Eur Arch Psychiatry Clin Neurosci (2012) 262:117–124
DOI 10.1007/s00406-011-0233-3

ORIGINAL PAPER

A promoter variant of *SHANK1* affects auditory working memory in schizophrenia patients and in subjects clinically at risk for psychosis

Leonhard Lennertz · Michael Wagner · Wolfgang Wölwer · Anna Schuhmacher · Ingo Frommann · Julia Berning · Svenja Schulze-Rauschenbach · Martin W. Landsberg · Anja Steinbrecher · Michael Alexander · Petra E. Franke · Ralf Pukrop · Stephan Ruhrmann · Andreas Bechdolf · Wolfgang Gaebel · Joachim Klosterkötter · Heinz Häfner · Wolfgang Maier · Rainald Mössner

Received: 10 February 2011 / Accepted: 26 July 2011 / Published online: 7 September 2011

Abstract Mutations in postsynaptic scaffolding genes contribute to autism, thus suggesting a role in pathological processes in neurodevelopment. Recently, two de novo mutations in SHANK3 were described in schizophrenia patients. In most cases, abnormal SHANK3 genotype was also accompanied by cognitive disruptions. The present study queries whether common SHANK variants may also contribute to neuropsychological dysfunctions in schizophrenia. We genotyped five common coding or promoter variants located in SHANK1, SHANK2 and SHANK3. A comprehensive test battery was used to assess neuropsychological functions in 199 schizophrenia patients and 206 healthy control subjects. In addition, an independent sample of 77 subjects at risk for psychosis was analyzed for replication of significant findings. We found the T allele of the SHANK1 promoter variant rs3810280 to lead to significantly impaired auditory working memory as assessed with digit span (12.5 ± 3.6 vs. 14.8 ± 4.1, $P < .001$) in schizophrenia cases, applying strict Bonferroni correction for multiple testing. This finding was replicated for forward digit span in the at-risk sample (7.1 ± 2.0 vs. 8.3 ± 2.0, $P = .044$). Previously, altered memory functions and reduced dendritic spines and postsynaptic density of excitatory synapses were reported in SHANK1 knock-out mice. Moreover, the atypical neuroleptic clozapine was found to increase SHANK1 density in rats. Our findings suggest a role of SHANK1 in working memory deficits in schizophrenia, which may arise from neurodevelopmental changes to prefrontal cortical areas.

Keywords SHANK · Schizophrenia · Prodrome · Cognition · Neuropsychology · Postsynaptic density · Prefrontal cortex · At-risk mental state

L. Lennertz · M. Wagner · A. Schuhmacher · I. Frommann · J. Berning · S. Schulze-Rauschenbach · M. W. Landsberg · A. Steinbrecher · W. Maier · R. Mössner (✉)
Department of Psychiatry and Psychotherapy, University of Bonn, Sigmund-Freud-Straße 25, 53105 Bonn, Germany
e-mail: rainald.moessner@ukb.uni-bonn.de

W. Wölwer · P. E. Franke · W. Gaebel
Department of Psychiatry and Psychotherapy, University of Düsseldorf, Düsseldorf, Germany

M. Alexander
Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany

M. Alexander
Institute of Human Genetics, University of Bonn, Bonn, Germany

R. Pukrop · S. Ruhrmann · A. Bechdolf · J. Klosterkötter
Department of Psychiatry and Psychotherapy, University of Cologne, Cologne, Germany

H. Häfner
Central Institute of Mental Health, Mannheim, Germany

Introduction

Most researchers consider schizophrenia a neurodevelopmental disorder, which is highly determined by genetic factors [29]. Several schizophrenia candidate genes have been identified with some of them being involved in neurodevelopmental processes and neural plasticity [3, 15, 30]. However, none of the investigated variants linked with schizophrenia did account for a substantial part of variance, thus underlining the complex and polygenic heredity of the disorder. Moreover, the exact functional role of putative risk genes remains largely unknown.

Recently, research on the genetic foundations of autism spectrum disorders (ASD) indicated a role of the SHANK genes as being relevant to neurodevelopmental disorders. These genes encode scaffolding proteins (SHANK proteins) involved in the construction of the postsynaptic density (PSD) and dendritic spines of excitatory synapses [5]. More precisely, SHANK proteins constitute master scaffolding proteins interconnecting proteins bound to neurotransmitter receptors or the cytoskeleton [39, 41]. The fundamental role of SHANK in the organization of the PSD is further highlighted by an increase in number and size of dendritic synapses due to SHANK expression [32]. With regard to psychopathology, several mutations of the SHANK genes have been linked with ASD. In addition, most published cases also exhibited speech difficulties, intellectual impairments or mental retardation [8, 13, 18, 26]. Recently, Gauthier and coworkers also presented data suggesting a role of SHANK in schizophrenia with two de novo mutations reported in SHANK3. One of these mutations was found in 3 siblings from one family, all affected with schizophrenia or schizoaffective disorder and presenting borderline mental retardation or mental retardation. The second mutation was identified in a woman with schizoaffective disorder, speech difficulties and poor intellectual abilities [17]. Investigating common variants of genes harboring disease-associated mutations is a promising approach to identify molecular etiological factors. This research strategy was successfully applied with regard to the transcription factor 4 gene (TCF4). Mutations of this gene were previously related to Pitt–Hopkins syndrome and mental retardation [23, 43]. Recently, a large genome-wide association study reported an association of a TCF4 variant (rs9960767) and schizophrenia [37]. Moreover, we found significantly reduced prepulse inhibition, a major schizophrenia endophenotype, in carriers of the disease-related C allele of TCF4 in independent samples of schizophrenia patients and healthy volunteers [28]. Thus, common variants of genes of which mutations lead to severely disrupted neuronal functions may also elicit more subtle effects contributing to neurodevelopmental disorders such as schizophrenia.

Following the approach outlined above, we queried whether common polymorphisms located within the genes SHANK1, SHANK2 and SHANK3 are related to schizophrenia psychopathology. We screened NCBI for all common coding or promoter variants within these genes and identified 5 common variants. Based on prior findings, we assumed that these variants play a role in cognitive functioning, thus contributing to an underlying deficit of schizophrenia patients.

Materials and method

Participants

The schizophrenia sample consisted of $n = 199$ patients diagnosed according to the criteria of DSM-IV or ICD-10 [1, 40]. Patients were recruited at the Departments of Psychiatry of the Universities of Bonn, Cologne, Munich, and at the Central Institute of Mental Health in Mannheim. With the exception of 2 schizophrenia cases with an ancestry from Turkey and 3 cases with uncertain ancestry, all were of Central European ancestry. The schizophrenia patients were in most cases treated with antipsychotics but had to be on stable medication for participation in the neuropsychological testing. Subjects with any neurological disturbance were excluded prior to testing.

The healthy control group consisted of 206 subjects recruited from the general population of Bonn, Cologne, and Mannheim, Germany. The control subjects had to be free of lifetime psychiatric or neurological disorders and were all of German ancestry. With regard to the psychiatric family background of the healthy control subjects, all but 2 subjects had a negative family history for a psychotic disorder.

The sample of 77 subjects clinically at risk for developing a psychosis was recruited within the early detection and intervention program of the German Research Network on Schizophrenia described in detail elsewhere [6]. Briefly, an early or late at-risk state [34] was assumed following detailed criteria including alternatively the presence of a cognitive-perceptive basic symptom criterion (i.e. cognitive disturbances, acoustic and visual perception disturbances); a combination of a marked functional decline with either a 1st degree relative with a schizophrenia spectrum disorder or pre- or perinatal complications; attenuated positive symptoms (ideas of reference; odd beliefs or magical thinking; unusual perceptual experiences; odd thinking and speech; suspiciousness; or paranoid ideations) several times a week over a period of 3 months; or brief limited intermittent psychotic symptoms resolving without treatment within 7 days. For operationalization, the early recognition inventory (ERIraos) was employed [25, 33]. Subjects who previously received antipsychotic treatment or fulfilled criteria of present or past diagnosis of schizophrenic, schizophreniform, schizoaffective, delusional or bipolar disorder, present or past diagnosis of a brief psychotic disorder with a duration of more than 1 week or within the last 4 weeks regardless of its duration, organic brain disease or alcohol or drug dependence within the last 3 months were excluded from the at-risk study.

Procedures

All subjects gave their written informed consent prior to inclusion in the study. The study was approved by the local ethics committees. All subjects were tested with a neuropsychological test battery tapping several cognitive domains known to be affected in schizophrenia. The digit span test of the Wechsler Adult Intelligence Scale [WAIS-R, 38] was used to assess auditory working memory with total repeated number sequences as the dependent measure. To assess processing speed, we used the digit symbol coding test from the WAIS-R with total coded symbols as the dependent measure. Parts A and B of the trail-making test were employed to measure viso-motor speed [31]. Verbal speech production was measured by a lexical fluency task asking the probands to produce as many words with a given initial letter (S, A, B, N) within 1 min with total correct words as dependent variable. Vigilance was assessed by the Continuous Performance Test—Identical Pairs with the deprime score *d'* reflecting a sensitivity measure composed of hits and false alarms as dependent variable [10]. The Rey Auditory Verbal Learning Test (RAVLT) was employed to assess verbal memory with learned words in the first trial, total learning in trials 1–5, delayed recall and recognition as dependent variables [21]. All neuropsychological tests and the cognitive functions measured are outlined in Table 1.

Genotyping

NCBI was screened for all common coding or promoter variants located in the SHANK1, SHANK2 and SHANK3 genes, and five identified polymorphisms were genotyped subsequently. DNA for SNP genotyping was isolated from EDTA anticoagulated blood using the QIAGEN protocol for Blood & Cell Culture DNA Maxi Kit (QIAGEN, Hilden, Germany). PCR was performed using 12.5 ng of DNA, the Taqman® Universal PCR MasterMix, No AmpErase® UNG and the Taqman® SNP genotyping assay for the SNP (all provided by Applied Biosystems, Foster City, CA, USA) according to the protocol for Taqman® SNP genotyping (Applied Biosystems). Each assay consisted of the unlabeled forward and reverse primers and the FAM and VIC dye-labeled MGB probes. Those assays are designed for allelic discrimination of specific SNPs. Both alleles were scored in a single well by measuring the fluorescence at the end of the PCR using a Tecan Ultra 384 reader (Tecan, Crailsheim, Germany). Excitation and emission wavelengths for the FAM-labeled probes were 485 and 535 nm and for the VIC-labeled probes 535 and 590 nm, respectively. Analyzed variants are described in Table 2. Detailed genotype information of the schizophrenia patients, the at-risk cases and the healthy control cases is presented in Table 3. In all samples, the TT genotype frequency of the rs3810280 variant was very low ($n = 5$ schizophrenia patients, $n = 1$ at-risk subjects, $n = 4$ in the healthy control group). Thus, T-allele carriers were combined and contrasted against the CC genotype in all subsequent analyses. In the at-risk cases, we also observed very low frequencies of the AA genotype of the rs3745521 ($n = 2$) and thus combined A-allele carriers of this variant.

Statistical analyses

The relationship of genetic variants and demographic characteristics was investigated using ANOVAs for continuous data and Pearson chi^2-Test for categorical data. We used the FAMHAP program [7] to compute case–control comparisons with regard to genotype distributions. For the analyses of a potential genetic association with cognitive performance, we used analyses of variances applying a strict significance level set at $P = .001$ (two-tailed), thus controlling for multiple testing by the Bonferroni method (5 variants × 10 test parameters). Separate analyses were conducted in the schizophrenia patients and the healthy

Table 1 Neuropsychological instruments with analyzed test parameters and presumed underlying cognitive functions

Neuropsychological instruments	Assessed cognitive functions	Test parameters
WAIS-R digit span	Auditory working memory	Repeated number sequences
WAIS-R digit symbol coding	Processing speed	Coded symbols
Trail-making test	Viso-motor speed and set-shifting	Time (s) part A
		Time (s) part B
Lexical fluency	Executive functioning, speech production	Produced words with letters S, A, B, N
Continuous performance test (CPT-IP)	Attention and vigilance	Deprime score (*d'*)
Rey Auditory Verbal Learning Test (RAVLT)	Verbal memory	Recalled words trial 1
		Recalled words all trials (1–5)
		Recalled words after delay (30 min)
		Recognized words

Table 2 Assessed SHANK variants in patients with schizophrenia and in subjects at risk of psychosis

Gene	rs number	Nucleotide exchange	Amino acid exchange	Chromosomal location of the variant
SHANK1	rs3810280	C-742 T	Promoter variant	Chr 19: 51220937
SHANK1	rs3745521	A4530G	Val1504Ala	Chr 19: 51170706
SHANK2	rs2509835	G -681 T	Promoter variant	Chr 11: 70508553
SHANK2	rs471931	C-1905 T	Promoter variant	Chr 11: 70509777
SHANK3	rs9616915	C734T	Thr245Ile	Chr 22: 51117580

Table 3 Demographic and clinical characteristics and genotype distribution of schizophrenia patients, subjects at risk of psychosis and the healthy control group

	Schizophrenia patients	At-risk subjects	Healthy control subjects
N	199	77	206
Mean age ± SD	36.3 ± 10.9	26.3 ± 7.1	44.8 ± 13.8
Sex (% male)	57.1%	61.0%	47.2%
Education, mean IQ ± SD	109.4 ± 16.3	106.6 ± 13.2	112.7 ± 13.5
Mean age at onset ± SD[a]	28.5 ± 9.1	–	–
Mean duration of illness ± SD[a]	7.3 ± 7.1	–	–
Family history of schizophrenia spectrum disorder, *N* (%)[a,b]	30 (15.1%)	–	–
rs3745521 genotype (AA/AG/GG)	10/70/113	2/27/48	14/80/108
rs3745521 allele frequencies (A/G)	23.3%/76.7%	20.1%/79.9%	26.7%/73.3%
rs3810280 genotype (CC/CT/TT)	145/48/5	55/21/1	139/59/4
rs3810280 allele frequencies (C/T)	85.4%/14.6%	85.1%/14.9%	83.4%/16.6%
rs2509835 genotype (GG/GT/TT)	53/108/36	26/32/19	63/100/39
rs2509835 allele frequencies (G/T)	54.3%/45.7%	54.5%/45.5%	55.9%/44.1%
rs471931 genotype (CC/CT/TT)	37/106/52	18/31/27	39/96/66
rs471931 allele frequencies (C/T)	46.2%/53.8%	44.1%/55.9%	43.3%/56.7%
rs9616915 genotype (CC/CT/TT)	45/106/45	20/40/17	45/95/61
rs9616915 allele frequencies (C/T)	50%/50%	51.9%/48.1%	46.0%/54.0%

Absolute numbers of successfully genotyped patients are given for each genotype. Genotyping success rate was 97–100%

[a] different *N* due to missing data

[b] positive family history of any disorder classified as F2X according to ICD-10

control group. In a second step, we attempted to replicate significant findings in the sample of subjects at risk for psychosis.

Results

The sample characteristics and genotypes are provided in Table 3. All common variants were in Hardy–Weinberg equilibrium in the schizophrenia patients and the healthy control subjects (P = ns) and were unrelated to disease status in the case–control comparisons (all P = ns). Clinical and demographic characteristics were unaffected by SHANK genotype in the schizophrenia, at-risk cases and healthy control subjects (P = ns), with the exception of the rs9616915, for which female schizophrenia cases were more likely to be heterozygote (65.9% vs. 34.1%) while male schizophrenia cases were predominantly homozygote (55% vs. 45%) [$chi^2(2) = 8.46$, $P = .015$]. We therefore introduced gender as a second factor in the analysis of rs9616915.

When analyzing neuropsychological data in the schizophrenia patients, a significant effect of the SHANK1 promoter variant rs3810280 on digit span was found which remained significant after correction for multiple testing. Cases with either a CT or TT genotype repeated only 12.5 ± 3.6 number sequences on average compared to 14.8 ± 4.1 sequences in carriers of the CC genotype [$F(1, 195) = 13.74$, $P < .001$, $eta_p^2 = .07$] (Fig. 1). Additional post hoc analyses indicated that both, forward and backward digit spans, contributed equally to this deficit with T-allele carriers achieving significantly lower scores

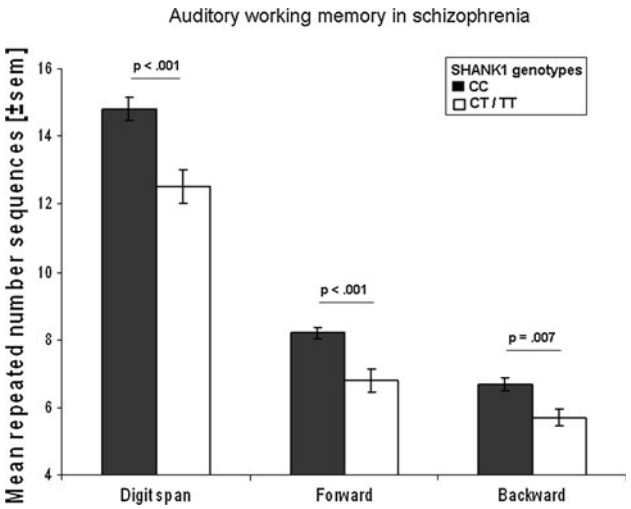

Fig. 1 Significantly altered auditory working memory in schizophrenia patients stratified for rs3810280 genotype applying Bonferroni correction. Numbers of repeated sequences in the WAIS-R digit span test are shown

(mean = 6.8 ± 2.4 vs. mean = 8.2 ± 2.2, [$F(1, 195) = 14.33$, $P < .001$, $\text{eta}_p^2 = .07$]; mean = 5.7 ± 1.8 vs. mean = 6.7 ± 2.3, [$F(1, 195) = 7.53$, $P = .007$, $\text{eta}_p^2 = .04$], respectively). These results remained significant after exclusion of schizophrenia patients with a non-Central European or uncertain ancestry ($n = 5$) (total digit span $P < .001$, forward digit span $P < .001$, backward digit span $P = .008$). In contrast to the significant finding in the schizophrenia patients, no effect of the SHANK1 promoter variant rs3810280 on digit span (CC: mean = 15.3 ± 3.8 vs. CT/TT: mean = 15.7 ± 3.9, [$F(1, 168) = .16$, $P = .692$]) or on any other neuropsychological test parameter was found in the healthy control group after correction for multiple testing. Combining the schizophrenia patients and the healthy control subjects, we still found an effect for the SHANK1 promoter variant rs3810280 on working memory. Thus, subjects with the CC genotype recalled 15.1 ± 3.9 number sequences on average compared to only 14.1 ± 4.1 sequences in T-allele carries. However, this finding was only nominally significant owing to the null effect of the SHANK1 variant in the healthy controls [$F(1, 369) = 4.78$, $P = .029$]. For the remaining SHANK variants, no association with neuropsychological performance was found in the schizophrenia patients and the healthy control subjects applying strict Bonferroni correction (P = ns).

We therefore aimed to confirm our finding for rs3810280 in the at-risk cases. A significant replication was found for forward digit span. Thus, at-risk subjects with a T allele showed a worse performance with 7.1 ± 2.0 recalled sequences on average compared to 8.3 ± 2.0 lines in carriers of the CC genotype [$F(1, 54) = 4.27$, $P = .044$, uncorrected P-value].

Discussion

This is to our knowledge the first study relating common variants of SHANK1, SHANK2, and SHANK3 to schizophrenia. The present work investigated 5 common coding or promoter variants and their effect on neurocognitive endophenotypes in a sample of schizophrenia patients, at-risk cases and healthy control subjects. The T allele of the promoter variant rs3810280 of the SHANK1 gene was significantly related to reduced auditory working memory capacity in schizophrenia patients, even after controlling for multiple testing. With regard to forward digit span, this finding was replicated in the sample of subjects clinically at risk for developing a psychosis, while no effect was found in the group of healthy volunteers.

Previously, several mutations were identified within the SHANK genes, which contribute to ASD. These mutations were also accompanied by severely disturbed mental abilities suggesting a role of SHANK on fundamental cognitive functions [8, 13, 18, 26]. With regard to schizophrenia, the chromosome 22q harboring the SHANK3 gene was previously related to the disorder by linkage analyses yielding significant loci at 22q13 and by a case report of a duplication on 22q in a girl with schizophrenia and borderline mental retardation [9, 14]. Direct evidence for a role of SHANK genes in the disorder was recently published by Gauthier et al.: They found two de novo mutations of SHANK3 in cases suffering from schizophrenia [17]. In one family, all of three affected brothers diagnosed with either schizoaffective disorder or schizophrenia but not their parents carried the same mutation suggesting germline mosaicism. The second mutation was found in a woman diagnosed with schizoaffective disorder, speech impairments and poor psycho-social functioning. Interestingly, all of the four cases with identified de novo mutations also showed mental retardation or borderline mental retardation, thus underlining the potential influence of SHANK genes on cognitive functioning [17]. SHANK1, which showed highly significant effects in our study, was previously not examined in schizophrenia patients. However, a study conducted by Hung and colleagues investigated SHANK1 knock-out mice to explore the effect of this gene on synaptic maturation and cognitive functioning [22]. In this study, SHANK1 knock-out mice showed impaired fear-related memory, enhanced spatial learning but impaired long-term retention of space. Moreover, SHANK1 deficiency led to reduced basal synaptic transmission primarily caused by a reduction in functional synapses. The authors suggest a role of SHANK1 in the maturation of small dendritic spines into larger, more stable spines, thus leading to cognitive alterations [22]. Indirect evidence of a role of SHANK1 density in the psychopathology of schizophrenia stems from a study conducted by Critchlow et al. [11]: After treatment with the atypical

neuroleptic clozapine, significantly increased SHANK1 density in primary and secondary dendritic spines of rat hippocampal neurons was observed while the classical antipsychotic drug haloperidol, in contrast, induced a reduction in SHANK1 density. Although it is far too early to draw any firm conclusions from this preliminary finding, it may be suggestive of a loss of SHANK1 proteins in the neurodevelopment of schizophrenia, which can be reversed by treatment with atypical antipsychotics.

Working memory is a highly replicable core feature of cognitive impairments in schizophrenia, which is found independently from presentation and task [12, 24]. As outlined by Silver et al. [35], evidence suggests that deficits in higher neuropsychological functions in schizophrenia may be partly caused by deficient working memory. For instance, successful in-depth processing or goal-oriented behaviors necessarily require retention of stimuli beyond visual or auditory presentation. In this view, the "patients' lower working memory capacity is rate limiting in performance of other cognitive operations" [p. 1813, 35]. In line with this assumption, Gold et al. [19] reported that the number of completed categories in the Wisconsin Card Sorting Test (WCST) was highly predicted by letter-number sequencing (LNS), a measure of working memory. Moreover, after controlling for LNS performance, WCST differences between schizophrenia patients and healthy controls were eliminated. Additionally, working memory impairment cannot be regarded as an epiphenomenon of schizophrenia symptoms. We observed reduced working memory capacity also in cases clinically at risk for psychosis [16], and meta-analyses replicated such deficits in unaffected first-degree relatives of schizophrenia patients [36]. Confirming the role of genetics in working memory dysfunction, it has been shown that verbal working memory declines with higher familial loading in schizophrenia patients [42]. Thus, working memory constitutes a highly suitable schizophrenia endophenotype for genetic study protocols [20].

The theoretical model of working memory elaborated by Baddeley [2] assumes a central executive system, which accesses three slave systems, the episodic buffer, the visuo-spatial sketch pad and the phonological loop. The latter refers to a system which is activated for phonological processing and auditory rehearsal of a given input such as digits [2]. While the forward digit span only requires maintenance of digit sequences, subjects have to store *and* mentally manipulate sequences in the backward condition. In our study, forward and backward digit spans were equally affected by SHANK1. Lesion studies found disrupted rehearsal of acoustic stimuli in patients with non-fluent aphasia commonly arising from damage to Broca's area [27]. In line with this, neuroimaging studies linked the articulatory rehearsal to the ventrolateral prefrontal cortex (Broca's area, BA 44, 45), while the phonological processing occurs in the left posterior parietal cortex [4]. With regard to the known neurobiological substrates of digit encoding and the finding of comparable effects of SHANK1 on forward and backward digit spans, the reported results might be suggestive of a role of SHANK1 in lateral prefrontal functions.

Based on the previous findings, our main hypothesis was that common SHANK variants would contribute to differential neuropsychological functioning. Although further studies are needed to clarify whether SHANK variants constitute schizophrenia susceptibility genes, our findings argue for a role of SHANK1 in aberrant cognitive functions, which are considered a core feature of schizophrenia. This main finding is further encouraged by the partial replication of SHANK1-affected impairments of working memory in cases at risk for psychosis. However, we did not observe any alterations due to SHANK variants in the healthy control cases. Thus, we cannot completely rule out potential gene–gene interactions of SHANK1 and other genetic variants conferring a risk for schizophrenia.

In conclusion, the SHANK1 promoter variant rs3818280 was found to contribute to deficient working memory in schizophrenia. Previously, it was shown that SHANK1 density increased after administration of the atypical antipsychotic clozapine indicating a role of SHANK1 in treatment response [11]. As has been demonstrated by Hung and coworkers, alterations of the SHANK1 gene may lead to reduced synaptic transmission probably due to synaptic loss [22]. Considering the neurobiological foundations of working memory in relation to our findings, we therefore suggest that future research should examine the role of SHANK variants on prefrontal structure and activation to further elucidate the exact mechanisms of altered working memory in schizophrenia.

Acknowledgments This study was part of the German Research Network on Schizophrenia and was funded by the German Federal Ministry for Education and Research BMBF (grants 01GI0501, 01GI0232, 01GI0234 and 01GV0907), the German Research Foundation DFG (grants Wa 731/6 and Wa 731/4), and by the 7th framework program of the European Union (ADAMS project, HEALTH-F4-2009-242257). We thank V. Guttenthaler, A. Petruschke and C. Hanses for expert technical assistance. The authors thank all clinicians, psychiatrists, psychologists and study nurses involved in the study.

Conflicts of interest The authors report no biomedical financial interests or potential conflicts of interest.

References

1. APA (1994) Diagnostic and statistical manual of mental disorders. American Psychiatric Association, Washington, DC
2. Baddeley A (2010) Working memory. Curr Biol 20:R136–R140
3. Balu DT, Coyle JT (2011) Neuroplasticity signaling pathways linked to the pathophysiology of schizophrenia. Neurosci Biobehav Rev 35:848–870

4. Barch DM, Smith E (2008) The cognitive neuroscience of working memory: relevance to CNTRICS and schizophrenia. Biol Psychiatry 64:11–17
5. Baron MK, Boeckers TM, Vaida B, Faham S, Gingery M, Sawaya MR, Salyer D, Gundelfinger ED, Bowie JU (2006) An architectural framework that may lie at the core of the postsynaptic density. Science 311:531–535
6. Bechdolf A, Ruhrmann S, Wagner M, Kuhn KU, Janssen B, Bottlender R, Wieneke A, Schulze-Lutter F, Maier W, Klosterkotter J (2005) Interventions in the initial prodromal states of psychosis in Germany: concept and recruitment. Br J Psychiatry Suppl 48:s45–s48
7. Becker T, Knapp M (2004) A powerful strategy to account for multiple testing in the context of haplotype analysis. Am J Hum Genet 75:561–570
8. Berkel S, Marshall CR, Weiss B, Howe J, Roeth R, Moog U, Endris V, Roberts W, Szatmari P, Pinto D, Bonin M, Riess A, Engels H, Sprengel R, Scherer SW, Rappold GA (2010) Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. Nat Genet 42:489–491
9. Condra JA, Neibergs H, Wei W, Brennan MD (2007) Evidence for two schizophrenia susceptibility genes on chromosome 22q13. Psychiatr Genet 17:292–298
10. Cornblatt BA, Risch NJ, Faris G, Friedman D, Erlenmeyer-Kimling L (1988) The continuous performance test, identical pairs version (CPT-IP): I. New findings about sustained attention in normal families. Psychiatry Res 26:223–238
11. Critchlow HM, Maycox PR, Skepper JN, Krylova O (2006) Clozapine and haloperidol differentially regulate dendritic spine formation and synaptogenesis in rat hippocampal neurons. Mol Cell Neurosci 32:356–365
12. Dickinson D, Ramsey ME, Gold JM (2007) Overlooking the obvious: a meta-analytic comparison of digit symbol coding tasks and other cognitive measures in schizophrenia. Arch Gen Psychiatry 64:532–542
13. Durand CM, Betancur C, Boeckers TM, Bockmann J, Chaste P, Fauchereau F, Nygren G, Rastam M, Gillberg IC, Anckarsater H, Sponheim E, Goubran-Botros H, Delorme R, Chabane N, Mouren-Simeoni MC, de Mas P, Bieth E, Roge B, Heron D, Burglen L, Gillberg C, Leboyer M, Bourgeron T (2007) Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. Nat Genet 39:25–27
14. Failla P, Romano C, Alberti A, Vasta A, Buono S, Castiglia L, Luciano D, Di Benedetto D, Fichera M, Galesi O (2007) Schizophrenia in a patient with subtelomeric duplication of chromosome 22q. Clin Genet 71:599–601
15. Fatemi SH, Folsom TD (2009) The neurodevelopmental hypothesis of schizophrenia, revisited. Schizophr Bull 35:528–548
16. Frommann I, Pukrop R, Brinkmeyer J, Bechdolf A, Ruhrmann S, Berning J, Decker P, Riedel M, Möller HJ, Wölwer W, Gaebel W, Klosterkotter J, Maier W, Wagner M (2011) Neuropsychological Profiles in different at-risk states of psychosis: executive control impairment in the early–and additional memory dysfunction in the late–prodromal state. Schizophr Bull 37:861–873
17. Gauthier J, Champagne N, Lafreniere RG, Xiong L, Spiegelman D, Brustein E, Lapointe M, Peng H, Cote M, Noreau A, Hamdan FF, Addington AM, Rapoport JL, Delisi LE, Krebs MO, Joober R, Fathalli F, Mouaffak F, Haghighi AP, Neri C, Dube MP, Samuels ME, Marineau C, Stone EA, Awadalla P, Barker PA, Carbonetto S, Drapeau P, Rouleau GA (2010) De novo mutations in the gene encoding the synaptic scaffolding protein SHANK3 in patients ascertained for schizophrenia. Proc Natl Acad Sci USA 107:7863–7868
18. Gauthier J, Spiegelman D, Piton A, Lafreniere RG, Laurent S, St-Onge J, Lapointe L, Hamdan FF, Cossette P, Mottron L, Fombonne E, Joober R, Marineau C, Drapeau P, Rouleau GA (2009) Novel de novo SHANK3 mutation in autistic patients. Am J Med Genet B Neuropsychiatr Genet 150B:421–424
19. Gold JM, Carpenter C, Randolph C, Goldberg TE, Weinberger DR (1997) Auditory working memory and Wisconsin card sorting test performance in schizophrenia. Arch Gen Psychiatry 54:159–165
20. Gur RE, Calkins ME, Gur RC, Horan WP, Nuechterlein KH, Seidman LJ, Stone WS (2007) The consortium on the genetics of schizophrenia: neurocognitive endophenotypes. Schizophr Bull 33:49–68
21. Helmstaedter C, Lendt M, Lux S (2001) VLMT—Verbaler Lern- und Merkfähigkeitstest Manual. Beltz, Göttingen
22. Hung AY, Futai K, Sala C, Valtschanoff JG, Ryu J, Woodworth MA, Kidd FL, Sung CC, Miyakawa T, Bear MF, Weinberg RJ, Sheng M (2008) Smaller dendritic spines, weaker synaptic transmission, but enhanced spatial learning in mice lacking Shank1. J Neurosci 28:1697–1708
23. Kalscheuer VM, Feenstra I, Van Ravenswaaij-Arts CM, Smeets DF, Menzel C, Ullmann R, Musante L, Ropers HH (2008) Disruption of the TCF4 gene in a girl with mental retardation but without the classical Pitt-Hopkins syndrome. Am J Med Genet A 146A:2053–2059
24. Lee J, Park S (2005) Working memory impairments in schizophrenia: a meta-analysis. J Abnorm Psychol 114:599–611
25. Maurer K, Hörrmann F, Trendler G, Schmidt M, Häfner H (2006) Identification of psychosis risk by the early recognition inventory (ERIraos)—description of the schedules and preliminary results on reliability and validity of the checklist. Nervenheilkunde 25:11–16
26. Mössner R, Marshall CR, Sutcliffe JS, Skaug J, Pinto D, Vincent J, Zwaigenbaum L, Fernandez B, Roberts W, Szatmari P, Scherer SW (2007) Contribution of SHANK3 mutations to autism spectrum disorder. Am J Hum Genet 81:1289–1297
27. Müller NG, Knight RT (2006) The functional neuroanatomy of working memory: contributions of human brain lesion studies. Neuroscience 139:51–58
28. Quednow BB, Ettinger U, Mössner R, Rujescu D, Giegling I, Collier DA, Schmechtig A, Kuhn KU, Moller HJ, Maier W, Wagner M, Kumari V (2011) The Schizophrenia Risk Allele C of the TCF4 rs9960767 polymorphism disrupts sensorimotor gating in schizophrenia spectrum and healthy volunteers. J Neurosci 31:6684–6691
29. Rapoport JL, Addington AM, Frangou S, Psych MR (2005) The neurodevelopmental model of schizophrenia: update 2005. Mol Psychiatry 10:434–449
30. Reif A, Schmitt A, Fritzen S, Lesch KP (2007) Neurogenesis and schizophrenia: dividing neurons in a divided mind? Eur Arch Psychiatry Clin Neurosci 257:290–299
31. Reitan RM (1979) Trail making test (TMT). Beltz, Weinheim
32. Roussignol G, Ango F, Romorini S, Tu JC, Sala C, Worley PF, Bockaert J, Fagni L (2005) Shank expression is sufficient to induce functional dendritic spine synapses in aspiny neurons. J Neurosci 25:3560–3570
33. Ruhrmann S, Bechdolf A, Kuhn KU, Wagner M, Schultze-Lutter F, Janssen B, Maurer K, Hafner H, Gaebel W, Möller HJ, Maier W, Klosterkötter J (2007) Acute effects of treatment for prodromal symptoms for people putatively in a late initial prodromal state of psychosis. Br J Psychiatry Suppl 51:s88–s95
34. Ruhrmann S, Schultze-Lutter F, Klosterkötter J (2003) Early detection and intervention in the initial prodromal phase of schizophrenia. Pharmacopsychiatry 36(Suppl 3):S162–S167
35. Silver H, Feldman P, Bilker W, Gur RC (2003) Working memory deficit as a core neuropsychological dysfunction in schizophrenia. Am J Psychiatry 160:1809–1816
36. Snitz BE, Macdonald AW 3rd, Carter CS (2006) Cognitive deficits in unaffected first-degree relatives of schizophrenia patients:

a meta-analytic review of putative endophenotypes. Schizophr Bull 32:179–194
37. Stefansson H, Ophoff RA, Steinberg S, Andreassen OA, Cichon S, Rujescu D, Werge T, Pietilainen OP, Mors O, Mortensen PB, Sigurdsson E, Gustafsson O, Nyegaard M, Tuulio-Henriksson A, Ingason A, Hansen T, Suvisaari J, Lonnqvist J, Paunio T, Borglum AD, Hartmann A, Fink-Jensen A, Nordentoft M, Hougaard D, Norgaard-Pedersen B, Bottcher Y, Olesen J, Breuer R, Moller HJ, Giegling I, Rasmussen HB, Timm S, Mattheisen M, Bitter I, Rethelyi JM, Magnusdottir BB, Sigmundsson T, Olason P, Masson G, Gulcher JR, Haraldsson M, Fossdal R, Thorgeirsson TE, Thorsteinsdottir U, Ruggeri M, Tosato S, Franke B, Strengman E, Kiemeney LA, Melle I, Djurovic S, Abramova L, Kaleda V, Sanjuan J, de Frutos R, Bramon E, Vassos E, Fraser G, Ettinger U, Picchioni M, Walker N, Toulopoulou T, Need AC, Ge D, Yoon JL, Shianna KV, Freimer NB, Cantor RM, Murray R, Kong A, Golimbet V, Carracedo A, Arango C, Costas J, Jonsson EG, Terenius L, Agartz I, Petursson H, Nothen MM, Rietschel M, Matthews PM, Muglia P, Peltonen L, St Clair D, Goldstein DB, Stefansson K, Collier DA (2009) Common variants conferring risk of schizophrenia. Nature 460:744–747
38. Tewes U (1991) Hamburg-Wechsler-Intelligenztest für Erwachsene–revision (HAWIE-R). Hogrefe, Göttingen
39. Valtschanoff JG, Weinberg RJ (2001) Laminar organization of the NMDA receptor complex within the postsynaptic density. J Neurosci 21:1211–1217
40. WHO (1992) International classification of disease, tenth revision. World Health Organisation, Geneva, Switzerland
41. Wouterlood FG, Bockers T, Witter MP (2003) Synaptic contacts between identified neurons visualized in the confocal laser scanning microscope. Neuroanatomical tracing combined with immunofluorescence detection of post-synaptic density proteins and target neuron-markers. J Neurosci Methods 128:129–142
42. Zilles D, Burke S, Schneider-Axmann T, Falkai P, Gruber O (2009) Diagnosis-specific effect of familial loading on verbal working memory in schizophrenia. Eur Arch Psychiatry Clin Neurosci 259:309–315
43. Zweier C, Peippo MM, Hoyer J, Sousa S, Bottani A, Clayton-Smith J, Reardon W, Saraiva J, Cabral A, Gohring I, Devriendt K, de Ravel T, Bijlsma EK, Hennekam RC, Orrico A, Cohen M, Dreweke A, Reis A, Nurnberg P, Rauch A (2007) Haploinsufficiency of TCF4 causes syndromal mental retardation with intermittent hyperventilation (Pitt-Hopkins syndrome). Am J Hum Genet 80:994–1001