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Partial Support for *ZNF804A* Genotype-Dependent Alterations in Prefrontal Connectivity

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Abstract: Genome-wide association studies identified the single nucleotide polymorphism rs1344706 in *ZNF804A* as a common risk-variant for schizophrenia and bipolar disorder. Whereas the molecular function of *ZNF804A* is yet unclear, recent imaging genetics studies have started to characterize the neural systems architecture linking rs1344706 genotype to psychosis. Carring rs1344706 risk-alleles was associated with a decrease in functional connectivity within the dorsolateral prefrontal cortices (DLPFCs) as well as an increase in connectivity between the DLPFC and the hippocampal formation (HF) in the context of a working memory task. The present study aimed at replicating these findings in an independent sample of 94 healthy subjects. Subjects were genotyped for rs1344706 and performed a working memory task during functional magnetic resonance imaging. Results indicate no support for a decrease of functional coupling between the bilateral DLPFCs at higher *ZNF804A* risk status. However, the current data show the previously described alteration in functional coupling between the right DLPFC and the HFs, albeit with weaker effects. Decoupled by default, the functional connectivity between the right DLPFC and anterior HFs increased with the number of rs1344706 risk alleles. The present data support fronto-hippocampal dysconnectivity as intermediate phenotype linking rs1344706 genotype to psychosis. We discuss the issues in replicating the interhemispheric DLPFC coupling in light of the effect sizes rs1344706 genotype has on brain function, concluding that further independent replication studies are fundamentally needed to ascertain the role of rs1344706 in the functional integration of neural systems. *Hum Brain Mapp 34:304–313, 2013.*

Key words: *ZNF804A*; fMRI; working memory; schizophrenia; bipolar disorder; prefrontal connectivity

Additional Supporting Information may be found in the online version of this article.

Frieder M. Paulus and Sören Krach contributed equally to this work.

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INTRODUCTION

Schizophrenia and bipolar disorder are among the most heritable common psychiatric disorders, with genetic influence explaining up to 85% of risk [Smoller et al., 2003; Sullivan et al., 2003]. Their etiology is complex, involving genetic and multiple environmental risk factors [Lang et al., 2007]. A genome-wide association study (GWAS) identified a single nucleotide polymorphism (SNP) in *ZNF804A*, rs1344706, as one genetic susceptibility factor for both schizophrenia and bipolar disorder [O'Donovan et al., 2008a]. This association has been replicated in independent samples [Purcell et al., 2009; Riley et al., 2010; Steinberg et al., 2010; Zhang et al., 2010], and a recent meta-analysis established rs1344706 as one of the best supported risk-variants for psychosis in GWASs [$P = 2.5 \times 10^{-11}$, Williams et al., 2010]. Thereby, carrying the adenine (A) risk allele is associated with schizophrenia with an odds ratio (OR) of 1.10 and an OR of 1.11 if schizophrenia and bipolar disorder are combined, $P = 4.1 \times 10^{-13}$. *ZNF804A* is known to be brain expressed and zinc-finger domains are associated with DNA-binding and enable various interactions with RNA, proteins, and small molecules. The molecular function of the gene, however, remains to be determined. Accordingly, the role of the SNP rs1344706 in the development of psychosis is still unclear. Studies suggested an influence on *ZNF804A* gene expression [Riley et al., 2010; Williams et al., 2010], but the functional mechanisms linking rs1344706 to clinical phenotypes are certainly more complex [Donohoe et al., 2010].

Imaging genetic approaches provide additional information on how risk-genes relate to alterations on the level of neural systems. By combining genetic assessment with multimodal neuroimaging numerous studies revealed specific structural and functional brain systems that mediate genetic vulnerability or liability to psychiatric disorders [O'Donovan et al., 2008b]. Imaging genetic studies thus have the potential to give valuable insight into the neural systems' architecture of illness at stages not affected by confounding factors like medical treatment, hospitalization, and differences in the lifestyle of patients [Meyer-Lindenberg, 2010]. Owing to the proximity to the genetic level, single genes very weakly associated with psychiatric disorders are assumed to have a higher penetrance on the neural systems level than on emergent mental or behavioral phenomena examined in GWASs [Meyer-Lindenberg, 2009]. Therefore, it was suggested that sample sizes larger than 70 provide enough statistical power to detect genetic risk-associated alterations in brain systems [Mier et al., 2010; Munafo et al., 2008].

Based on the results of the first studies on rs1344706, hypotheses have been formulated on how rs1344706 genotype in *ZNF804A* relates to alterations in brain function. Specifically, the functional integration of the frontal cortex has been targeted by these analyses. Applying different cognitive and emotional paradigms, three recent functional resonance imaging (fMRI) studies examined the effects of rs1344706 on brain function in a large sample of healthy subjects. First, Esslinger et al. found that the number of rs1344706 risk alleles was associated with reduced functional connectivity both within and between the bilateral dorsolateral prefrontal cortex (DLPFC) areas and an increase in connectivity between the right DLPFC and the hippocampal formation (HF) during a working memory task [Esslinger et al., 2009]. Moreover, the amygdala showed stronger connectivity with the hippocampus, orbitofrontal cortex, and medial prefrontal cortex during a face-matching task. Second, in a follow-up analysis, the sample was reanalyzed on how the effects of rs1344706 generalize across different experimental settings. The reduced interhemispheric DLPFC connectivity at higher rs1344706 risk status was shown to persist in cognitive states induced by an emotion recognition task or resting state. In contrast, the increase in prefrontal-hippocampal connectivity was exclusively observed while subjects engaged working memory [Esslinger et al., 2011]. This indicates that rs1344706 relates to both a chronic decrease in DLPFC connectivity and a diminution of cognitive state-dependent regulation in connectivity between the DLPFC and HF. Third, the same subjects were also examined during the execution of an affective "Theory of Mind" task. This study revealed a negative association of the rs1344706 risk-allele with neural activity in the medial prefrontal cortex, the left temporoparietal cortex, the left inferior parietal cortex, and left inferior frontal cortex. Further, altered functional connectivity between frontal and temporoparietal regions was reported in rs1344706 risk allele carriers [Walter et al., 2010].

In sum, this empirical evidence suggests several pathways of how rs1344706 genotype may affect brain function in healthy individuals, predominantly through alterations in functional coupling of prefrontal cortex areas. Albeit limited to a single sample of healthy subjects, these findings contributed to establish the dysconnectivity hypothesis as an intermediate phenotype linking rs1344706 genotype to psychosis. This is plausible in so far as dysconnectivity hypotheses of schizophrenia [Stephan et al., 2009] and patient studies [Schlösser et al., 2003a,b] indicate comparable alterations in the functional integration of neural systems. The empirical evidence for rs1344706 effects on brain function, however, was acquired in a single sample of healthy individuals and has not yet been replicated. In the present study, we thus investigated the effects of rs1344706 on brain connectivity—as assessed by fMRI—in a large sample of healthy subjects during a working memory task, aiming to replicate the findings of rs1344706 genotype-related alterations in functional coupling. To our knowledge, this is the first effort of this kind conducted by an independent group in an independent sample.

METHODS

Subjects

As part of an ongoing study on the genetic basis of schizophrenia and bipolar disorder, $N = 94$ healthy subjects were

TABLE I. Sample characteristics

	rs1344706 genotype			
	CC ($n = 21$)	CA ($n = 46$)	AA ($n = 27$)	$P =$
Sex ratio (m/w)	17/4	30/16	19/8	0.426[a]
Age (years)	23.2 ± 2.7	23.2 ± 2.7	23 ± 3.3	0.879[b]
Education (years)	15.6 ± 2.5	15.5 ± 2.4	16 ± 2.9	0.695[b]

[a]Pearson chi-square for differences in frequencies.
[b]One-way ANOVA for mean differences.
Subjects were divided into three groups according to their rs1344706 genotype with CC = homozygote Cytosin, AA = homozygote Adenine, and CA = heterozygote; A = risk allele.

included in the present study. Subjects were recruited through advertisements in local newspapers, postings at the campus of the University of Aachen. Inclusion criteria were age (18–55 years), right-handedness [as assessed by the Edinburgh Inventory, Oldfield, 1971], no psychiatric disorders according to ICD-10, no family history of schizophrenia or bipolar disorder, and Western- or Middle-European descent. Twenty-one subjects were rs1344706 homozygote CC, 46 heterozygote CA, and 27 homozygote AA (C = Cytosine, A = Adenine = risk allele). The distribution of the different allelic variants did not significantly deviate from the Hardy–Weinberg equilibrium, $P = 0.839$ (Wigginton et al., 2005). Rs1344706 genotype groups did not significantly differ with regards to age, education, and sex ($ps > 0.426$, Table I). Participants gave written informed consent and the study protocol was approved by the local ethics committee according to the declaration of Helsinki.

Genetic Analysis

Genomic DNA was extracted from ethylenediaminetetraacetic acid anticoagulated venous blood according to standard procedures. The SNP rs1344706 was genotyped on an Applied Biosystems 7900HT Fast Real-Time PCR System, using a TaqMan 5′ nuclease assay (TaqMan® SNP Genotyping Assay ID C_2834835_10; Applied Biosystems). Genotyping accuracy was assessed by running 15% of the sample in duplicates. Reproducibility was 100%. Overall, 10 different SNPs were genotyped.

FMRI Paradigm

Working memory was assessed by a letter variant of the *n*-back task previously applied in imaging genetics [Jansen et al., 2009] with letter fixation as a high-level baseline, 0-back, and 2-back. In each condition, pseudorandomized sequences of 19 red Latin letters were presented on a black background using the Presentation software package (Neurobehavioral Systems, San Francisco, CA). During 0-back, responses were required for the target letter "X" and in the 2-back condition, target letters were defined as all letters that were identical to the one presented two steps before. Responses were made with the right index finger on a response button (LUMItouch™ Lightwave Technologies, Richmond, B.C., Canada). However, due to irregularities in recordings behavioral responses of 13 subjects had to be excluded from the analyses of behavioral data. During each nonbaseline block, seven targets required a response (target rate = 0.37). Each letter presentation trial consisted of a blank screen that was presented for 500 ms, followed by the letter presentation of 500 ms and a blank screen of 400-ms duration. In all three conditions, the blocks lasted 27.4 s. The instruction screens announcing the next condition were presented for 2.1 s. Four 0-back blocks (selective attention) were alternated with four 2-back blocks (working memory) with eight baseline blocks (letter fixation) in between conditions. Including an initial pause of 6.8 s, the whole fMRI task lasted 8.13 min. Subjects were thoroughly instructed before the scanning procedure.

MRI Data Acquisition

Data were acquired on a 3 Tesla TIM-Trio MR scanner (Siemens Medical Systems) at the Forschungszentrum Jülich. Functional images were collected with a T2*-weighted echo planar imaging sequence sensitive to BOLD contrast (64 × 64 matrix, FOV 200 mm, in plane resolution 3.13 mm, 36 slices, slice thickness 3 mm, TR = 2.25 s, TE = 30 ms, flip angle 90°). Slices covered the whole brain and were positioned transaxially parallel to the anterior–posterior commissural line. Two hundred seventeen functional images were collected, and the initial three images were excluded from further analysis to remove the influence of T1 saturation effects.

Data Analyses

SPM5 (www.fil.ion.ucl.ac.uk/spm) standard routines and templates were used for the fMRI data analysis. After slice-timing, functional images were realigned, normalized (resulting voxel size 2 × 2 × 2 mm), smoothed (8-mm isotropic Gaussian filter), and high-pass filtered (cut off period 128 s). The preprocessed images were statistically analyzed for rs1344706 genotype related (i) neural activation and (ii) functional connectivity of the right DLPFC in a two-level mixed effects procedure.

Working memory-related neural activation

To identify task-related hemodynamic responses on the subject-level, a fixed-effects general linear model (GLM) included three epoch regressors, modeling the 2-back condition, the 0-back condition, and the instructions, as well as six regressors modeling head movement parameters. Parameter estimate (β-) and *t*-statistic images were calculated for each subject. To confirm the validity of our

working memory paradigm, individually weighted β-maps contrasting the 2-back with the 0-back condition were analyzed on the group-level with a one-sample *t*-test. Resulting activation (2-back > 0-back) and deactivation (0-back > 2-back) images were thresholded at $P < 0.05$, corrected for multiple comparisons (applying the familywise error, FWE, correction implemented in SPM5). Rs1344706 genotype effects were assessed in a random-effects GLM with the number of rs1344706 risk alleles as covariate of interest (CC < CA < AA). Resulting *t*-maps were thresholded at $P < 0.05$, corrected. We conducted region of interest (ROI) analyses based on the Wake Forest University PickAtlas software (www.fmri.wfubmc.edu) with smoothed masks (9 mm) for left and right HFs and DLPFCs created with the Marina toolbox (http://www.bion.de/index.php?title = MARINA/= eng). Localization of activation maxima are reported as MNI-coordinates.

Functional connectivity analysis

For the analysis of functional connectivity, we applied a seed region approach, analogous to the procedure described by Esslinger et al. [2009]. Accordingly, the right DLPFC was selected as seed region[1]. To determine seed voxels located within the right DLPFC, we constrained the search space to a right DLPFC mask with the lateral sections of Brodmann area (BA) 9 and BA 46 forming the DLPFC. Starting at the peak activation on the group level at MNI coordinates 52, 30, 30, we identified the next local maximum within each subject for the 2-back vs. 0-back contrast at $P < 0.01$ uncorrected. Additionally, we limited the next local maximum to clusters extending 20 voxels. One subject (CC) did not show DLPFC activation within this cluster at this threshold and was therefore excluded from the connectivity analysis. Seed time series were extracted as the first eigenvariate in a sphere of 6-mm radius as implemented in SPM5. Task-related variance was removed by applying an effects-of-interest correction with the *F*-contrast set on the six movement parameters [Esslinger et al., 2009]. The seed regions' localization in the right DLPFC approximated normal distribution and the center of the seed time series (49.2, 30.8, 29.2) was comparable to the one described by Esslinger et al. (43.5, 34.5, 31.4) with a total Euclidian distance of 7.14 mm (see Fig. S1 for histograms of the seed voxel localization and Fig. S2 for visualization in the three-dimensional space). Seed localizations did not significantly differ across rs1344706 genotype groups ($Fs(2, 90) < 1.92$, $ps < 0.152$). To account for noise, two additional time series were extracted for each subject from the first eigenvariates of all voxels within masks covering medial cerebrospinal fluid regions (CSF) or white matter (WM) [Esslinger et al., 2009].

The fixed-effects GLM on the subject-level included the extracted seed time series of the right DLPFC, the two WM and CSF noise regressors, one regressor modeling the instruction period and six regressors modeling head movement parameters. Regressors for the 2-back and 0-back conditions explained variance due to hemodynamic responses induced by the experimental task. These additional task regressors reduce potential inflation of residuals and the influence of group-differences in neural responses to the experimental conditions on connectivity profiles. Parameters of the GLM were calculated for each subject and the β-maps of the DLPFC seed time series (connectivity maps) were analyzed on the group-level. To visualize the connectivity profile with the right DLPFC, a one-sample *t*-test was conducted. Further, rs1344706 genotype effects were assessed in a random-effects GLM with the number of rs1344706 risk alleles as covariate of interest (CC < CA < AA). Whole brain and ROI analyses for the bilateral HFs and DLPFCs were conducted.

RESULTS

Behavioral Data

Rs1344706 genotype groups were comparable in their performance during the *n*-back task. Hits, misses, false alarms, and reaction times during the 2-back and 0-back condition did not significantly differ with rs1344706 genotype, $Fs(2,78) < 1.86$, all $ps > 0.115$, uncorrected. Overall, response times, amount of misses, and false alarms were significantly higher in the 2-back condition compared to the 0-back condition $ts(80) > 3.22$, $ps < 0.002$, uncorrected and amount of hits were significantly lower $t(80) = 7.52$, $P < 0.001$ uncorrected (see Table S1 for detailed description of subjects' performance). On average, the hit rate was above 91% in the 2-back condition ($M = 25.59$, SD = 2.75) and above 99% in 0-back condition ($M = 27.85$, SD = 0.48), indicating a high level of performance.

Neuroimaging Data

The working memory task activated (2-back > 0-back) a widespread network encompassing the bilateral DLPFCs, superior medial frontal cortex, the inferior parietal lobule, basal ganglia, and the cerebellar hemispheres, $t(93) > |4.96|$, $P < 0.05$, corrected. Deactivations (0-back > 2-back) were observed in the ventromedial prefrontal cortex, as well as middle temporal and parahippocampal areas (Fig. 1A). No significant positive or negative association of rs1344706 risk alleles with working memory-related

[1]To control for potential methodological confounds, we established two alternate procedures in seed time series extraction. The analyses described in the following sections refer to the one with the best approximation of the seed time series distribution described in previous studies. For detailed descriptions of the second analysis, see Supporting Information Methods and Supporting Information Results.

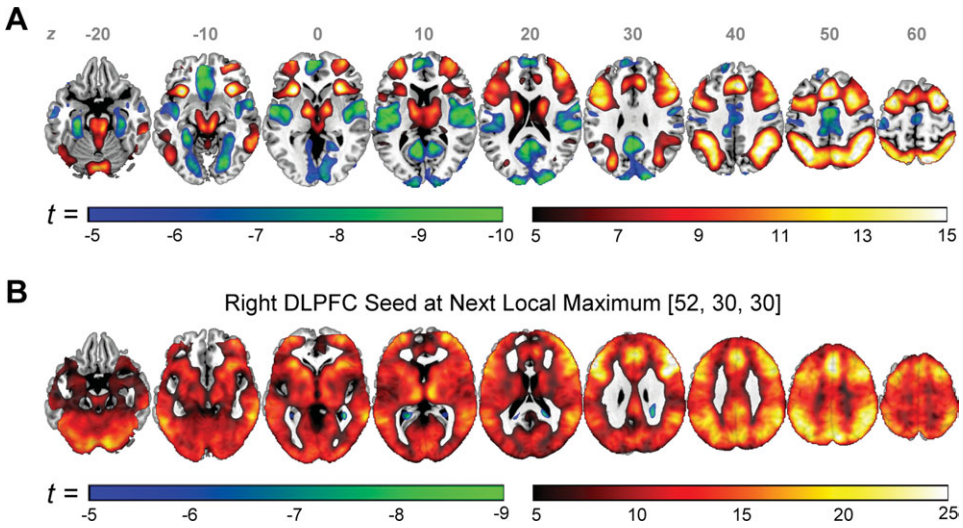

Figure 1.

Working memory task-related activation and functional integration with the right dorsolateral prefrontal cortex. (**A**) Activated (2-back > 0-back, red-yellow) and deactivated (0-back > 2-back, blue-green) networks during the working memory task. Axial slides display the results of a one-sample *t*-test contrasting 2-back vs. 0-back thresholded at $t(93) > |4.96|$, $P < 0.05$, corrected. (**B**) Correlation pattern of the functional connectivity analysis applied in the between-subject analyses of associations with number of rs1344706 risk alleles. Axial slides display results of a one-sample *t*-test of correlations with the seed time series in right dorsolateral prefrontal cortex (DLPFC) thresholded at $t(92) > |5.04|$, $P < 0.05$, corrected.

neural activation was observed for whole brain and ROI analyses in the DLPFCs and HFs at $P < 0.05$, corrected.

Across all subjects, most time series within cortical as well as subcortical areas were positively correlated with the DLPFC seed time series after removal of task-related variance $T(92) > |5.04|$, $P < 0.05$, corrected (Fig. 1B). Nonsignificant or negative correlations were found for bilateral HFs, and WM as well as CSF regions. The correlation profile, however, well reflected the activated network during the working memory task. Besides the bilateral DLPFCs, strongest associations with the seed time series emerged in the bilateral inferior parietal lobules, superior medial frontal cortex, structures of the basal ganglia, and cerebellar hemispheres. This distinctive correlation pattern demonstrates a higher level of functional integration within the functional network commonly active during the working memory task (compare Fig. 1A,B), basically validating the current approach of seed-region connectivity analyses. However, we did not find any significant association of rs1344706 genotype with alterations in functional connectivity at thresholds corrected for multiple comparisons in a whole brain analysis or ROI analyses within bilateral HFs and DLPFCs at $P < 0.05$, corrected.

One reason why we did not replicate previous findings might be that effects did not survive the stringent α-correction levels. In an exploratory analysis, we therefore lowered the statistical threshold and also examined the effects of rs1344706 genotype on functional connectivity at an uncorrected level $t(91) > |1.66|$, $P < 0.05$, cluster size ≥ 20. In line with our a priori hypothesis, the search space was limited to the bilateral HFs and DLPFCs. Within bilateral HFs, several clusters showed an increase of functional coupling with increasing number of rs1344706 risk alleles (CC < CA < AA). In contrast, no voxel was found in support of a negative association at a threshold of $P < 0.05$, uncorrected (Table II). Effects of rs1344706 genotype on functional coupling were less uniform within the bilateral DLPFC ROIs. One cluster in the right DLPFC ($k = 21$ voxels) showed the expected negative effect of decreased coupling with increasing number of rs1344706 risk alleles at $P < 0.05$ (uncorrected) and four clusters in bilateral DLPFCs masks displayed the opposite positive effects (Table II).

To better estimate the potential influences of rs1344706 genotype on functional coupling between the right DLPFC and HFs, we additionally visualized the net effects for the whole HF volumes. As illustrated in Figure 2, there was an observable shift toward positive effects for the majority of voxels in the HF volumes. Voxels indicating negative effects were rare in numbers and associated with weaker

TABLE II. Positive and negative effects of rs1344706 risk alleles on functional coupling of the right DLPFC with bilateral HFs and DLPFCs at uncorrected thresholds

	MNI coordinates					
	x	*y*	*z*	Cluster size	*T*	*P* <
Positive effects						
Left HF	−26	−14	−16	44	2.30	0.011
	−32	−38	−4	26	2.22	0.013
Right HF	36	−36	−6	124	2.85	0.003
	26	−8	−22	35	2.19	0.016
Left DLPFC	−42	14	40	253	3.42	0.001
	−40	40	12	107	2.26	0.006
Right DLPFC	46	12	30	54	2.39	0.009
	46	34	16	82	2.33	0.011
	42	48	0	36	2.33	0.011
Negative effects						
Right DLPFC	22	48	40	21	2.43	0.009

Cluster size at $P < 0.05$ (uncorrected). $t(91)$ and P values are displayed for the peak-voxel within a cluster.

effect sizes within both ROIs. Of the total of 1,194 voxels in the right HF mask, 1,096 were associated with positive effects, whereas 98 had negative effects, a ratio of 11.2 to 1. The ratio of positive effects to negative effects within left HF ROI was 3.5 to 1. Although the effect sizes illustrated in Figure 2 by no means allowed for controlling false positive findings and were nonsignificant for most of the volume even at uncorrected thresholds ($ps > 0.05$), they provided insight on the distribution of effects in a priori defined regions of interest. The strongest positive associations of rs1344706 genotype with DLPFC coupling were almost symmetrically found in the bilateral anterior HFs (Fig. 2, Table II). The average connectivity (β-) parameters of the clusters within the HFs surviving $P < 0.05$, uncorrected, are plotted in Figure 3 for illustration.

DISCUSSION

Recent imaging genetics studies suggested strong effects of the SNP rs1344706 in the gene *ZNF804A* on brain function, particularly through altered functional coupling of the DLPFC regions [Esslinger et al., 2009, 2011]. Albeit limited to a single sample of healthy subjects, these findings contributed to establish the dysconnectivity hypothesis as an intermediate phenotype linking *ZNF804A* genotype to psychosis. The aim of the present study was to replicate these findings in an independent sample to support the existing evidence for potential neurogenetic mechanisms. However, the results of our sample are inconsistent with the previous studies demonstrating rs1344706 related alterations in brain function.

In contrast to previous studies, we did not find the negative association of functional coupling both within and between the bilateral DLPFCs with the number of rs1344706 risk alleles. Even the exploration at uncorrected thresholds showed no evident support for the strong a priori hypothesis. This nonfinding was unexpected, since particularly the attenuation of interhemispheric prefrontal connectivity has repeatedly been found in patients with schizophrenia [e.g., Schlösser et al., 2003a,b] and—as a central feature of rs1344706 genotype-related alterations—generalized across different cognitive states [Esslinger et al., 2011]. However, our exploratory analyses at more liberal thresholds within the HFs did support previous findings for rs1344706 risk status-dependent alterations in functional coupling between the right DLPFC and the HFs [Esslinger et al., 2009]. Decoupled by default, functional connectivity between the right DLPFC and anterior HFs increased with the number of rs1344706 adenine alleles. Effects were not as strong as previously reported, but the uniformity and symmetry of the effects' distribution within the bilateral HF volumes corroborated this assumption. This finding is particularly important since the risk-dependent increase of DLPFC and HF coupling was previously found to be exclusively present under the influence of a working memory task [Esslinger et al., 2011] and confirms the fronto-hippocampal dysconnection hypothesis of schizophrenia [Meyer-Lindenberg, 2010].

The current sample is a comparably large imaging genetics sample and, as indicated in previous studies, holds potential to reveal risk-type dependent changes in brain function [e.g., Jansen et al., 2009, Krach et al., 2010; Krug et al., 2009, 2010]. Since it is unlikely that the divergent results to previous studies can be attributed to methodological differences (see Supporting Information discussion on methodological issues[2]), the most likely explanation for the inconsistent findings is that population effects are smaller than generally expected. A reoccurring difficulty in the field of psychiatric genetics is the nonreplication of initially promising findings. This is partly caused by the small effects of single genes not only on complex behavioral phenotypes but possibly also on intermediate phenotypes such as brain connectivity [Munafò and Flint, 2004, 2010]. However, two recent meta-analyses reported large effect sizes several 10 times stronger than found in GWASs [Mier et al., 2010; Munafo et al., 2008]. How well these estimates represent the population effects of all genetic risk variants has not yet been addressed adequately. It is likely that effects of different genes vary largely not only in their magnitude but also with regards to their specific localization in the neural system. Both meta-analyses published so far report the effects of two well-characterized risk variants, 5-HTTLPR and COMT

[2]In the Supporting Information discussion, we address three potential issues related to differences in timing of the experimental design, the difficulty of the implemented working-memory task, and sample characteristics.

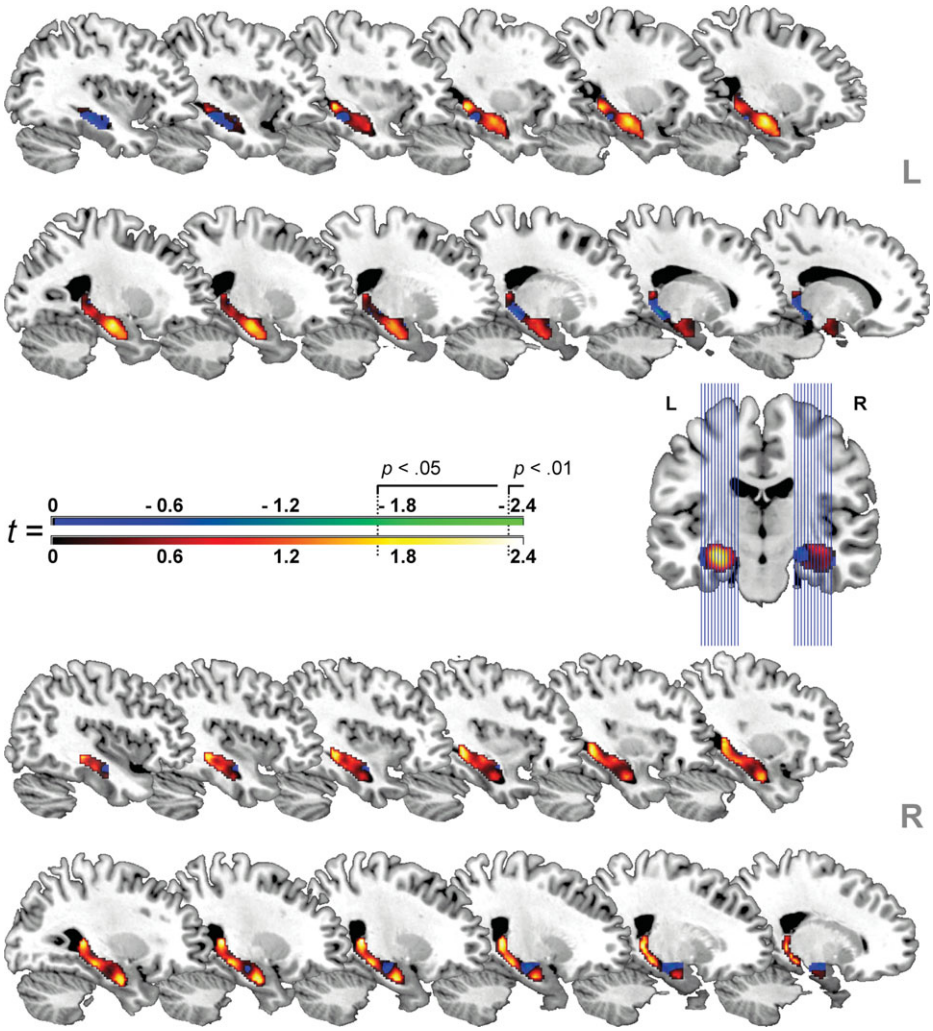

Figure 2.

Positive and negative effects of number of rs1344706 risk alleles on functional connectivity between the right dorsolateral prefrontal cortex and bilateral hippocampal formations. Sagittal planes display the results of a random-effects GLM analysis with rs1344706 genotype (CC < CA < AA) as covariate of interest within left and right masks covering the HF. Positive effects for the number rs1344706 risk alleles are coded in red-yellow, negative effects in blue-green colors.

rs4680 in two specific brain regions associated with their molecular function, the amygdala, and the DLPFC. Rather than providing the expected value of the SNPs' effects on the level of neural systems, they more likely represent upper bounds for population effects and are therefore not necessarily representative for other risk variants such as rs1344706.

Further, in the case of fMRI studies or other imaging procedures that deal with numerous multiple comparisons, estimations of population effects based on sample parameters are even more challenging and not trivial to solve [Kriegeskorte et al., 2010][3]. Although inferential statistics are controlled for false-positive findings and support the presence of rs1344706 genotype-dependent in

[3]This issue may have also affected the meta-analyses provided by Mier et al. [2010]. The reported estimates of population effects are likely inflated by aggregating the effect sizes surviving stringent α correction procedures at variable locations within the right DLPFC. The selection of the most significant cluster in case when several clusters were reported for the DLPFC in primary studies has additionally strengthened this bias and may limits the value of the results.

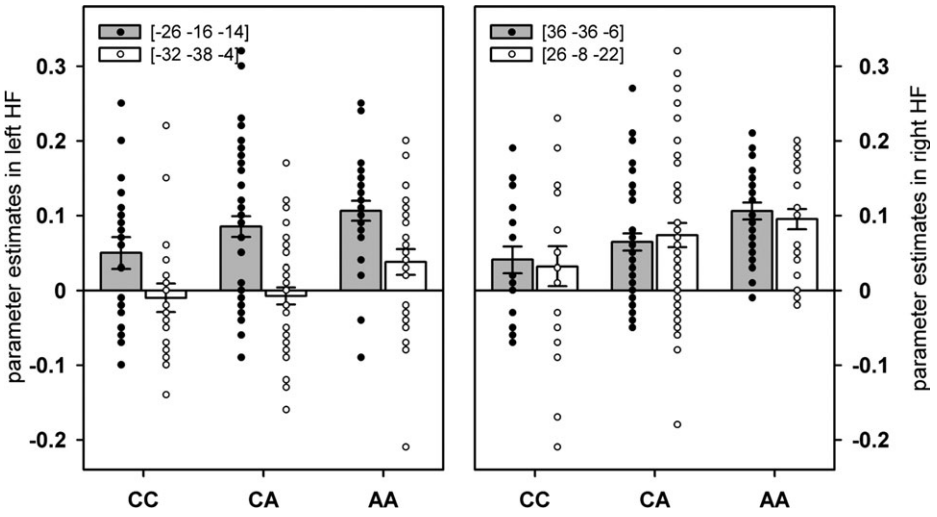

Figure 3.

Means and standard errors of connectivity parameters with the right DLPFC within the left hippocampal formation (HF, left panel) and right HF (right panel) at different rs1344706 genotype (CC = homozygote Cytosin, AA = homozygote Adenine, and CA = heterozygote). Summary statistics are plotted together with individual parameters for the average within the largest (gray) and second largest cluster (white) at $t(91) > 1.66$, $P < 0.05$, uncorrected, cluster size ≥ 20. Numbers in brackets denote coordinates for the peak voxel of the corresponding cluster.

functional coupling, the size of the underlying population effect remains unknown. Even if the penetration of rs1344706 on the neural systems level is multiple times stronger than on emergent mental or behavioral phenomena, the absolute population effects may still be small in their magnitude. With regards to the statistical power, this implies that even with sample sizes of ~ 100 subjects the probability to obtain statistically significant rs1344706 effects is not necessarily sufficient. This holds true especially if stringent α correction procedures are required to control for false positive findings. Especially imaging data have to deal with this fact that has not yet been accounted for in the power analyses provided in meta-analyses calculated only for uncorrected α thresholds [Mier et al., 2010; Munafo et al., 2008]. This rationale could explain the present difficulties to find rs1344706 risk-dependent effects on the interhemispheric prefrontal coupling and the smaller effect sizes for the altered prefrontal-hippocampal connectivity in our study.

In the long run, meta-analyses offer the appropriate tools to further combine effects of SNPs on brain function across different studies and samples. To do so, it is important to avoid publication bias and to report effect sizes of replication studies at a lower statistical threshold; meta-analytical estimates will be biased if empirical findings that fail to achieve statistical significance or are of contradictory nature are not adequately represented in the body of empirical literature [Munafo et al., 2008]. Particularly in the field of imaging genetics this might become a problem: due to the enormous amount of structural, functional and genetic data, tendencies to selectively publish positive results can get easily reinforced[4]. For replication studies the situation faces even more of a dilemma, since inferential statistics should not only minimize false-positive findings by all means but at the same time also need to be sensitive in identifying potentially small population effects. Else, accumulated evidence will suffer from non-replication and prevents meta-analyses to accumulate gene-dependent effects across different samples and, most importantly, brain regions. However prone to false-positive errors, the current approach in visualizing net effects in focal volumes of interest (i.e., for the bilateral HF volumes) could offer a reasonable approach for replication studies to estimate less biased effects of genetic-risk variants on brain function and to better understand the variability of results in empirical findings.

In conclusion, the present study aimed to support recent findings of rs1344706 genotype-dependent alterations in functional coupling and the developing hypotheses on the neural systems' architecture of psychotic illness. Although the functional connectivity analysis provided support for a gene dosage-dependent increase in fronto-hippocampal coupling, we failed to replicate previously described changes in interhemispheric prefrontal connectivity. In addition to presenting results after applying inferential statistics controlling for false-positive findings, replication

[4]To our knowledge, there exist only two studies at present that explicitly reported negative findings in imaging genetics regardless of candidate genes [Jansen et al., 2010; Tost et al., 2010].

studies in the field of imaging genetics should also allow access to effects at more liberal thresholds. As it is common practice in GWASs, any data on effect sizes generated within the analyses should get published together with appropriate information on standard errors and confidence intervals to support meta-analyses in aggregating findings [de Bakker et al., 2008]. The latter might imply, for example, to make raw β images available together with publication. Leaving this aside, the current study demonstrates that replication and publication of negative findings are crucially needed in the field of imaging genetics and will contribute to better estimates of the true effect of rs1344706 and other SNPs in the long run.

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