNA extraction and genotyping

Genomic DNA was automatically extracted from the EDTA-treated venous blood using the Chemagic Magnetic Separation Module I (Chemagen Biopolymer-Technologie AG, Baesweiler, Germany). Genotyping was carried out on a 7900HT Fast Real-Time PCR System (Applied Biosystems, Darmstadt, Germany), using TaqMan SNP Genotyping Assays (Applied Biosystems). Genotype accuracy was assessed by running 15% of the sample in duplicate, and reproducibility was routinely greater than 99.9%. SNP and the sample call rates were 100%.

Statistical analysis

Statistical analysis of the demographic and clinical parameters was performed using the statistical package for social sciences (IBM SPSS version 18.0: predictive analytics software PASW), the PLINK toolset (version 1.07; *http://pngu.mgh.harvard.edu/~purcell/plink/*) and the R software package (version 2.7.2; *http://www.r-project.org/*). Non-normally distributed parameters were compared between groups using nonparametric χ^2-tests, whereas other characteristics were analysed using unpaired t-tests.

For statistical evaluations of genotypic and allelic distributions, we used Fisher's exact test and applied logistic regression to test for an association. According to the procedure of Kwon and colleagues, we assumed a dominant model of inheritance. The evaluations were performed for the entire sample ($N = 103$) and for the subgroup of clozapine-treated patients ($N = 40$).

We controlled for the possible confounding effects of sex, age and type of antipsychotic medication using these variables as covariates. Because of the high diversity of SGA treatment (number of substances: seven), we grouped amisulpride, aripiprazole, risperidone, ziprasidone, quetiapine and olanzapine ($N = 63$) against clozapine ($N = 40$) in the haplotype analysis of the complete sample. The level of significance was set at P less than or equal to 0.05.

Results

Sociodemographic characteristics and clinical assessments

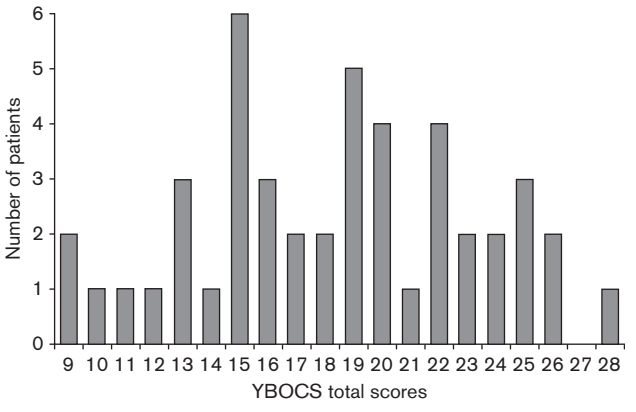
A total of 103 patients with schizophrenia according to DSM IVR and receiving stable, monotherapeutic SGA treatment were investigated. According to the YBOCS results, we assigned the patients to group I (OCS, YBOCS score ≥ 8) or group II (non-OCS, YBOCS score ≤ 7). The sociodemographic characteristics and clinical variables are presented in Table 1. The two groups did not differ in terms of sex, years of education or age at onset of schizophrenia. Patients with comorbid OCS, however, were significantly older ($T = -3.210$, $P < 0.01$) and ill for a significantly longer period of time ($T = -5.459$, $P < 0.01$). Significant differences were found in terms of the severity of OCS, as documented by the YBOCS obsession and compulsion subscores in Table 1. All patients showed an insight into their OCS, allowing for a clear-cut distinction from psychotic symptoms such as delusions or hallucinations. The OCS group showed a mean YBOCS score of 18.4 (±4.9). Twenty-nine (65.9%) of these patients reported a total score of at least 16, representing a clinically relevant severity of illness. Figure 1 shows the distribution of individual YBOCS scores within group I.

Table 1 The study sample was separated according to the presence or absence of obsessive–compulsive symptoms

	Group I OCS	Group II Non-OCS	Between-group differences
Sociodemographics			
N	44	59	
Age	41.2±11.1	34.2±10.8	$T = -3.210$, $P = 0.002$
Male/female ratio	35 : 9	38 : 21	$X = -1.665$, $P > 0.05$
Age of onset	26.2±10.1	28.1±9.7	$T = 0.924$, $P > 0.05$
Duration of illness	16.5±10.1	7.1±6.8	$T = -5.459$, $P < 0.001$
Education in years	10.4±1.7	10.3±1.8	$T = -0.243$, $P > 0.05$
YBOCS			
Obsession	8.5±3.8	0.3±1.4	$T = -15.162$, $P < 0.001$
Compulsion	9.7±3.3	0.9±2.2	$T = -16.264$, $P < 0.001$
Total	18.4±4.9	1.3±2.6	$T = -22.823$, $P < 0.001$

Further significant differences were observed in terms of age and the duration of illness ($P < 0.01$).
OCS, obsessive–compulsive symptoms; YBOCS, Yale-Brown Obsessive–Compulsive Scale.

Fig. 1



The histogram shows the frequencies of individual YBOCS scores in the group with obsessive–compulsive symptoms. YBOCS, Yale-Brown Obsessive–Compulsive Scale

Genetic analysis

None of the SNPs in *SLC1A1* (rs2228622, rs3780412 or rs3780413) showed significant deviation from Hardy–Weinberg equilibrium. We analysed the allelic associations using Fisher's exact tests and did not observe significant group differences for the SNPs (Table 2). Minor allele frequencies in the controls are reported in Table 2. These differed significantly from the frequencies reported by Kwon and colleagues with respect to SNP rs3780413 (G > C 0.34 vs. C > G 0.27). Associations between the three SNPs and secondary OCS were investigated using logistic regression analysis and a dominant model of inheritance. Neither SNP-based nor additional haplotype-based logistic regression analyses showed significant associations (Table 3). Separate subgroup evaluations were carried out for the group of clozapine-treated patients. No marker reached the level of significance. In addition, no significant differences were observed after adjusting for the possible confounding effects of age, sex and type of medication with individual substances as group determinants (Table 4).

The haplotype-based analyses showed no significant associations of the A/C/G haplotype block and OCS, either in the complete sample or in the subgroup of clozapine-treated patients (Table 5). Adjustment for the variables age, sex and groups of medication (clozapine vs. nonclozapine) did not alter the results significantly (Table 6).

Despite the nonsignificant results obtained, ORs were calculated to account for the allele distribution between the two groups. Analyses of the entire sample yielded odds between 0.92 and 1.85 for the three investigated SNPs and 1.10 for the A/C/G haplotype. Within the clozapine-subgroup, odds for the SNPs were between 1.65 and 6.92 and the confidence intervals were noticeably larger (Tables 3–5). This also applied for the haplotype analysis (OR: 1.56, NS).

Pharmacological effects on obsessive–compulsive symptoms prevalence and severity

Significant differences were found between the two groups in terms of the duration of treatment with SGAs in general and the specific SGA at the time of assessment. In addition, the SGAs were distributed differentially between groups. Most importantly, clozapine was more frequent within group I and amisulpride, aripiprazole and quetiapine within group II. Group I showed significantly higher daily treatment doses of clozapine and lower doses of quetiapine (Table 7).

To account for the order of occurrence, three events were assessed retrospectively: (a) first psychotic manifestation; (b) start of SGA treatment; and (c) onset of OCS. Stability versus dynamic increase in OCS over time was also explored. In the vast majority of cases, a sequential order of these events was observed, strongly suggesting SGA-induced OCS (Fig. 2). A minority of patients ($n = 7$) reported OCS before or simultaneously with the psychotic manifestation; all except two, however, recalled worsening of symptoms during SGA treatment.

Furthermore, correlation analyses support the assumption of causal interactions between SGA treatment and OCS: the duration of antipsychotic treatment correlated significantly with the severity of OCS as measured using the YBOCS total score ($r = 0.39$, $P < 0.01$) and the subscale obsessions ($r = 0.50$, $P < 0.001$).

Within the subgroup of clozapine-treated patients, these correlations were more prominent and highly statistically significant: YBOCS total score ($r = 0.67$, $P < 0.001$), obsessions ($r = 0.74$, $P < 0.001$) and compulsions ($r = 0.48$, $P < 0.01$). Subgroup analyses for olanzapine-treated patients also reached statistical significance for the total YBOCS scores ($r = 0.46$, $P < 0.05$) and the subscale obsessions ($r = 0.75$, $P < 0.001$). The duration of illness was integrated as a regressor. Partial correlation analyses for all associations between the severity of OCS and duration of treatment remained significant, except for the correlation between compulsions and duration of clozapine treatment.

Analyses of the clozapine subgroup also showed significant associations between the dose of medication and the subscale compulsion ($r = 0.33$, $P < 0.05$). The YBOCS total score ($r = 0.31$, $P = 0.058$) and the subscale obsessions

Table 2 Allelic association analysis of the single nucleotide polymorphisms of interest between groups according to Fisher's exact test

SNP	Genotype	Group I OCS	Group II Non-OCS	Allele	MAF	Fisher's exact test *P* value	OR (95% CI)
rs2228622				G>A	0.42	0.20	1.47 (0.85–2.57)
	AA	13	8				
	AG	19	33				
	GG	12	18				
rs3780413				G>C	0.34	1.00	1.00 (0.55–1.79)
	CC	6	5				
	CG	17	29				
	GG	21	25				
rs3780412				A>G	0.41	0.40	1.29 (0.74–2.24)
	GG	12	10				
	GA	18	29				
	AA	14	20				

CI, confidence interval; MAF, minor allele frequency in controls; OCS, obsessive–compulsive symptoms; OR, odds ratio; SNP, single nucleotide polymorphism.

Table 3 Association analysis of obsessive–compulsive symptoms with single nucleotide polymorphisms on SLC1A1 using logistic regression and a dominant model of inheritance

SNP	*P* value	OR (95% CI)
Total sample		
rs2228622	0.73	1.17 (0.49–2.78)
rs3780413	0.59	0.81 (0.37–1.77)
rs3780412	0.82	1.10 (0.48–2.53)
Clozapine subgroup		
rs2228622	0.43	1.83 (0.41–8.23)
rs3780413	0.21	0.38 (0.08–1.73)
rs3780412	0.56	1.56 (0.35–6.88)

CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

Table 4 Logistic regression analysis with a dominant model of inheritance adjusted for age, sex and type of medication

SNP	*P* value	OR (95% CI)
Total sample		
rs2228622	0.32	1.85 (0.55–6.20)
rs3780413	0.87	0.92 (0.29–2.83)
rs3780412	0.36	1.72 (0.54–5.43)
Clozapine subgroup		
rs2228622	0.1121	6.92 (0.64–75.2)
rs3780413	0.6990	1.65 (0.13–20.6)
rs3780412	0.2342	3.63 (0.43–0.41)

CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

Table 5 Haplotype analysis between groups with and without obsessive–compulsive symptoms using logistic regression and a dominant model of inheritance

Haplotype	*P* value	OR (95% CI)
Total sample		
A/C/G	0.82	1.10 (0.48–2.53)
Clozapine subgroup		
A/C/G	0.56	1.56 (0.35–6.90)

CI, confidence interval; OR, odds ratio.

Table 6 Haplotype analysis between groups with and without obsessive–compulsive symptoms using logistic regression and a dominant model of inheritance adjusted for the interfering variables age, sex and groups of medication (clozapine against nonclozapine treatment)

Haplotype	*P* value	OR (95% CI)
Total sample		
A/C/G	0.62	1.30 (0.46–3.68)
Clozapine subgroup		
A/C/G	0.24	3.63 (0.43–30.50)

CI, confidence interval; OR, odds ratio.

($r = 0.29$, $P = 0.073$) showed trends of a dose-related association. No significant associations were found between blood serum levels and the severity of OCS.

Discussion

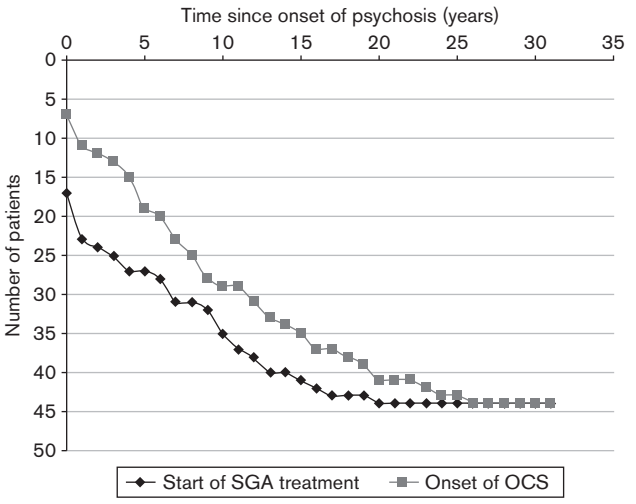
Growing evidence reports the de-novo occurrence of OCS as a side effect of SGA treatment in schizophrenic patients; yet, only limited research exists exploring the mechanisms of this co-occurrence. Both genetic and pharmacological factors have been proposed, but the supposed interplay of genes and environment has not been unravelled clearly.

Table 7 Psychopharmacological properties of the study sample

	Group I OCS ($N=44$)	Group II Non-OCS ($N=59$)	Between-group differences
Duration of medication (months)			
SGA's	121.6±72.0	50.3±54.7	***T*= −5.497, *P*<0.001**
Index (currently applied)	101.8±78.4	20.2±26.7	***T*= −7.449, *P*<0.001**
Type of antipsychotics			
Clozapine	29	10	***X*=25.679, *P*<0.001**
Olanzapine	8	12	$X=0.075$, $P>0.05$
Amisulpride	1	13	***X*=8.380, *P*=0.004**
Aripiprazole	1	14	***X*=9.325, *P*=0.002**
Quetiapine	3	9	$X=1.743$, $P>0.05$
Ziprasidone	1	1	$X=0.044$, $P>0.05$
Risperidone	1		NA
Dose of antipsychotics			
Clozapine (SD)	417.2 (201.1)	282.5 (139.5)	***T*= −2.331, *P*=0.029**
Olanzapine	20.6 (6.8)	20.7 (9.5)	$T=0.029$, $P>0.05$
Amisulpride	1200	530.8 (275.0)	NA
Aripiprazole	25.0	18.9 (5.9)	NA
Quetiapine	500.0 (173.2)	777.8 (156.4)	***T*=2.986, *P*=0.024**
Ziprasidone	320	160	NA
Risperidone	7	–	NA

The groups differ in terms of the duration of treatment with SGA therapy in general and currently applied SGA. The frequencies of individual substances were unevenly distributed between groups. Differences were tested for each substance against all others by χ^2 testing. *t*-tests were performed to analyse the differences in the daily treatment dose. Because of small groups, some comparisons were not tested (NA). Significant differences are shown in bold.
NA, not applicable; OCS, obsessive–compulsive symptoms; SGA, second-generation antipsychotics.

Fig. 2



The survival curve shows the events 'start of SGA treatment' and 'onset of OCS' on an individual basis related to the first manifestation of psychosis within group I (with OCS). Seven of 44 patients suffered from OCS before or at manifestation of psychosis. The vast majority reported onset of this comorbid syndrome during treatment with SGAs. OCS, obsessive–compulsive symptoms; SGA, second-generation antipsychotics.

Kwon and colleagues were the first to report associations between SNPs of the glutamate transporter gene *SLC1A1* and an increased susceptibility to develop secondary OCS

under treatment with SGAs. In a sample powered to detect the effects reported by Kwon and colleagues we used a replication approach with 103 schizophrenic patients of European descent (44 of whom were suffering from comorbid OCS). We were unable, however, to detect associations between *SLC1A1* and OCS: neither did single marker nor haplotype analyses yield any significant results for the overall sample or the subgroup of patients receiving clozapine treatment.

To determine the impact of genetic variation on the occurrence of OCS in our sample, calculations of ORs for the investigated SNPs and haplotype were carried out. Effect size analyses showed odds that were noticeably smaller than those reported by Kwon *et al.* (2009) (0.92–1.85 for the three single markers and 1.30 for the haplotype).

As our study provides no evidence for associations, it should be noted that the power analysis was based on the reported OR of 3.96, with a power of 0.85 and a significance level of P less than or equal to 0.05. Because of an ascertainment bias known as the 'winner's curse', the genetic effect might have been overestimated and may have thus contributed to our nonreplication (Zoellner *et al.*, 2007).

Compared with our study (17%), Kwon and colleagues. included significantly more clozapine-treated patients in their non-OCS group (80%). As the majority of clozapine-treated schizophrenic patients develop second-onset OCS (Schirmbeck and Zink, 2011b), it may be possible that their non-OCS group contained OCS-resistant patients, probably due to protective genetic factors. This may have contributed to the high OR of 3.96. Assuming a smaller effect of 1.30 (on the basis of the haplotype upper estimate from our own data), sample size calculation indicates the need to include over 1000 in a subsequent study to achieve sufficient power. As monocentric investigations usually cannot cover such sample sizes, multicentre studies should be carried out. We further consider the publication of results obtained in small samples as important to facilitate meta-analytic approaches. More generally, the lack of an association may also indicate major genetic differences between the two ethnic groups. The findings obtained in one group may have fundamental limitations for detecting common SNPs in other populations (Ju *et al.*, 2011).

Differences in allele frequencies may have also contributed to group differences between the Asian and the European sample as has been described for some common diseases, for example, hypertension or diabetes (Tate and Goldstein, 2008). However, in none of the reported cases has allelic variation in a susceptibility gene been shown to account for a significant fraction of the difference in prevalence among groups.

The present study has methodological limitations. Some degree of phenotype heterogeneity within our sample may have reduced penetrance. A small minority of our patients (seven of 44) experienced OCS before or simultaneously with psychotic manifestation and treatment with SGAs.

In addition, given the high likelihood of a combined effect of multiple genes in the aetiology of OCS, it is desirable to examine sufficiently large samples of individuals so that homogeneous clinical subgroups can be identified and additional functional candidate genes can be investigated.

With respect to OCS development under SGA treatment, our data strengthen evidence from previous studies. Within antiserotonergic substances, induction of secondary OCS occurs most likely under clozapine (Schirmbeck and Zink, 2011b) represented by the high proportion of clozapine-treated patients in group I and significant correlations of the severity of OCS with the duration and the dose of clozapine treatment. Further evidence also hints towards a causal effect of SGA treatment on OCS development. The vast majority of patients in group I reported de-novo occurrence after the onset of SGA treatment and/or a noticeable aggravation of symptoms (Fig. 2).

Pathomechanism

As a pharmacodynamic contribution to OCS development by antiserotonergic effects seems very likely, we could not replicate the genetic association found by Kwon and colleagues. Therefore, the proposed gene–environment interplay, which might predispose schizophrenic patients with genetic risk factors to develop OCS under pro-obsessive pharmacological treatment such as clozapine, requires further clarification. The calculated OR of 3.63 within the clozapine-treated subgroup hints towards such a pharmacogenetic effect. However, due to the lack of significance and the large confidence interval (0.433–30.5), we cannot draw any conclusions. Investigations with larger samples are needed to prove a predisposing importance of *SLC1A1* for clozapine-induced OCS.

Therapeutic implications

The clinical constellation of comorbid OCS in schizophrenia still awaits therapeutic interventions with demonstrated effectiveness (Hwang *et al.*, 2009). Assuming pro-obsessive effects of SGAs suggests the use of combination and augmentation strategies aimed at minimizing their antiserotonergic effects. In case of future defined genetic risk factors, clinical consequences might result in preventive genetic screening and close monitoring of patients at risk for secondary OCS. As recent findings suggest a differential profile of neuropsychological deficits in schizophrenic patients with and without comorbid OCS, careful cognitive testing with sensitive batteries

should be considered for SGA-treated schizophrenic patients (Schirmbeck *et al.*, 2011a).

Conclusion

Our results did not replicate previous findings, but support a pharmacodynamic mechanism of OCS in schizophrenia and highlight the need for further investigations on possible gene–environment interplays. Forthcoming, large multicentre studies should broaden the perspective to additional candidate genes (Nicolini *et al.*, 2009; Pauls, 2010), interactions of polymorphisms and imaging genetics to examine the attribution of genetic markers to the functional properties of the brain (Meyer-Lindenberg, 2010). Insights into the differential pathogenesis of OCS in schizophrenia might have important implications for antipsychotic and antiobsessive treatment in combination with cognitive behavioural therapy.

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Conflicts of interest

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