

Further Evidence for the Impact of a Genome-Wide-Supported Psychosis Risk Variant in ZNF804A on the Theory of Mind Network

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The single-nucleotide polymorphism (SNP) rs1344706 in ZNF804A is one of the best-supported risk variants for psychosis. We hypothesized that this SNP contributes to the development of schizophrenia by affecting the ability to understand other people's mental states. This skill, commonly referred to as Theory of Mind (ToM), has consistently been found to be impaired in schizophrenia. Using functional magnetic resonance imaging, we previously showed that in healthy individuals rs1344706 impacted on activity and connectivity of key areas of the ToM network, including the dorsomedial prefrontal cortex, temporo-parietal junction, and the posterior cingulate cortex, which show aberrant activity in schizophrenia patients, too. We aimed to replicate these results in an independent sample of 188 healthy German volunteers. In order to assess the reliability of brain activity elicited by the ToM task, 25 participants performed the task twice with an interval of 14 days showing excellent accordance in recruitment of key ToM areas. Confirming our previous results, we observed decreasing activity of the left temporo-parietal junction, dorsomedial prefrontal cortex, and the posterior cingulate cortex with increasing number of risk alleles during ToM. Complementing our replication sample with the discovery sample, analyzed in a previous report (total $N=297$), further revealed negative genotype effects in the left dorsomedial prefrontal cortex as well as in the temporal and parietal regions. In addition, as shown previously, rs1344706 risk allele dose positively predicted increased frontal-temporo-parietal connectivity. These findings confirm the effects of the psychosis risk variant in ZNF804A on the dysfunction of the ToM network.

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INTRODUCTION

The single-nucleotide polymorphism (SNP) rs1344706 in ZNF804A is one of the best-supported risk variants for schizophrenia with evidence stemming from genome-wide association studies, independent replications, and meta-analyses (O'Donovan *et al*, 2008; Riley *et al*, 2010; Williams *et al*, 2011; Zhang *et al*, 2012). The gene ZNF804A is located on the chromosome 2q32.1 encoding zinc finger protein 804A. Until now, little was known about its specific

molecular effects. Zinc finger proteins are known to be expressed especially during the development of the cortex, hippocampus, and in the adult cerebellum (Johnson *et al*, 2009). In line with this, Hill and Bray (2012) found evidence that the risk SNP exerts effects on ZNF804A allelic expression at a fetal stage of brain development. Furthermore, first results suggest a role in the regulation of expression of other genes associated with schizophrenia (Girgenti *et al*, 2012) as well as genes involved in cell adhesion (Hill *et al*, 2011).

A promising approach to elucidate the neurophysiological impact of risk genes are imaging genetics studies, which have the potential to uncover intermediate phenotypes, that is genetic effects on the neural level that may mediate the vulnerability to psychiatric disorders. Detection of such effects should be more likely as genetic architecture ought to be associated more closely with structural and functional brain systems than with complex behavioral phenotypes (Meyer-Lindenberg and Weinberger, 2006; Rose and Donohoe, 2013). In case of rs1344706, previous studies have consistently shown impact on aberrant connectivity between the bilateral dorsolateral prefrontal cortices (DLPFC) as well as between the DLPFC and the hippocampal formation (Esslinger *et al*, 2009, 2011; Paulus *et al*, 2011; Rasetti *et al*, 2011), abnormalities previously observed in patients with schizophrenia (Meyer-Lindenberg *et al*, 2005).

We previously investigated the effects of rs1344706 on neural activation and connectivity in healthy subjects who performed a Theory of Mind (ToM) task (Walter *et al*, 2011). Social cognitive deficits are a major clinical and empirically well-supported problem in schizophrenia (Couture *et al*, 2006), and ToM (or mentalizing), the ability to infer other people's feelings, intentions, beliefs, and thoughts (Walter, 2012), has consistently been shown to be impaired in patients with schizophrenia (Bora *et al*, 2009; Brüne, 2005; Sprong *et al*, 2007). Motivated by these findings and empirical evidence that mentalizing is influenced by genetic variation (Knafo *et al*, 2008), we assumed in our earlier study (Walter *et al*, 2011) that the link between the genetic architecture and schizophrenia might be mediated by ToM functioning. We previously observed decreasing activation in crucial areas of the ToM network (Carrington and Bailey, 2009; Van Overwalle and Baetens, 2009; Van Overwalle, 2009), the left dorsomedial and dorsolateral prefrontal and temporo-parietal cortices, with increasing number of risk alleles (Walter *et al*, 2011). In patients with manifest schizophrenia, these genotype-affected areas have been shown to exhibit abnormal activation patterns (Sugranyes *et al*, 2011; Walter *et al*, 2009). Additionally, we found genotype-dependent fronto-temporo-parietal connectivity changes. Further support for a role of ZNF804A in social cognition comes from a study showing behavioral effects of the risk variant (Hargreaves *et al*, 2012). Carriers of at least one risk allele exhibited a stronger tendency to attribute negative events to other people rather than to situational factors. This propensity could be of relevance for the development of delusions, which are hypothesized to be the consequence of inappropriate inferences regarding mental states of others (Frith and Frith, 1999).

In light of this evidence for impact of rs1344706 on social information processing and because of the need to replicate

imaging genetic findings, we aimed to confirm our previous findings in an independent sample. We expected to find decreasing activation with increasing number of risk alleles in those areas of the ToM network reported before (Walter *et al*, 2011): the bilateral dorsomedial (DMPFC) and left DLPFC, left temporo-parietal junction (TPJ), left inferior parietal cortex, and left posterior cingulate (PCC). Likewise, we intended to confirm risk allele dose-dependent effects on aberrant functional connectivity for the left TPJ and right DLPFC, as we found these to be altered by the rs1344706 genotype. Additionally, to further explore possible impact of rs1344706 on a network level, functional connectivity was analyzed for areas for which genotype-altered functional activity was confirmed in the independent sample.

Apart from including a third data acquisition site, we used identical data collection procedures (including genotyping, the imaging paradigm, scanner hardware, and protocols) and data analysis strategies as before. In addition, as it is a necessary prerequisite to compare results from both of our samples, we analyzed test-retest reliability of the mentalizing paradigm in an independent sample to ensure stability of task-evoked brain activation over time.

MATERIALS AND METHODS

Subjects

A total of 188 German volunteers from Berlin ($n = 78$), Bonn ($n = 80$), and Mannheim ($n = 30$) with grandparents of European ancestry were genotyped for rs1344706 (Table 1). Participants did not report any lifetime axis 1 disorder or had a family history of psychotic or affective disorders as evidenced by a structural clinical interview (SCID-I, Wittchen *et al*, 1997). Our sample consisted of 38 rs1344706 CC homozygotes, 87 CA heterozygotes, and 63 AA homozygotes (frequencies in Hardy-Weinberg equilibrium, $\chi^2 = 0.04$, $df = 1$, $p = 0.84$; A = risk allele). There were no genotype effects on gender, age, handedness, or level of education. We additionally carried out analyses in a combined sample consisting of the sample from our previous (Table 1; for a detailed description, see Walter *et al*, 2011) and the present analyses. These analyses were carried out to increase statistical power, as single-SNP effects are usually small. They cannot be regarded as replication, as this larger cohort lacks independence from the subsamples. This total sample comprised 297 subjects ($n = 56$ CC, $n = 136$ CA, $n = 105$ AA; HWE $\chi^2 = 0.03$, $df = 1$, $p = 0.85$; no genotype-related differences according to site, demographic variables, or handedness). All participants gave prior written informed consent. The study was approved by local ethics committees of the Universities of Bonn and Heidelberg and Charité—Universitätsmedizin Berlin.

Theory of Mind Task

The experimental task was part of a larger imaging genetics battery and has been published before, demonstrating that it robustly activates the ToM network (Schnell *et al*, 2011; Walter *et al*, 2011). Two alternating conditions were presented with eight trials each: a ToM (mentalizing) and a control (non-mentalizing) condition. Each trial started

Table 1 Sample Characteristics by rs1344706 Genotype

	CC	CA	AA	
Replication sample (<i>N</i> = 188)	<i>n</i> = 38	<i>n</i> = 87	<i>n</i> = 63	
Site (Berlin/Bonn/Mannheim)	21/14/3	32/42/13	25/24/14	$\chi^2 = 6.66$, NS
Sex (male/female)	14/24	49/38	31/32	$\chi^2 = 4.04$, NS
Age (years)	34.68 ± 10.30	34.01 ± 9.36	33.75 ± 10.40	<i>F</i> = 0.11, NS
School education (years)	15.79 ± 2.49	15.69 ± 2.78	15.33 ± 2.23	<i>F</i> = 0.52, NS
Handedness (right/left/both) ^a	31/4/3	80/3/2	54/6/1	$\chi^2 = 7.72$, NS
Joint sample (<i>N</i> = 297)	<i>n</i> = 56	<i>n</i> = 136	<i>n</i> = 105	
Site (Berlin/Bonn/Mannheim)	21/22/13	32/67/37	25/42/38	$\chi^2 = 7.41$, NS
Sex (male/female)	24/32	66/70	52/53	$\chi^2 = 0.70$, NS
Age (years)	34.14 ± 10.32	32.91 ± 9.60	34.23 ± 10.12	<i>F</i> = 0.62, NS
School education (years)	15.58 ± 2.54	15.52 ± 2.58	15.34 ± 2.55	<i>F</i> = 0.21, NS
Handedness (right/left/both) ^b	48/5/3	123/8/2	93/9/1	$\chi^2 = 5.77$, NS

Abbreviations: A, adenine = risk allele; C, cytosine; *N/n*, (sub-) sample size; ±, SD.

^aData missing for four participants.

^bData missing for five participants.

with an instruction (6.53 s) followed by a cartoon story (22.58 s) consisting of three consecutive pictures (7.53 s per picture). In the ToM condition, subjects had to judge changes in affective states of the protagonist by pressing one of three buttons indicating whether the protagonist felt better, equal, or worse than in the picture shown before. All pictures were free of externally visible signs of characters' emotions such as facial expressions, therefore affective states had to be inferred by taking the person's perspective. In the control condition, subjects were asked to evaluate via button presses whether there were more, less, or just as much living beings compared with the picture before (Supplementary Figure S1).

DNA Extraction and Genotyping

Genomic DNA was extracted from whole blood according to the standard procedures. Rs1344706 was genotyped using a TaqMan 5' nuclease assay (Life Technologies Corporation, Carlsbad, California). Genotype accuracy was assessed by duplicating 15% of the sample, showing reproducibility of 100%.

Imaging Parameters

BOLD fMRI was performed on three Siemens Trio 3T MR scanners at the Charité—Universitätsmedizin Berlin, the Life and Brain Center of the University of Bonn, and the Central Institute of Mental Health Mannheim. At all sites, identical scanners, sequences, and scanner protocols as in our previous study were used (echoplanar imaging sequence with 240 volumes, 28 slices of 4 mm + 25% gap, TR 2 s, TE 30ms, flip angle 80%, FOV 192 mm, descending slice order). Quality control measurements were conducted at all sites on every day of data collection according to a multicenter quality assurance protocol (Friedman and Glover, 2006), revealing stable signals over time and

comparable quality between sites. In order to account for any remaining differences in hardware parameters (eg, mean signal, drift, signal-to-noise ratio, spatial noise), site was used as a covariate for all statistical analyses.

Functional Image Processing

Image processing and statistical analyses were conducted as described previously (Walter *et al*, 2011) using statistical parametric mapping methods as implemented in SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>). Briefly, images were corrected for acquisition delay, realigned to a mean image (movement parameters were confined to <3 mm translation and <3° rotation between volumes; note that movement did not vary across genotype groups, Supplementary Table S4), spatially normalized to a standard echoplanar imaging template (created by the Montreal Neurological Institute) with volume units (voxels) of 2 × 2 × 2 mm³, smoothed with a 9-mm FWHM (full width at half maximum) Gaussian filter, and ratio normalized to the whole-brain global mean. Five epoch regressors (for stories, instructions, both experimental conditions, and button presses) as well as six regressors modelling head motion were included in the first-level analyses for each participant. The individual ToM > control contrasts modelled at the first level were then entered into a multiple regression analysis with number of risk alleles as regressor and site as covariate of no interest. In the replication sample, the significance threshold was set to *p* < 0.05, corrected for multiple comparisons using family-wise error (FWE) correction across the whole brain and within *a priori* defined anatomical regions of interest (ROIs) defined based on previous findings (Walter *et al*, 2011; for description see Supplementary Table S2). Statistical inferences in the combined sample were *p* < 0.05 corrected for multiple comparisons across the whole brain using FWE.

Table 2 Behavioral Results in the Replication Sample

	Effects of rs1344706 genotype					
	CC	CA	AA	df	F	P
<i>RT (s)</i>						
ToM condition	3.16 ± 0.63	3.05 ± 0.64	3.11 ± 0.65	2	0.38	NS
Control condition	2.61 ± 0.66	2.52 ± 0.59	2.50 ± 0.61	2	0.42	NS
<i>Errors</i>						
ToM condition	1.92 ± 1.60	1.94 ± 1.57	1.86 ± 1.44	2	0.06	NS
Control condition	0.87 ± 0.93	1.01 ± 0.90	1.16 ± 1.05	2	1.12	NS
Effects of condition						
	Mean ± SD	df	T	P		
RT (s): ToM—control condition	0.56 ± 0.43	186	17.82	<0.001		
Errors: ToM—control condition	0.88 ± 1.56	186	7.68	<0.001		

Abbreviations: A, adenine = risk allele; C, cytosine; RT, reaction times; ToM, Theory of Mind. N = 188.

Functional Connectivity Analyses

Functional connectivity was analyzed for the two seed regions, where we found aberrant functional connectivity previously, the left TPJ and right DLPFC (Walter *et al*, 2011) as well as for those areas for which we could replicate rs1344706 impact on functional activity, the right DMPFC and left PCC (see below). Time series from the seed regions were extracted by centering spheres with a 6-mm radius on the group maxima of the task main effect (ToM > control). For time series extraction from the right DLPFC, as in our previous work, a mask adopted from analyses of rs1344706 effects on connectivity during a working memory task (Esslinger *et al*, 2009) was used (Automated anatomical labeling: Brodmann areas 9, 46 with exclusion of the medial brain surface), centering the sphere on the individual regional maximum of the mentalizing main effect within this mask. The first eigenvariate of all voxels surpassing a nominal threshold of $p = 0.025$ (ToM > control contrast) were extracted. This nominal threshold was used to exclude noise from the time series, not for statistical inference. White matter and cerebrospinal fluid noise was accounted for by extracting averaged time series from respective masks. Subsequently, the time series from the respective ROI were entered as covariate, in addition to the five experimental regressors, the six head movement regressors, and white matter and cerebrospinal fluid regressors, in new first-level models for each subject. Task conditions were included in these models as described above to rule out covariation between regions attributable to temporal correlations with the experimental paradigm only. To test for genotype effects on functional connectivity, the resulting individual contrast images were entered into multiple regression analyses with number of risk alleles as regressor and site as covariate of no interest. For affirmative analyses within the replication sample, the significance threshold was set to $p < 0.05$, corrected for multiple comparisons (FWE) across the whole brain as well as within *a priori* defined anatomical ROIs for areas in which we found

connectivity effects before (Supplementary Table S2). For exploratory functional connectivity analyses within the replication sample, we applied uncorrected significance levels of $p < 0.001$ at the voxel level and $p < 0.05$ at the cluster level. Analyses within the combined sample were carried out using a significance threshold of $p < 0.05$, corrected for multiple comparisons (FWE) across the whole brain.

Test–Retest Reliability of the ToM Task

To examine stability of task-evoked brain activation, 25 healthy subjects (mean age: 24.4 years, 10 males) were scanned twice, usually with a retest interval of 14 days (mean: 14.6 days) using the original task version (A) as used in the general analyses as well as a parallelized version (B), which included different but similar cartoon stories. Thirteen subjects performed version A at the first scanning date and version B at the second time, 12 subjects performed the task versions in the opposite order. Note that 11 of those subjects who performed task version A first were also included in further analyses reported in this paper (genotypes were missing for 2 subjects), whereas those subjects who performed version B first were included in reliability analyses exclusively. Test–retest reliability was assessed using intraclass correlation coefficients (ICC), a widely accepted measure to quantify agreement of observed brain activity between sessions (Bennett and Miller, 2010). The analyses were restricted to regions, consistently shown to be implicated in ToM, the medial prefrontal cortex (MPFC), TPJ and posterior cingulate/precuneus (PCC/Pcu) (Van Overwalle and Baetens, 2009). ROIs were created from the task main effect (ToM > control contrast) observed in a one-sample *T*-test with the full sample ($N = 297$, thresholded at $p < 0.01$ FWE) within unilateral anatomical masks (provided by the Wake Forest University PickAtlas, Maldjian *et al*, 2003) of the respective regions (Supplementary Table S1). ICCs of mean BOLD amplitudes of voxels

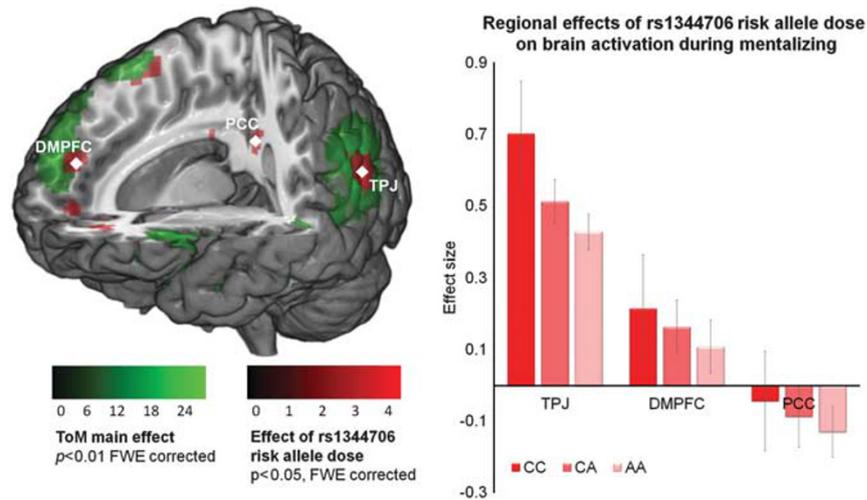


Figure 1 Left: Functional risk allele dose effect (red) superimposed on main effects of mentalizing (green). Green: Main effect of Theory of Mind > control condition across the replication sample ($N = 188$; $p < 0.01$, FWE corrected for whole brain). Red: Negative risk allele dose effect ($N = 188$; $p < 0.05$, FWE corrected). Effect on the left TPJ is significant at $p < 0.05$ FWE correction for the whole brain; effects on the left PCC and right DMPFC at $p < 0.05$ FWE correction for the respective region of interest. Right: Effect sizes (beta values) for risk allele dose effect at the left TPJ ($-57, -64, 21$), left PCC ($-3, -49, 33$), and right DMPFC ($12, 47, 24$) in the replication sample ($N = 188$). A, adenine = risk allele; C, cytosine; DMPFC, dorsomedial prefrontal cortex; FWE family-wise error correction; PCC, posterior cingulate cortex; ToM, Theory of Mind; TPJ, temporo-parietal junction.

Table 3 rs1344706 Risk Allele Dose Effect on Brain Activation During Mentalizing

Region		MNI coordinates x, y, z (mm)			k	T	Z
<i>Replication sample (N = 188)</i>							
Middle temporal gyrus (TPJ)	L	-57	-64	21	73	4.56	4.56 ^a
Superior frontal gyrus	L	-15	38	54	116	4.24	4.13
Supplementary motor area	L	-12	5	66		3.29	3.24
Middle temporal gyrus	L	-57	-10	-24	22	3.99	3.90
Medial frontal gyrus	L	-21	47	3	22	3.76	3.69
Medial frontal gyrus	R	12	47	27	18	3.74	^b 3.67
Anterior Cingulate Cortex	L/R	0	35	3	39	3.55	3.48
Cerebellum (Declive)	R	18	-76	-21	24	3.48	3.42
Posterior Cingulate Cortex	L	-3	-49	33	15	3.37	^b 3.32
<i>Joint sample (N = 297)</i>							
Middle temporal gyrus (TPJ)	L	-57	-61	18	29	5.28	5.16
Medial frontal gyrus	R	12	38	27	25	5.06	4.95
Inferior temporal gyrus	L	-57	-10	-27	3	4.82	4.73
Medial frontal gyrus	L	-6	38	39	9	4.74	4.65
Inferior parietal lobule	L	-51	-43	45	8	4.72	4.63
Posterior cingulate cortex	L	-6	-46	33	3	4.65	4.56
Supramarginal gyrus	L	-63	-40	36	4	4.58	4.50

Abbreviations: k, cluster size; L, left; MNI, coordinates Montreal Neurological Institute (MNI) space coordinates; N, sample size; R, right; TPJ, temporo-parietal junction; x, y, z = location in mm with the three axes.

Brain regions showing a significant negative rs1344706 risk allele dose effect for the Theory of Mind > control contrast. Replication sample: Reported results are $p < 0.001$ uncorrected. Joint sample: Reported results are $p < 0.05$ FWE for the whole brain.

^aRegion significant at $p < 0.05$ FWE (family-wise error correction) across the whole brain.

^bRegion significant at $p < 0.05$ FWE for respective region of interest.

within these masks were calculated as described by Shrout and Fleiss (1979) using SPSS Statistics 21 (IBM, Armonk, NY, USA). As proposed by Bennett and Miller (2010), we

considered ICCs lower than 0.40 as 'poor', values between 0.40 and 0.58 as 'fair', between 0.59 and 0.75 as 'good', and results exceeding these values as 'excellent'.

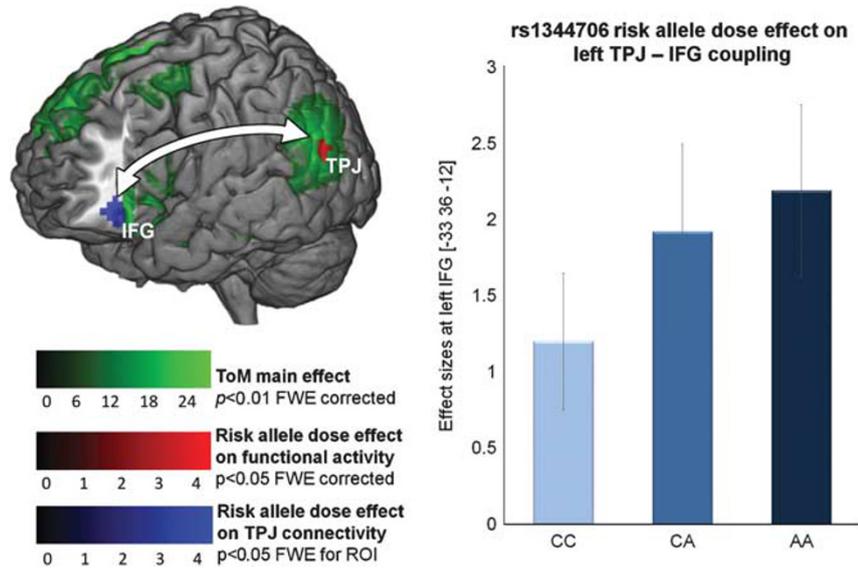


Figure 2 Functional connectivity between the left TPJ ($-57, -64, 21$) and left IFG ($-33, 38, -9$) in the replication sample ($N = 188$; $p < 0.05$, FWE small volume corrected). Left: Green: Main effect of Theory of Mind > control condition across the replication sample ($p < 0.01$, FWE corrected for whole brain). Red: Negative risk allele dose effect on functional TPJ activity ($p < 0.05$ FWE corrected for whole brain). Blue: Positive risk allele dose effect on functional connectivity between left TPJ and left IFG ($p < 0.05$ FWE small volume corrected). Right: Effect sizes (beta values) for the risk allele dose effect on left TPJ–IFG connectivity demonstrate stronger coupling between the two regions with increasing number of risk alleles. A, adenine = risk allele; C, cytosine; FWE, family-wise error correction; IFG, inferior frontal gyrus; ToM, Theory of Mind; TPJ, temporo-parietal junction.

RESULTS

Behavioral Data

Similar to our previous study, we found a significant effect of task condition (Table 2). Subjects made more errors and had longer reaction times in the ToM compared with the control condition. There were no genotype effects on these task performance measures.

Test–Retest Reliability of the Theory of Mind Task

Analyses of stability measures showed ICC values ranging from 0.76 to 0.82 (Supplementary Table S1). These results indicate excellent reliability of brain activity evoked by the fMRI paradigm (ToM > control contrast) within all ROIs.

Neuroimaging Data

For the main effect of mentalizing (comparing the ToM with the control condition), we found significant effects in the ToM network, including prefrontal, temporal, and parietal brain structures (Figure 1; Supplementary Table S3) in the replication sample. Within the same sample, we detected significant negative effects of rs1344706 risk allele dose on the left TPJ at a corrected level across the whole brain (Figure 1; Table 3). Small volume corrections also revealed decreasing activity within the right DMPFC and left PCC, albeit these effects would not withstand Bonferroni correction for the number of ROI analyses carried out. Additionally, at an uncorrected level we observed further support for genetic influence on activation of the left DMPFC during mentalizing, though these effects were located more superior than those reported before and did not overlap with previous observations in this region.

Repeating this analyses in the joint sample, we observed negative whole-brain effects of genetic load on the left TPJ, bilateral DMPFC, left inferior parietal lobule, left supra-marginal gyrus, left PCC, and the junction between left middle and inferior temporal gyrus (Table 3).

Functional Connectivity Effects

In the replication sample, we found a significant genotype effect on increased functional connectivity for the left TPJ. There was a positive association of risk allele number and connectivity with the left IFG ($x = -33, y = 38, z = -9, Z = 3.98$; $p < 0.05$, FWE, small volume corrected; Figure 2). However, we did not observe this effect in the joint sample. Also, we could not find rs1344706 impact on any other investigated region in the replication and combined sample.

DISCUSSION

We aimed to replicate effects of the genome-wide-supported schizophrenia risk SNP rs1344706 in the gene ZNF804A on brain function during mentalizing. Consistent with previous findings (Walter *et al*, 2011), analyses in an independent sample support the impact of rs1344706 on functional activity of core ToM regions at the left TPJ, right DMPFC, and left PCC. When combining the discovery and replication sample to increase statistical power, we observed genotype impact on even more mentalizing areas: the left DMPFC, left anterior temporal, and inferior parietal cortex. Furthermore, our analyses partly confirm genotype-dependent alterations of TPJ–IFG coupling, which also has been implicated in social-cognitive processes before (Decety and Sommerville, 2003).

Behavioral Results

As in our earlier study, we found condition but no genotype effects on task performance measures. This might be due to the fact that our paradigm was not specifically designed to detect behavioral effects but is supposed to be particularly sensitive to changes at the neural level. Moreover, this further supports the concept of endophenotypes according to which one would expect more pronounced genetic effects on biological than on behavioral parameters.

Reliability of the Theory of Mind Task

As good reliability of task-induced brain activity is crucial in order to investigate replicability of genotype effects, we examined the stability of brain function during mentalizing within crucial ToM areas. Our results showed excellent reliability of activation estimates within all ROIs, the MPFC, TPJ, and PCC/Pcu. The high values obtained are particularly noteworthy as a previous meta-analysis reported considerably lower mean retest reliability of fMRI studies and found that tasks involving higher cognition typically showed lower reliability than motor and sensory tasks (Bennett and Miller, 2010). A limitation of these results is that reliability analyses were carried out with subjects from Mannheim exclusively, while genotype effects were investigated with participants from Berlin and Bonn as well. However, as standardized data collection procedures were used (additionally controlled for by site visits) and hardware parameters were stable across time and sites (see Materials and methods section), we assume that site effects should have been kept minimal (although we cannot prove this empirically at this point). Nevertheless, the reliability data support the employment of the paradigm for future investigations involving ToM and validate the replication analyses of rs1344706 genotype impact.

Risk Allele Dose Effects on Functional Brain Activation

In accordance with our previous results, we found significant negative risk allele dose effects on the left TPJ as well as the right DMPFC and left PCC, that is, decreasing activation with increasing number of risk alleles. It has to be noted, that the two latter effects would not survive Bonferroni correction for the number of statistical tests carried out (we applied a whole brain as well as four ROI analyses). Still, the effects were observable in the replication sample and reached whole-brain significance in the joint sample. Hence, they likely reflect smaller but true genotype effects, which are more reliably detectable with a larger sample size. The TPJ and the DMPFC have been described as the most specific regions subserving ToM abilities, being involved in representing the intentions of others, goals of behavior, or the extraction of social scripts (Carrington and Bailey, 2009; Van Overwalle and Baetens, 2009; Van Overwalle, 2009). Although repeatedly implicated to be part of the mentalizing circuit, the role of the PCC appears less clear. In addition to a potential role in reasoning about mental states of others (Saxe and Powell, 2006), it has been hypothesized to be part of self-referential processing or to have a relay function, forwarding input from the TPJ to limbic and paralimbic structures for further emotional processing (Abu-Akel and Shamay-Tsoory, 2011). All of

these mentalizing areas have frequently been found to show abnormal activation in fMRI studies on patients with manifest schizophrenia (Das *et al*, 2012; Park *et al*, 2011; Sugranyes *et al*, 2011; Walter *et al*, 2009).

Complementing these findings, in our joint sample negative effects of risk allele dose were found within further regions initially observed in the discovery sample: the left DMPFC, inferior parietal lobule, and supramarginal gyrus. As the joint sample included the discovery cohort, these results cannot be regarded as replication, although the fact that these were not washed out in a sample almost three times as large speaks against their randomness. Nevertheless, confirmation from an independent sample is required to assert reliability. Additionally, genotype impact on the junction between left middle and inferior temporal gyrus activity was observable, an effect that did not survive statistical correction for multiple comparisons in the separate independent cohorts. This new finding as well as the greater number of significantly impacted brain regions in the joint data set underline the importance of collecting larger samples to increase statistical power, as single-SNP effects might be too small to be detected with a limited sample size. This is further supported by the fact that rs1344706 risk allele dose-affected regions detected in the subsamples were observable more reliably at an even more conservative statistical threshold in the joint sample. Although this does not mean that our subsamples were underpowered for the analyses conducted, as we observed consistent effects using appropriate statistical thresholds in them (Meyer-Lindenberg *et al*, 2008), future imaging genetics studies should take care of collecting sufficiently large samples, preferably estimated by *a priori* power analyses.

The inferior parietal cortex has been described to be part of the human mirror neuron system (Van Overwalle and Baetens, 2009; Rizzolatti and Fabbri-Destro, 2008) and has a crucial role in the understanding of observed actions. Abnormal activation has repeatedly been observed to be associated with misinterpretations of other's actions in schizophrenia (Brunet-Gouet and Decety, 2006) and to correlate with typical psychotic symptomatology, that is, the experience of alien control over self-initiated movements (Schnell *et al*, 2008). The anterior temporal cortex has frequently been implicated in ToM abnormalities in schizophrenia patients as well (Lee *et al*, 2006; Park *et al*, 2009, 2011). Its involvement in mentalizing is supported by several studies (Farrow *et al*, 2001; Johnson *et al*, 2002) as well as by the overlap we observed with the task main effect. Functional imaging studies show that this area is also implicated in episodic and autobiographical memory retrieval, action understanding, and self-reflection (Decety *et al*, 1997; Johnson *et al*, 2002; Lee *et al*, 2002). These processes might be part of ToM processing: self-reflection could, for example, include the recollection of personal episodic memory content, which would aid in the identification of actions, their goals, and accompanying mental states. One could speculate that disruption of this function could lead both to misinterpretations of actions or that difficulties in self-reflection could result in disturbed self-other distinction.

Risk Allele Dose Effects on Functional Connectivity

We could confirm ZNF804A impact on aberrant functional connectivity of the left TPJ with the left IFG. However,

although this effect was observable in the discovery and replication samples, it could not be detected in the joint sample. A likely explanation for this discrepancy is that we found connectivity with a different subregion of the IFG than before, namely the pars orbitalis in the replication sample as opposed to the pars triangularis in the discovery sample. Hence, these effects might be distinct and too subtle to be of statistical significance in the joint sample. Still, as analyses in both subsamples indicate genotype-altered TPJ–IFG connectivity, further examination of this effect would be desirable. At this point, interpretations about the meaning of this alteration in functional connectivity have to remain speculative, but involvement in social cognition seems possible. The IFG is also part of the human mirror system (Van Overwalle and Baetens, 2009), subserving the identification of goals and intentions of actions, presumably by comparison with stored representations of them. Therefore a functional coupling with other regions occupied with inferring mental states is conceivable. Genotype impact on increased TPJ–IFG connectivity in the face of its effects on decreased TPJ activity could represent a compensational mechanism. Dampened TPJ activity might lead to difficulties in inferring goals and intentions of others, which could therefore entail additional recruitment of further resources for action understanding like the IFG.

Alternatively, a role for TPJ–IFG coupling in self-other distinction might be possible. The right IFG has been assumed to exert inhibitory control by suppressing the self-perspective in order to support the decoding of another person's mental states (Decety and Sommerville, 2003). Consequently, altered connectivity could represent a neural correlate of passivity symptoms like thought insertion or transference, which are hypothesized to stem from problems in self-other distinction. In addition, an excessive suppression of the self in favor of the other-perspective could also point to the 'Hyper-ToM' hypothesis, according to which patients could have a hyperactive intention detector, which is believed to underlie delusional ideation (Walter *et al*, 2009). However, TPJ–IFG coactivation has also been implicated in more general attentional control (Shulman *et al*, 2009), and it is unclear whether a function in self-other distinction would apply to the left hemisphere in the same way.

We could not confirm genotype effects on functional connectivity for any other region analyzed. This could imply that our previous uncorrected results have been incidental and that there are no further rs1344706-affected alterations on the network level during ToM. Although the possibility remains, it appears unlikely that genetic effects were too subtle to be detected in this cohort, because a larger sample was investigated here. Still, effects could also be left undetected due to the limitations of the seed-based approach utilized.

Further Evidence for ZNF804A Effects on Social Cognition

The ZNF804A risk variant has been implicated in social cognition before. Hargreaves *et al* (2012) found that healthy risk allele carriers exhibited a stronger tendency to attribute negative events to other people rather than to situational factors, pointing to a stronger 'personalizing bias'. As this is

associated with paranoid ideation, it undermines the notion that ZNF804A-mediated ToM disturbances might represent one mechanism in the development of delusional symptomatology. Backing up this assumption, Park *et al* (2009) reported altered ToM network activity in patients with schizophrenia using an fMRI paradigm to evoke referential ideas. Compared with healthy controls, patients showed aberrant activation of the TPJ, middle temporal gyrus, IFG, MPFC, and the anterior cingulate while watching video scenes of people having conversations suggesting to refer to the viewer compared with non-referential conversations. In addition, TPJ activity correlated with delusional and hallucinatory symptomatology in patients. These results point to potential mechanisms by which alterations in the mentalizing network may affect typical psychotic symptomatology.

Limitations

A few points have to be regarded that might limit our findings. First, we only included non-affected healthy subjects. On the one hand, this offers the advantageous possibility of investigating isolated variables in the absence of other confounding effects arising from the onset of a disease. On the other hand, this prohibits undoubtedly valuable comparisons with affected subjects. Investigating patients would provide additional interesting insights into the psychopathological significance of the alterations observed on the functional brain level, eg, by differentiating between subgroups of patients with schizophrenia (paranoid vs disorganized) or by examining associations with symptom measures. Moreover, it cannot be safely stated whether rs1344706 exhibits wider-spread effects on connectivity patterns within the mentalizing network than our results show. Our seed-based connectivity approach was limited to a small number of circumscribed ROIs and does not rule out more genotype-altered associations. As the method used might not have been sensitive enough to find these effects, more sophisticated approaches to analyze brain connectivity should be used in future studies. Finally, our results point to only one mechanism by which the risk variant might contribute to psychosis risk. Further effects on cognitive variables as well as further brain systems have been reported (Esslinger *et al*, 2009, 2011; Walters *et al*, 2010, Van Den Bossche *et al*, 2012), showing that it very likely impacts on multiple processes. Upcoming investigations should therefore also focus on characterizing the affected psychological and biological variables as well as their interplay more widely.

CONCLUSIONS

Our findings clearly strengthen previous observations of rs1344706 effects on activity patterns of key ToM structures, while we could only partially replicate rs1344706 impact on functional connectivity during mentalizing. This implicates that one mechanism by which the ZNF804A risk SNP increases the likelihood for schizophrenia might be by altering brain function central to mentalizing, which is well known to be disrupted in patients. These findings suggest an intriguing interplay of genetic, neurobiological, and psychosocial aspects in the etiology of schizophrenia, which could stimulate important future research. In the long term,

this could prove beneficial not only for a better understanding of the disease but also for the development of advanced biological and psychosocial interventions.

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