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Complexin2 modulates working memory-related neural activity in patients with schizophrenia

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Abstract The specific contribution of risk or candidate gene variants to the complex phenotype of schizophrenia is largely unknown. Studying the effects of such variants on brain function can provide insight into disease-associated mechanisms on a neural systems level. Previous studies found common variants in the complexin2 (*CPLX2*) gene to be highly associated with cognitive dysfunction in schizophrenia patients. Similarly, cognitive functioning was found to be impaired in Cplx2 gene-deficient mice if they were subjected to maternal deprivation or mild brain trauma during puberty. Here, we aimed to study seven common *CPLX2* single-nucleotide polymorphisms (SNPs) and their neurogenetic risk mechanisms by investigating their relationship to a schizophrenia-related functional neuroimaging intermediate phenotype. We examined functional MRI and genotype data collected from 104 patients with DSM-IV-diagnosed schizophrenia and 122 healthy controls who participated in the Mind Clinical Imaging Consortium study of schizophrenia. Seven SNPs distributed over the whole *CPLX2* gene were tested for association with working memory-elicited neural activity in a frontoparietal neural network. Three *CPLX2* SNPs were significantly associated with increased neural activity in the dorsolateral prefrontal cortex and intraparietal sulcus in the schizophrenia sample, but showed no association in healthy controls. Since increased working memory-related neural activity in individuals with or at risk for schizophrenia has been interpreted as 'neural inefficiency,' these findings suggest that certain variants of CPLX2 may contribute to impaired brain function in schizophrenia, possibly combined with other deleterious genetic variants, adverse environmental events, or developmental insults.

Keywords Complexin2 · Imaging genetics · Intermediate phenotype · Working memory · Frontoparietal circuit · Schizophrenia

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Introduction

The neurobiological underpinnings of severe mental disorders such as schizophrenia are still poorly understood. Twin studies suggest that certain genes predispose individuals to schizophrenia, but a concordance rate of 40–65 % in monozygotic twins [1, 2] indicates the importance of epigenetic or environmental risk factors. The widely accepted diathesis-stress model [3–5] proposes that susceptibility to psychological disorders is due to a pre-existing vulnerability that emerges after exposure to deleterious psychological states and environmental events. The diathesis-stress conceptual model is the basis for the 'two (or second)-hit' hypothesis of schizophrenia. First, described by Bayer et al. [6], the two-hit hypothesis states that a genetic defect leads to deficiencies in neuronal networks (first hit), and a second hit (e.g., an environmental hazard) further perturbs the functioning of the network, perhaps through modulation of gene activity, which then leads to the emergence of an ongoing psychotic illness.

Since there is considerable evidence for synaptic dysfunction playing a central role in the etiology of schizophrenia [7, 8], synaptic molecules such as synapsins and complexins might be of great importance for the disease process. The complexin family of presynaptic regulatory proteins consists of four members, of which only complexin1 and complexin2 (CPLX2) are strongly expressed in the central nervous system [9]. Complexins regulate synaptic neurotransmitter release and thereby influence synaptic signaling [10], synaptic plasticity [11], and neuronal network function [12]. Dysregulation of complexin expression occurs in neurodegenerative disorders such as Huntington's, Parkinson's, and Alzheimer's disease [13–15], as well as in psychiatric disorders such as schizophrenia [16, 17]. Although results from case–control genetic association studies have been inconsistent [18, 19], Eastwood and Harrison [20] found markedly reduced CPLX2 expression in the dorsolateral prefrontal cortex (DLPFC) and the superior temporal cortex (STC) in schizophrenia patients.

Recent animal models allow us to better understand how genetic and environmental risk factors for schizophrenia interact [21]. Yamauchi et al. [22] examined the physiological characteristics of Cplx2 gene-deficient mice subjected to maternal deprivation stress and found a significant decrease in posttetanic potentiation and LTP induction in the knockout mice compared to the wild type, suggesting that in the setting of an environmental insult (maternal deprivation), variation in the Cplx2 gene becomes a critical determinant of synaptic functioning. Radyushkin et al. [23] designed a similar experimental approach for a schizophrenia-like phenotype in mice to specifically test the aforementioned 'second-hit' hypothesis. They studied

behavior and cognitive functioning (which is typically impaired in schizophrenia) in Cplx2-null mutant ($Cplx2^{-/-}$) mice jointly with a mild parietal neurotrauma, applied during puberty. Consistent with the 'second-hit' hypothesis, they found reduced prepulse inhibition and deficits of spatial learning as well as decreased hippocampal volume in $Cplx2^{-/-}$ mice after the neurotrauma had been applied, but not in lesioned wild-type mice or nonlesioned $Cplx2^{-/-}$ mice.

Subsequently, Begemann et al. [24] tested for associations between CPLX2 single-nucleotide polymorphisms (SNPs) and several domains of cognitive functioning (executive functioning, reasoning, and verbal learning/memory) in a large sample of patients with schizophrenia ($N_{SZ} = 1,071$). They found associations between six CPLX2 SNPs and impaired neurocognitive performance in patients. Assuming that schizophrenia patients have been exposed to additional environmental risk, they interpreted CPLX2 as a modifier of cognitive functioning in individuals which were subjected to adverse environmental events or developmental insults ('second hit'). Although CPLX2 has not been shown to be a risk gene for a diagnosis of schizophrenia in a recent GWA study/meta-analysis [25, 26], the results presented by Begemann et al. illustrate the impact of CPLX2 risk variants on the severity of cognitive dysfunction in patients with schizophrenia. However, the precise roles of these variants in disease-associated mechanisms on a neural systems level are unknown. Impaired working memory processing and functional abnormalities of a lateral frontoparietal network, which mediates working memory functions, are established intermediate phenotypes of schizophrenia [27–29]. Therefore, the aim of our study was to investigate the neurogenetic risk mechanisms of CPLX2 SNPs by examining their associations with frontoparietal network efficiency during working memory. Based on the aforementioned studies in rodents and in patients with schizophrenia [24], we hypothesized the presence of such associations in patients with schizophrenia, assuming that they have been exposed to additional environmental risk factors.

Materials and methods

Participants

Imaging, genetic, and behavioral data from participants of the multisite Mind Clinical Imaging Consortium (MCIC) study of schizophrenia [30, 31] were used for analyses. All subjects gave written informed consent prior to study enrollment. The human subjects' research committees at each of the four sites (Massachusetts General Hospital (MGH) and the Universities of Iowa (UI), Minnesota

(UMN), and New Mexico (UNM)) approved the study protocol. The patient group (SZ) consisted of subjects with a DSM-IV diagnosis of schizophrenia established using structured clinical interviews and review of case files by trained clinicians (see also Online Resource 1.1). Healthy controls (HC) were included if they had no history of a medical or Axis I psychiatric diagnosis. All participants were required to be at least 18 years of age and no older than 60 and had to be fluent in English. Participants were excluded if they had a history of neurologic disease, or psychiatric disease other than schizophrenia, history of a head injury with loss of consciousness, history of substance abuse or dependence within the past month, severe or disabling medical conditions, any contraindication to MR scanning, or an IQ less than 70 (based on the reading subtest from the Wide Range Achievement Test (WRAT3)). For details on clinical measures, see Online Resource 1.1.

Sternberg Item Recognition Paradigm

The Sternberg Item Recognition Paradigm (SIRP) is a working memory (WM) task, previously shown to activate the DLPFC in healthy controls and schizophrenia patients [32]. The SIRP was administered during six 46 s blocks per run for three 360 s runs. In each block, a memory set, composed of one (load1), three (load3), or five (load5) digits, was presented (two blocks per load condition). The Encode phase was followed by a presentation of 14 digits, one at a time (the Probe phase), and participants responded to each probe to indicate whether or not the probe digit was in the memory set. For additional details about the paradigm, see Roffman et al. [33] and Online Resource 1.2. The stimuli and responses were presented and collected using E-prime software (EPrime v1.1, Psychology Software Tools, Inc., Pittsburg, PA) during fMRI scanning. Participants were excluded from further analysis if they completed a block with less than a 80 % accuracy rate and/or with more than 6 probes not answered within a block.

Image acquisition and processing

Structural MRI data were acquired with either a 1.5-T Siemens Sonata (UNM, MGH, UI) or a 3-T Siemens Trio (UMN) and analyzed in an automated manner with atlas-based FreeSurfer parcellation and segmentation software version 4.0.1 (http://surfer.nmr.mgh.harvard.edu) to generate cortical and subcortical volumetric measures of regions of interest (ROIs) using each participant's individual anatomy [34]. We focused on two ROIs, the intra-parietal sulcus (IPS), which is a standard output of FreeSurfer parcellation, and the DLPFC. DLPFC ROIs were derived from a combination of several FreeSurfer

cortical parcellations as described previously [35, 36]. For details, see Online Resource 1.3.

Functional MRI data were acquired with either a 1.5-T Siemens Sonata (UNM) or a 3-T Siemens Trio (UMN, MGH, UI) and analyzed using the Function Biomedical Informatics Research Network (FBIRN) Image Processing Stream (FIPS), a pipeline using the Functional MRI of the Brain (FMRIB) Software Library of FSL [37]. Variability due to different scanners and acquisition protocols was analyzed using ten MCIC subjects who were scanned at all four sites [36]. Variability of neural activity due to site differences was negligible small compared to variability due to subject differences. For additional information about data acquisition and processing, see Online Resource 1.3.

A functional imaging linear model [38] was fit to model the Probe phases of each subject's preprocessed functional time series. We used the linear Contrasts of Parameter Estimate (COPE) Probe-load 5 versus Probe-load 1 and refer to responses to this condition as 'load-dependent' activation [33]. We obtained indices of activation for the left and right IPS and left and right DLPFC ROIs using the COPE obtained from the second-level fixed-effects analysis for each participant. We applied an additional functional mask, based on the COPE of all loads (average of Probe-load 1, Probe-load 3 and Probe-load 5) versus fixation exceeding a threshold of $Z = 2.3$ and extracted the mean percentage signal change. The use of a functional mask (within anatomical ROIs) from all working memory loads protected against biases in signal change calculations derived from individual conditions [39].

Genotyping and imputation

Blood samples were obtained from 255 MCIC participants and sent to the Harvard Partners Center for Genetics and Genomics for DNA extraction. All DNA extraction and genotyping was done blind to group assignment. Genotyping was performed at the Mind Research Network Neurogenetics Core Laboratory using the IlluminaHumanOmni-Quad BeadChip. Quality control steps included common standard procedures [40] using PLINK, 1.07 [41]. SNPs on the X or Y chromosome, or those with a genotyping rate of less than 90 % or a minor allele frequency of less than 5 % were excluded from the analysis. We removed seven participants with extreme heterozygosity values (±3SD). Another 22 subjects were excluded because of low quality or missing fMRI data (for imaging control steps, see Online Resource 1.3). The final sample with complete and high-quality structural and functional MRI and genetic data comprised of 122 HC and 104 SZ.

Additional SNPs were imputed based on the Hapmap3 data set. Imputation was done using IMPUTE2 [42] with a probability threshold of 0.95. The imputed data set was

then again filtered for a minor allele frequency of 5 %, a genotyping rate of less than 90 %, and a Hardy–Weinberg equilibrium in controls with a threshold of 10^{-6}. Using this data set, seven *CPLX2* SNPs (rs2443541, rs2243404, rs4242187, rs10072860, rs4868539, rs3892909, and rs3822674) were extracted, five of those being significantly associated with current cognition in patients with schizophrenia in a recent candidate gene study [24]. General information and quality control measures including details about SNP location, an LD plot, and allele frequencies according to diagnostic group for all seven SNPs are given in Online Resource Table 1 and 2 and Online Resource Fig. 1. Because of the low frequency of T/T genotypes for both rs2243404 ($N = 6$) and rs2443541 ($N = 6$), T/T and C/T participants were combined into a T allele carrier group ($N = 37$ and $N = 38$, respectively).

Statistics and principal component analysis (PCA)

Given our strong hypothesis, we primarily analyzed the patient sample ($N = 104$). Additional (separate) analyses were carried out for the control group ($N = 122$). Basic demographic characteristics were compared across genotype group and across sites with different scanner field strengths using one-way ANOVA and subsequent Bonferroni post hoc tests where necessary. Chi-square statistics were used to examine differences in categorical variables. Alpha was set to 0.05 for all analyses.

Each of the seven *CPLX2* SNPs was tested for association with neural activity in the a priori defined working memory-related brain regions using a linear mixed model and controlling for age, gender, and scanner field strength. We assumed a compound symmetry covariance structure for the load-dependent change of neural activity in the four brain regions (DLPFC and IPS mean activity in the left and right hemisphere). Population stratification was addressed using ten principal components as additional covariates calculated by EIGENSTRAT of the EIGENSOFT 3.0 software package [43, 44]. Details are given in Online Resource 1.6. We ran separate mixed models for the two diagnostic groups (SZ and HC). Mixed models for rs2243404 and rs2443541 were performed contrasting C allele homozygotes and T allele carriers, respectively, because of the small number of participants in the TT group. Multiple testing was addressed by Bonferroni correction ($p < 0.007$; corrected for the number of tested SNPs). All statistical analyses were carried out in IBM SPSS Statistics 19.

To verify our results in an ethnically homogeneous sample we re-ran the analyses in a subsample defined by stringent genetic criteria, including individuals of European descent only ($N_{SZ} = 68$; $N_{HC} = 95$; see Online Resource 1.7 for further details). Additional analyses were carried out on a whole-brain level using FSL (FMRIB Software Library; version 4.1.7; Smith et al. [37]). Please refer to Online Resource 1.8 for methodological details.

Results

Sample characteristics

In the patient group, there were no differences among genotype groups (seven *CPLX2* SNPs) with respect to gender, age, parental SES, handedness, length of illness, and negative or positive symptoms. Rs3892909 T allele homozygotes and rs4868539 A allele homozygotes showed significantly higher WRAT3-RT scores; rs3892909 C allele homozygotes had higher lifetime antipsychotic exposure; and rs4242187 C/T heterozygotes showed a higher working memory performance than C/C or T/T homozygotes (see Online Resource 2.1, Online Resource Table 3). Also, patients from acquisition sites with different scanner field strengths did not differ in any demographic or clinical variables. In the control group, there were no differences among genotype groups for any of the tested variables (data not shown). An overview about the demographic (and clinical) variables of patients with schizophrenia and healthy controls, respectively, is displayed in Table 1. As expected, schizophrenia patients had a significantly lower performance on the Sternberg Paradigm when compared to healthy controls ($F = 44.26$; $p < 0.001$).

Functional MRI

Three *CPLX2* SNPs (rs2443541, rs2243404, and rs4868539) were associated with load-dependent neural activity in working memory-related brain regions (DLPFC and IPS; see Fig. 1) in the schizophrenia sample. For both, rs2443541 and rs2243404, C allele homozygotes showed an increased neural activity when compared to T allele carriers [$F(df = 1,104) = 12.387$, $p = 0.001$ and $F(df = 1,104) = 12.972$, $p < 0.001$, respectively]. The same was found for rs4868539 G allele carriers compared to A allele homozygotes [$F(df = 2,104) = 5.530$, $p = 0.005$; see Table 2]. According to Bonferroni-corrected post hoc tests, the estimated residuals of the neural activity differed significantly between G allele homozygotes and A allele homozygotes ($p = 0.024$), as well as between heterozygotes and A allele homozygotes ($p = 0.005$), but not between G allele homozygotes and heterozygotes. We found no evidence for associations between working memory-related neural activity and any of the seven *CPLX2* SNPs in healthy participants. Table 3 displays the test statistics and p values for the linear mixed models of each SNP. For *CPLX2* rs2443541 and

Table 1 Demographic and clinical variables

Scanner field strength	N	Sex (female) N	Sex (female) %	Age (years) Mean	Age (years) SD	WRAT3-RT Mean	WRAT3-RT SD	Parental SES Mean	Parental SES SD	Handedness Mean	Handedness SD
Schizophrenia patients											
1.5 T	19	5	26.3	36.26	13.82	45.28	6.92	2.88	1.11	1.32	3.06
3 T	85	21	24.7	34.19	10.26	46.65	6.63	2.81	0.98	1.13	2.99
Total	104	26	25.0	34.57	10.95	46.40	6.67	2.82	0.99	1.17	2.99
Healthy controls											
1.5 T	22	4[a]	18.2	30.27	12.84	51.50	3.79	2.14[b]	0.77	1.05	2.42
3 T	100	42[a]	42.0	33.06	10.87	50.75	4.08	2.80[b]	0.68	0.85	2.62
Total	122	46	37.7	32.56	11.25	50.89	4.03	2.68	0.74	0.89	2.58

Scanner field strength	WM performance Mean	WM performance SD	Length of illness (years) Mean	Length of illness (years) SD	Negative symptoms Mean	Negative symptoms SD	Positive symptoms Mean	Positive symptoms SD	Medication (cum. dose years) Mean	Medication (cum. dose years) SD
Schizophrenia patients										
1.5 T	95.47	3.98	11.71	12.53	8.58	3.58	4.74	2.66	32.99	26.66
3 T	95.58	4.50	11.56	9.78	7.84	3.83	4.82	2.84	134.65	63.85
Total	95.56	4.39	11.58	10.27	7.97	3.78	4.81	2.79	122.86	56.85
Healthy controls										
1.5 T	98.90	0.70	–	–	–	–	–	–	–	–
3 T	98.32	1.72	–	–	–	–	–	–	–	–
Total	98.42	1.61	–	–	–	–	–	–	–	–

Means and standard deviations (SD) are given. SZ = patient with schizophrenia; HC = healthy control. WRAT3-RT = Wide Range Achievement Test 3—Reading Test. Parental SES (socioeconomic status) was classified according to Hollingshead, and handedness determined using the Annett Scale of Hand Preference. Working memory (WM) performance represents the mean accuracy during SIRP across all loads (1, 3, 5). Severity of positive and negative symptoms was rated using the Scale for the Assessment of Positive Symptoms (SAPS) and the Scale for the Assessment of Negative Symptoms (SANS). Cumulative antipsychotic exposure was calculated using the chlorpromazine (CPZ) conversion factors of Woods et al. [61]. [a] Significantly different between 1.5-T and 3-T sites on basis of chi square ($p < 0.05$). [b] Significantly different between 1.5- and 3-T sites on basis of Welch ($p < 0.05$). The patient group comprised more females [$\chi^2(1) = 4.17$, $p = 0.041$], showed lower WRAT3-RT scores [$F(1,220) = 38.23$, $p < 0.001$] and a lower performance in the WM task [$F(1,221) = 44.26$, $p < 0.001$]

rs2243404, the aforementioned effects were also determinable on a whole-brain level (see Online Resource 2.2).

In a subsample of participants of European descent, we found similar results for rs2443541 and rs2243404 in schizophrenia patients. Again, none of the tested polymorphisms showed any association with neural activity in the DLPFC or IPS in the subsample of healthy participants with European ancestry (Table 3). In subsequent models,
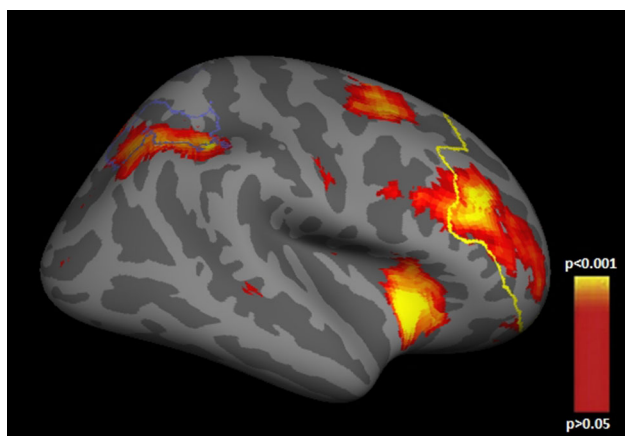
we also covaried for the amount of lifetime antipsychotic exposure, working memory performance, and WRAT3-RT scores. All main effects remained robust (Online Resource 2.3, Online Resource Table 4).

Discussion

Our analysis revealed an association between *CPLX2* polymorphisms and working memory load-dependent neural activity in a frontoparietal brain network in schizophrenia patients but not in healthy controls. Results were strengthened by subsequent analyses including potentially confounding variables such as lifetime antipsychotic exposure and working memory performance. Our findings are in line with the results from Cplx2$^{-/-}$ rodent models and consistent with the 'second-hit' hypothesis of schizophrenia, i.e., *CPLX2* risk variants increase neural inefficiency if the individual has experienced an additional risk factor. Second-hit risk factors such as prenatal infections, perinatal complications, early insults to the brain, stressful life events, or drug abuse are more prevalent in people affected by schizophrenia than demographically similar control samples (for a review, see Vilain et al. [45]).

The risk alleles of two (rs2443541 and rs2243404; C allele for both SNPs) out of the three genetic variants which were associated with working memory-related neural activity in our study had previously been related to cognitive functioning specifically in schizophrenia patients [24]. As described in the Supplementary Online

Fig. 1 FreeSurfer cortical parcellation of the DLPFC and IPS in the schizophrenia sample. Cortical statistical map illustrating regions of working memory-related load-dependent neural activity in the dorsolateral prefrontal cortex (anterior outline) and the intraparietal sulcus (posterior outline) in the schizophrenia sample. This map is shown on the inflated surface of the standard average subject allowing visualization of data across the entire cortical surface without interference from cortical folding

Table 2 Associations between working memory-related load-dependent neural activity and *CPLX2* SNPs in schizophrenia patients

	rs2443541		rs2243404		rs4242187			rs10072860		
	CC	T carrier	CC	T carrier	CC	CT	TT	GG	GT	TT
N	67	37	67	37	43	47	14	30	55	19
Left DLPFC	**0.072**	**0.043**	**0.072**	**0.042**	0.062	0.063	0.055	0.084	0.053	0.049
Right DLPFC	**0.069**	**−0.024**	**0.069**	**−0.028**	0.063	0.033	−0.047	0.027	0.024	0.078
Left IPS	**0.087**	**0.044**	**0.087**	**0.043**	0.077	0.077	0.035	0.076	0.068	0.072
Right IPS	**0.058**	**0.030**	**0.057**	**0.030**	0.049	0.050	0.035	0.044	0.046	0.059

	rs4868539			rs3892909			rs3822674		
	GG	AG	AA	CC	CT	TT	CC	CT	TT
N	20	44	40	19	44	41	25	45	34
Left DLPFC	**0.085**	**0.068**	**−0.003**	0.072	0.070	0.047	0.056	0.050	0.080
Right DLPFC	**0.044**	**0.068**	**−0.054**	0.025	0.068	0.003	0.004	0.049	0.039
Left IPS	**0.076**	**0.076**	**0.053**	0.067	0.083	0.061	0.086	0.077	0.053
Right IPS	**0.044**	**0.058**	**0.035**	0.049	0.063	0.033	0.051	0.053	0.038

Working memory-related mean neural activity (% BOLD change) for corresponding genotype groups of each *CPLX2* SNP in the schizophrenia sample; significant differences according to the linear mixed models ($p < 0.007$; Bonferroni corrected for number of tested SNPs) are printed in bold face. *DLPFC* dorsolateral prefrontal cortex, *IPS* intraparietal sulcus. *N* = number of schizophrenia patients carrying the particular genotype

Table 3 Test statistics and p values for the mixed models testing associations between *CPLX2* variants and load-dependent working memory-related neural activity in schizophrenia patients and healthy controls

SNP	SZ			SZ (Europ. descent)			HC			HC (Europ. descent)		
	F	*df*	*p*	*F*	*df*	*p*	*F*	*df*	*p*	*F*	*df*	*p*
rs2443541	**12.387***	**1, 104**	**0.001**	**5.278**	**1, 68**	**0.025**	1.233	1, 122	0.269	3.127	1, 95	0.080
rs2243404	**12.972***	**1, 104**	**<0.001**	**5.349**	**1, 68**	**0.024**	1.454	1, 122	0.230	3.274	1, 95	0.074
rs4242187	2.327	2, 104	0.103	0.535	2, 68	0.588	0.426	2, 122	0.654	1.380	2, 95	0.257
rs10072860	0.849	2, 104	0.431	0.802	2, 68	0.453	1.095	2, 122	0.338	2.212	2, 95	0.115
rs4868539	**5.530***	**2, 104**	**0.005**	1.638	2, 68	0.202	1.241	2, 122	0.293	1.981	2, 95	0.144
rs3892909	1.890	2, 103	0.156	2.388	2, 67	0.100	0.007	2, 122	0.993	0.201	2, 95	0.819
rs3822674	0.410	2, 104	0.665	0.012	2, 68	0.988	0.024	2, 122	0.976	0.661	2, 95	0.519

Single-locus association analyses of working memory-related load-dependent neural activity (left and right DLPFC and left and right IPS) using mixed models and covarying for age, gender, scanner field strength, and population stratification. Significant test statistics and p values are displayed in bold face ($p < 0.05$) indicating differences between the genotype groups of each SNP. * p value survives Bonferroni correction ($p < 0.007$; corrected for number of tested SNPs). Additional models were carried out in healthy controls and in a subset of each participants group of European descent. SNP = single-nucleotide polymorphism; SZ = patient with schizophrenia; HC = healthy control

content of Begemann et al. (eFigure 1A), for both SNPs, T homozygotes performed significantly better compared to C allele carriers regarding executive functioning and verbal learning/memory, which is in line with our results. Increased load-dependent neural activity in a frontoparietal network in individuals with *CPLX2* risk variants can be interpreted as 'neural inefficiency,' i.e., the additional recruitment of neural resources to achieve a comparable task performance as nonrisk carriers or healthy controls [32, 46, 47]. Based on similar findings in unaffected siblings, prodromal, and medication-naïve and first-episode patients with schizophrenia, 'neural inefficiency' during working memory has been proposed to reflect an expression of genetic liability to schizophrenia [48–53]. A meta-analysis showed that working memory-related neural networks are consistently 'disrupted' in schizophrenia [54]. It has been hypothesized that this putative neural signature of schizophrenia may arise from altered signal transduction and synaptic functioning and plasticity [28, 55]. Synaptic proteins that are involved in the presynaptic secretory machinery such as complexins appear possible molecular candidates.

According to the Expression Atlas of EMBL-EBI (http://www.ebi.ac.uk/gxa/; last accessed on 2013-03-15), *CPLX2* is primarily expressed in the human brain, but in the DLPFC and STC of schizophrenia patients, *CPLX2* expression is abnormally reduced [20]. The altered expression profile of *CPLX2* and other synaptic proteins in schizophrenia suggests that there are aberrations in the precise organization and functioning of neural circuits, indicating that *CPLX2* polymorphisms may contribute susceptibility to schizophrenia [19, 20]. Results of the current study provide a link between frontoparietal cortical function in schizophrenia and variation in the *CPLX2* gene in schizophrenia.

The effects of *CPLX2* are different from common risk genes of schizophrenia since *CPLX2* seems to moderate the expression of the disease but not the general risk for schizophrenia. Other possible explanations for the case-only effect described here include gene X gene and gene X genome interactions. A variety of environmental and other factors can alter the influence of mutant alleles on organismal phenotypes, and the impact of these factors can vary with genetic background [56]. All explanations have in common that *CPLX2* is not an independent risk factor for schizophrenia.

The findings of this study should be interpreted in light of the following limitations. First, we focused on seven *CPLX2* SNPs that had been previously investigated by other researchers. These SNPs may be in high linkage disequilibrium with other functional variants that are responsible for the effects described above. Second, we had an only medium-sized and rather heterogeneous sample, but subsequent models in European ancestry subsamples of patients and controls confirmed our initial results. Furthermore, results of additional whole-brain analyses support our main findings and the assumption that the observed SNP effects are rather specific to working memory-related brain regions. Third, we did not measure environmental factors in this study; we based our hypothesis on previous studies that have consistently shown a significantly higher prevalence of a variety of pre-, peri- and postnatal environmental risk factors for patients with schizophrenia compared to healthy subjects [57–59], which may represent a 'second hit.' Finally, although we covaried for the amount of lifetime antipsychotic exposure in a secondary model and found our results to be essentially the same, we cannot completely exclude a potential effect of antipsychotic medication on our results in patients with schizophrenia. Sawada et al. [60] studied the effect of

antipsychotic drug treatment on the expression of complexin and other presynaptic proteins in rats and observed no differences between treated and control animals for either *Cplx1* or *Cplx2* immunostaining in frontal cortex.

Taken together, our results indicate that *CPLX2* may be involved in the etiology of schizophrenia and we identified one of its potential biological mechanisms by studying the effects of *CPLX2* risk variants on working memory load-dependent neural activity in a frontoparietal network. Since the reported associations were restricted to the patient group, we interpret them in the framework of the 'second-hit' hypothesis and assume that certain *CPLX2* variants only confer risk for schizophrenia if other risk factors are also present. Although not directly related to the risk of the disorder, *CPLX2* variants may moderate its expression. Our findings may help to understand the heterogeneity of schizophrenia and its underlying complex genetic architecture. However, additional studies are needed to replicate the findings and to fully untangle the effects of such genetic variants on brain function.

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