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ZNF804A and Cortical Structure in Schizophrenia: In Vivo and Postmortem Studies

Carl Christoph Schultz*[,1,13], Igor Nenadic[1,13], Brien Riley[2], Vladimir I. Vladimirov[3], Gerd Wagner[1], Kathrin Koch[1,4], Claudia Schachtzabel[1], Thomas W. Mühleisen[5], Buket Basmanav[6,7], Markus M. Nöthen[6–8], Thomas Deufel[9], Michael Kiehntopf[9], Marcella Rietschel[10], Jürgen R. Reichenbach[11], Sven Cichon[5–7,12], Ralf G. M. Schlösser[1], and Heinrich Sauer[1]

[1]Department of Psychiatry and Psychotherapy, Jena University Hospital, Jena, Germany; [2]Department of Psychiatry and Human Genetics, Molecular Genetics Lab, Virginia Institute of Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, VA; [3]Department of Psychiatry, Pharmacotherapy & Outcomes Science, Virginia Institute for Psychiatry and Behavioral Genetics and School of Pharmacy, Virginia Commonwealth University, Richmond, VA; [4]Department of Neuroradiology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany; [5]Institute of Neuroscience and Medicine (INM-1), Research Center Juelich, Juelich, Germany; [6]Institute of Human Genetics, University of Bonn, Bonn, Germany; [7]Department of Genomics, Life and Brain Center, University of Bonn, Bonn, Germany; [8]German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany; [9]Department of Clinical Chemistry and Laboratory Diagnostics, Jena University Hospital, Jena, Germany; [10]Division of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany; [11]Medical Physics Group, Institute for Diagnostic and Interventional Radiology, Jena University Hospital, Jena, Germany; [12]Division of Medical Genetics, University of Basel, Basel, Switzerland;
[13]These authors contributed equally to this article.

*To whom correspondence should be addressed; Department of Psychiatry and Psychotherapy, Jena University Hospital, Philosophenweg 3, 07740 Jena, Germany; tel: +49-3641-9-35665, fax: +49-3641-9-35444, e-mail: christoph.schultz@med.uni-jena.de

Recent evidence indicated that the *ZNF804A* (rs1344706) risk allele A is associated with better cognitive performance in patients with schizophrenia. Moreover, it has been demonstrated that *ZNF804A* may also be related to relatively intact gray matter volume in patients. To further explore these putatively protective effects, the impact of *ZNF804A* on cortical thickness and folding was examined in this study. To elucidate potential molecular mechanisms, an allelic-specific gene expression study was also carried out. Magnetic resonance imaging cortical thickness and folding were computed in 55 genotyped patients with schizophrenia and 40 healthy controls. Homozygous risk allele carriers (AA) were compared with AC/CC carriers. *ZNF804A* gene expression was analyzed in a prefrontal region using postmortem tissue from another cohort of 35 patients. In patients, AA carriers exhibited significantly thicker cortex in prefrontal and temporal regions and less disturbed superior temporal cortical folding, whereas the opposite effect was observed in controls, ie, AA carrier status was associated with thinner cortex and more severe altered cortical folding. Along with this, our expression analysis revealed that the risk allele is associated with lower prefrontal *ZNF804A* expression in patients, whereas the opposite effect in controls has been observed by prior analyses. In conclusion, our analyses provide convergent support for the hypothesis that the schizophrenia-associated *ZNF804A* variant mediates protective effects on cortex structure in patients. In particular, the allele-specific expression profile in patients might constitute a molecular mechanism for the observed protective influence of *ZNF804A* on cortical thickness and folding and potentially other intermediate phenotypes.

Key words: rs1344706/ZNF804A/schizophrenia/cortical thickness/cortical folding/susceptibility gene/gene expression/psychosis

Introduction

ZNF804A (rs1344706) was the first common genetic risk variant associated with schizophrenia and bipolar disorder on a genome-wide level.[1] Since the initial finding, an intensive exploration of its neurobiological underpinnings has begun. Genetic knockdown of *ZNF804A* in neural progenitor cells provided important clues to molecular mechanisms of the zinc finger protein 804A, suggesting a crucial role of *ZNF804A* in neural migration, neurite outgrowth, and synapse formation.[2] At a macroscopic level, imaging genetics revealed that *ZNF804A* influences prefrontomedial temporal connectivity in healthy controls,[3–5] reflecting a major intermediate phenotype of schizophrenia.[6] Moreover, in healthy controls, the genetic risk variant was associated with reduced gray matter volume of the parahippocampal gyrus, posterior cingulate,

and medial orbitofrontal gyrus[7] and reduced cortical thickness of the superior temporal, anterior, and posterior cingulate cortex.[8] Surprisingly, in patients, *ZNF804A* risk status was associated with increased cortical volume of the superior temporal gyrus, insula, and hippocampus.[9] At a behavioral level corresponding to this initial neuroimaging findings in schizophrenia, patients with *ZNF804A* risk carrier status, but not healthy controls, demonstrated better episodic and working memory performance in 2 large and independent samples including more than 500 cases and 1600 controls.[10] In contrast to the initial structural neuroimaging findings of Donohoe and colleagues, a large study of Wassink et al[11] demonstrated no effect of the *ZNF804A* risk genotype on global lobar gray matter volume (but an effect on white matter volume) in patients with schizophrenia and healthy controls. This discrepancy might in parts be explainable by methodical differences (eg, voxel based vs lobar level analysis).

Taken together, several aspects of *ZNF804A* effects in schizophrenia remain poorly understood. On the one hand, there is strong evidence that the genotype-cognitive phenotype relationship between patients with schizophrenia and healthy controls is indeed distinctive, ie, the *ZNF804A* risk allele has protective effects on cognitive measures in patients with schizophrenia but not controls. On the other hand, a distinctive effect on a neuroimaging phenotype has not yet been clearly demarcated. In particular, case-control studies, which might shed light on potentially distinctive genotype-phenotype relationships regarding *ZNF804A*, are rare and conflicting with regard to cortical volume findings.

Measures derived from cortical surface analysis, such as cortical thickness and folding, might be better suited markers to study such effects.[12] They are replicated as biomarkers of the disease[13–20] and show robust heritability.[12,21,22] Furthermore, cortical thickness reduction demonstrates a clear association with cognitive performance deficits[23–27] and disturbed neuronal activation in schizophrenia.[28]

On a molecular level, recent work made it most plausible that variation in *ZNF804A* indirectly increases schizophrenia risk through one of several possible transcriptional or downstream mechanisms. The risk allele A is expected to maintain binding sites for brain-expressed transcription factors MYT1 and POU3F1/Oct-655, which are both implicated in neurodevelopmental processes, particularly differentiation and proliferation of oligodendrocytes.[29,30] Chung et al[31] identified the mouse homologues of *ZNF804A* as a downstream target gene of Hoxc8. Binding of Hoxc8 to an intronic region of *ZNF804A* led to an upregulation of ZNF804A mRNA levels. Hoxc8 regulated genes are known to be involved in neurodevelopmental processes such as cell adhesion and migration,[32] providing further support for a potential role of *ZNF804A* in molecular mechanisms of brain development. As shown, knockdown of *ZNF804A* expression of neural progenitor cells derived from human cortical neuroepithelium leads to decreased expression of genes involved in cell adhesion, migration, and synapse formation.[2] First evidence for an effect of the *ZNF804A* single-nucleotide polymorphism (SNP) on cerebral gene expression was provided by the study of Riley et al,[33] demonstrating a trend for higher *ZNF804A* gene expression in postmortem prefrontal brain tissue of patients with schizophrenia and significantly higher expression depending on the risk allele counts in healthy controls. A recent study exploring the fetal brain expression suggests that *ZNF804A* exerts its disease-related effects mainly in prenatal stages,[34] indicating that imaging phenotypes with a strong linkage to neurodevelopment such as cortical thickness and folding[14,35,36] might represent sensitive markers for deviations of brain structure mediated by *ZNF804A*.

Thus, because it has repeatedly been shown that the *ZNF804A* SNP influences cerebral gene expression, differences of expression profiles in patients with schizophrenia and healthy controls might be one potential clue for distinctive genotype-phenotype interactions and putatively protective effects on cortical structure in patients.

Objectives and Hypothesis

The aim of our study was to assess the effects of *ZNF804A* rs1344706 in both in vivo imaging and postmortem gene expression, respectively, as follows:

1. Delineation of a valid neuroimaging phenotype for the detection of putatively distinctive effects of *ZNF804A* in a case-control design.
2. Characterization of a molecular mechanism underlying putatively distinctive effects of *ZNF804A* by investigation of postmortem *ZNF804A* gene expression of patients with schizophrenia as an extension of the results in healthy controls.[33]

Thus, in the first part of the study, we investigated 2 intermediate phenotypes—cortical thickness and cortical folding—in a cohort of patients with schizophrenia and healthy controls genotyped for the *ZNF804A* (rs1344706). Based on the current data regarding *ZNF804A* associations with behavioral and neuroimaging phenotypes, we hypothesized that (1) risk carrier status in patients with schizophrenia is related to increased cortical thickness and lower cortical folding compared with nonrisk patients; (2) risk carrier status in healthy controls is associated with reduced cortical thickness and higher cortical folding; and (3) *ZNF804A* genotype is associated with a significant genotype × diagnosis interaction with regard to cortical thickness and folding in patients and healthy controls, indicating putatively differing genotype-intermediate phenotype relationships.

In the expression study, we performed *ZNF804A* allelic gene expression analysis in postmortem brains of patients

with schizophrenia with the aim of revealing a molecular mechanism explaining putatively distinctive effects of *ZNF804A*.

Subjects and Methods

Participants

For the neuroimaging analysis, we studied 55 patients with schizophrenia and 40 healthy controls. All participants were right handed.[37] Diagnoses were established by a clinical psychiatrist (C.C.S.) based on the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) and were confirmed by 2 independent psychiatrists (R.G.M.S. and I.N.). All patients met DSM-IV criteria for schizophrenia and had no second psychiatric diagnosis. Of the 55 patients, 3 patients were unmedicated, 6 patients were medicated with typical antipsychotics (5 × haloperidol, 1 × flupentixol), and 46 patients were medicated with atypical antipsychotics (7 × amisulpride, 3 × aripiprazole, 8 × clozapine, 12 × olanzapine, 6 × quetiapine, and 10 × risperidone). There were no significant differences ($P > .978$) regarding the distribution of the type of medication (unmedicated, typical, and atypical antipsychotic) between the ZNF genotype groups (AC/CC vs AA).

Healthy controls were recruited by newspaper advertisement and blackboard ads.

Healthy volunteers were screened for major medical, neurological, and psychiatric history. None of the healthy subjects had a current or history of a psychiatric disorder or first-degree relatives with a psychiatric disorder according to DSM-IV. Exclusion criteria for all participants were neurological disease or damage and medical disorders potentially influencing neurocognitive function. All participants were Caucasian. All participants gave written informed consent to the study approved by the Ethics Committee of the Friedrich-Schiller University. Sociodemographic and psychopathological data are given in table 1.

For the allelic *ZNF804A* gene expression study, brain tissue of the dorsolateral prefrontal cortex of 35 patients was analyzed as described in Riley et al.[33] Postmortem brain tissue was provided by The Stanley Medical Research Institute. For characteristics of the sample and exclusion criteria, see Riley et al.[33]

Magnetic Resonance Imaging Acquisition

We acquired high-resolution anatomical T1-weighted scans on a 1.5 T Siemens Magnetom Vision whole-body system (for details, see online supplementary information).

Magnetic Resonance Scan Processing and Calculation of Cortical Thickness, Folding, and Area

We used the FreeSurfer software package (version 4.0.5, http://surfer.nmr.mgh.harvard.edu) for processing of images[38,39] (for details, see online supplementary information). Cortical thickness is computed by finding the shortest distance between a given point on the estimated pial surface and the gray/white matter boundary and vice versa and averaging these 2 values.[41]

As a highly local measure for cortical folding, absolute mean curvature has been used.[42,43] This measure allows the exact in vivo quantification of cortical folding at about 300 000 points of the whole cortical mantle. Mean curvature has already been used in several studies in schizophrenia[13,44–46] (for a detailed methodical description, see Schultz et al).[44]

As a measure for cortical area, we post hoc computed vertex-by-vertex estimates of the relative areal expansion or contraction of each location in atlas space by using implemented FreeSurfer tools.

Table 1. Demographic and Clinical Data

	Genetic Groups		
	AA	AC/CC	*P*
Healthy controls			
N	15	25	
Age (y)	28.1 (9.1)	26.8 (6.92)	.625
Patients			
N	20	35	
Age (y)	29.6 (12.2)	29.2 (9.3)	.915
PANSS total	68.8 (24.3)	70.0 (27.2)	.884
PANSS positive	18.3 (9.7)	17.0 (7.0)	.614
PANSS negative	15.9 (8.0)	16.9 (7.9)	.676
CPZE	485.0 (339.5)	511.1 (373.5)	.798

Note: PANSS, Positive and Negative Syndrome Scale (Kay et al 1987[40]); CPZE, chlorpromazine equivalents. Participants were grouped according to their *ZNF* risk status, ie, AA genotype vs CC + AC genotype of single-nucleotide polymorphism rs1344706. There were no significant differences with regard to age and gender between the resulting genotype groups (*P* values > .510). Data expressed as mean (SD). *P* values resulting from 2-sample *t* test.

Genetic Analyses and Identification of Subgroups

Lymphocyte DNA was isolated from venous blood samples using standard methods. To target SNP rs1344706, a primer pair was designed. Amplicons were generated using standard polymerase chain reaction conditions. These were then sequenced using the Sanger method and a 3130XL Genetic Analyzer (Applied Biosystems). Electropherograms were analyzed by 2 independent investigators using SeqMan II (DNAStar).

Depending on the *ZNF804A* genotype, patients with schizophrenia and healthy controls were divided in subgroups resulting in 2 genotype subgroups per diagnostic group, ie, 2 × A/A and 2 × A/C and C/C, as done in previous *ZNF804A* imaging genetic studies.[7–9] There were no significant differences with regard to age and gender between the resulting genotype groups (*P* values > .510).

Statistical Analysis

Statistical Cortical Maps. Each cortical thickness and folding measurement of each vertex of the subjects' surface was mapped on a common spherical coordinate system using a spherical transformation. Maps were smoothed using a Gaussian kernel of 10 mm. A general linear model (GLM) controlling for the effect of age was used to estimate differences in cortical thickness and folding between the genotype groups in patients and healthy controls separately at each vertex of the surface.

We, furthermore, performed a genotype × diagnosis interaction analysis with the same 4 groups as defined for the comparisons of cortical thickness and folding between the genotype groups (HC:AA; HC:AC/CC; SZ:AA; SZ:AC/CC).

Right and left hemispheres were tested separately.

Monte Carlo Simulation and Clustering. For the imaging analysis, as a conservative procedure for the correction of multiple comparisons, a Monte Carlo simulation with 10 000 iterations and cluster analysis was performed to identify contiguous clusters of significant cortical thickness and folding differences between the genotype groups in patients and healthy controls and the genotype × diagnosis interaction analyses ($P < .05$).

Postmortem ZNF804A Allelic Gene Expression Analysis. The expression values were binned by rs1344706 allele, and the means of the bins were compared using the unpaired *t* test with Welch's correction (for methodical details, see Riley et al[33]).

By ANOVA, we assessed the effect of potential confounder variables (age, gender, pH, postmortem interval, refrigerator interval, smoking, drug abuse, and antipsychotic medication).

Influence of ZNF804A on Cortical Thickness and Cognitive Functioning. To test for a potential association of cognitive functioning and cortical thickness reduction related to the *ZNF804A* risk variant, we performed partial correlations controlling for the effect of age between the premorbid intelligence level and the mean cortical thickness values of the significant clusters, which result from the whole cortex diagnosis × genotype interaction analysis. Premorbid intelligence level was measured in subjects ($n = 91$) by the multiple choice vocabulary intelligence test,[47] which is a valid test for measuring the premorbid intelligence level.

Results

Cortical Thickness

ZNF804A homozygote risk carrier status (AA) demonstrated extensive effects on cortical thickness in patients. In comparison with patients with the AC/CC genotype, patients with AA genotype exhibited significantly (corrected for multiple comparisons) thicker cortex in left dorsolateral prefrontal, ventrolateral prefrontal, Broca's area, orbitofrontal, superior temporal, middle and inferior temporal regions and the inferior parietal cortex, medial superior frontal, and anterior medial temporal cortex. On the right hemisphere, risk carrier status affected dorsolateral prefrontal, inferior frontal, superior temporal, middle and inferior temporal, inferior parietal, lateral occipital, medial superior frontal cortices, and the parahippocampus (figure 1). Online supplementary table 2 summarizes the characteristics of the clusters (see online supplementary information).

To test for a potential influence of the current antipsychotic medication and cortical thickness, we performed a partial correlation between the mean cortical thickness values of the significant clusters and chlorpromazine equivalents (CPZE). There were no significant correlations ($P < .05$) between CPZE and cortical thickness, which is in line with our and other previous findings of antipsychotic medication and cortical thickness correlations in schizophrenia.[17,48]

In healthy controls, the AA carriers demonstrated no significant differences in cortical thickness in comparison with healthy AC/CC carriers.

To evaluate the effect of diagnostic group in these clusters, we extracted mean cortical thickness values of the significant clusters resulting from the genotype groups in patients as described in Schultz et al.[19] Direct comparison (GLM controlling for the effect of age) of cortical thickness values yielded significant left and right hemispheric frontotemporal cortical thinning in patients with schizophrenia (all *P* values < .0072). Focusing the comparisons on the *ZNF804A* risk patients (AA genotype), these differences were no more significant ($P < .05$), demonstrating the protective effect of the AA genotype on cortical thinning in patients.

The genotype × diagnosis interaction analysis demonstrated significantly divergent effects of genotype risk status between patients and controls on cortical thickness. This was significant for left and right hemispheric superior, middle and inferior temporal regions (see figure 2 and online supplementary table 3 for the cluster characteristics). To a smaller extent, right inferior frontal areas (pars opercularis; BA 44) were affected.

Cortical Folding

AA risk allele carriers in patients demonstrated significantly lower ($P < .05$, corrected for multiple comparisons) cortical folding in a right superior temporal region encompassing 577 mm^2 (figure 3).

No effects were found in healthy controls.

In order to evaluate the effect of diagnostic group on cortical folding, we extracted the mean curvature values in patients and healthy controls. A GLM analysis of mean curvature values controlling for the effect of age

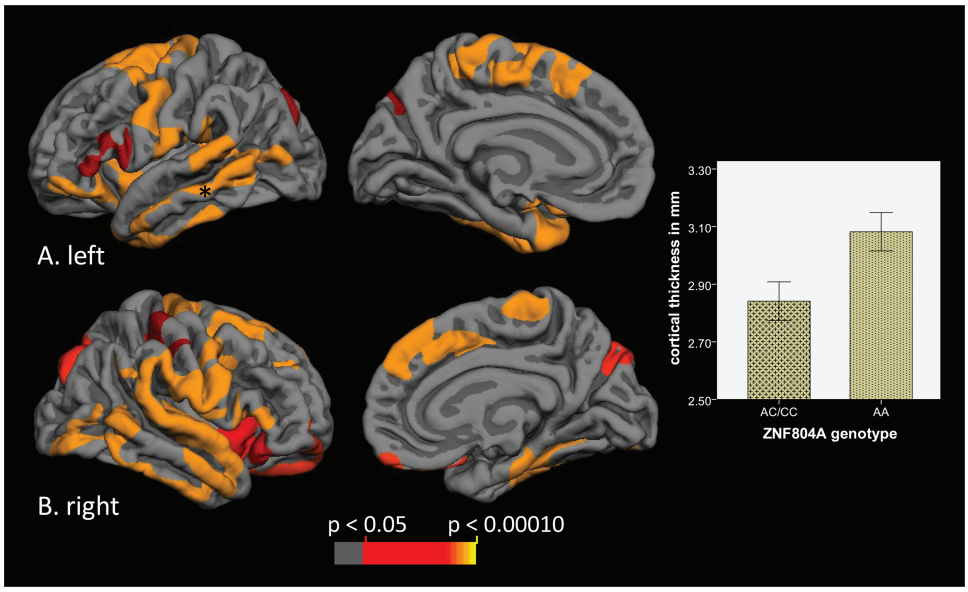

Fig. 1. Cortical statistical maps displaying cortical thickness differences in schizophrenia patients between carriers of AA and AC/CC. In these regions, AA carriers exhibit a significantly thicker cortex compared with AC/CC carriers. Displayed is the lateral and medial surfaces of both hemispheres, (A) left hemisphere and (B) right hemisphere, pial view, and *P* values corrected for multiple comparisons. Bar diagram illustrates the cortical thickness difference between the genotype groups and mean values exemplarily extracted from the marked (*) cortical region.

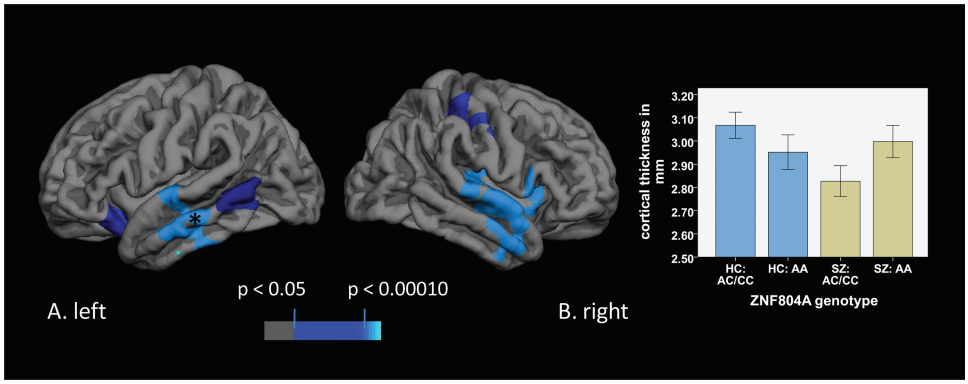

Fig. 2. Cortical statistical maps displaying significant cortical thickness clusters from the *ZNF804A* genotype × diagnosis interaction analysis between patients with schizophrenia (SZ) and healthy controls (HC). Shown are the surfaces of both hemispheres, (A) left hemisphere and (B) right hemisphere, pial view, and *P* values corrected for multiple comparisons. Bar diagram illustrates the genotype × diagnosis interaction on cortical thickness and mean values exemplarily extracted from the marked (*) cortical region.

showed significantly increased folding in patients in this superior temporal cluster ($P < .026$).

Again, comparing only the AA patients group and the whole healthy control group, this folding difference was not longer significant ($P > .430$), underlining the "protective" effect of *ZNF804A* homozygote A status on cortical folding in schizophrenia.

Furthermore, there was an opposite but not significant ($P > .130$) effect of the *ZNF804A* genotype on cortical folding in healthy controls, ie, AA vs AC/CC was associated with higher cortical folding.

The genotype × diagnosis analysis revealed a significant ($P < .05$, corrected for multiple comparisons) interaction in the left medial cortical surface covering parts of the precuneus.

Cortical Area

In patients, we found significantly (cluster-wise probability < .05) decreased cortical area in AA carriers in several regions, including left middle and superior temporal regions, left precentral and dorsolateral prefrontal areas, right dorsolateral prefrontal, inferior frontal, lateral, and occipital cortical areas. In controls, AA carriers showed decreased surface area in a left medial superior frontal area and smaller parts of the anterior cingulate cortex.

Allele-Specific Expression Analysis in Patients

The analysis revealed a significant difference in *ZNF804A* expression mediated by the risk allele A compared with the nonrisk C ($P < .027$). Presence of C led to significantly

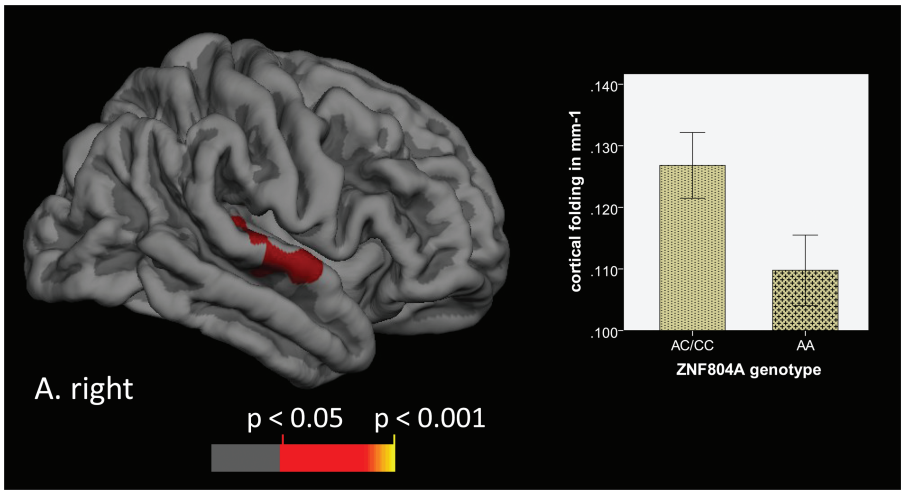

Fig. 3. Cortical statistical maps displaying significant cortical folding differences in schizophrenia patients between carriers of AA and AC/CC. In this region, AA carriers exhibit a significantly lower cortical folding compared with AC/CC carriers. Displayed is the lateral surface, (A) right hemisphere, pial view, and *P* values corrected for multiple comparisons. Bar diagram illustrates the cortical folding difference between the genotype groups and mean values extracted from the affected cortical region.

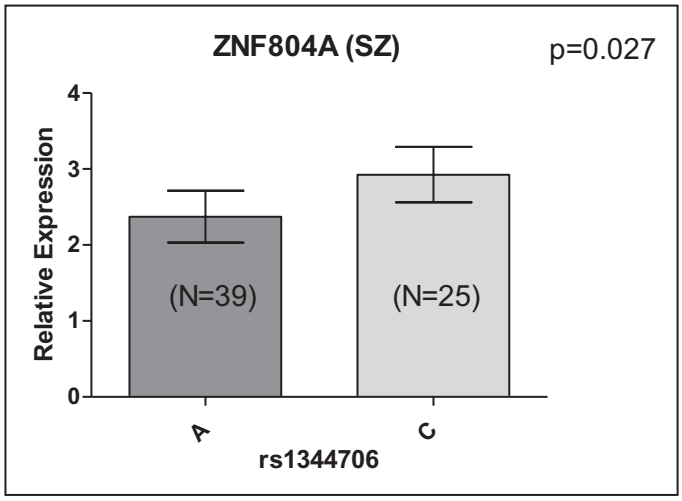

Fig. 4. Relative *ZNF804A* gene expression depending on the allelic counts of A and C. Analysis from postmortem prefrontal brain tissue of patients with schizophrenia. *P* value resulting from unpaired *t* test with Welch's correction.

elevated expression levels compared with A, ie, the schizophrenia-associated allele is associated with significantly lower *ZNF804A* gene expression in the prefrontal cortex of patients with schizophrenia (for illustration, see bar diagram of figure 4).

The observed differences in allelic-specific gene expression was not due to potential confounder variables (age, gender, pH, postmortem interval, refrigerator interval, smoking and drug abuse, and fluphenazine units) as assessed by the ANOVA (*F* ratio: 2.052, *P* value > .108).

Influence of ZNF804A on Cortical Thickness and Cognitive Functioning

We found significantly positive correlations between the premorbid intelligence level and cortical thickness for left superior, middle, and inferior temporal regions ($r = .234$, $P < .027$ for the left posterior middle temporal cluster and $r = .258$, $P < .014$ for the left superior-middle-inferior temporal cluster) in patients and controls. Testing patients and controls separately did not yield significant results.

Discussion

Our study has 3 main findings:

(1) We demonstrate first evidence for an extensive influence of *ZNF804A* risk status on prefrontotemporal cortical thickness in schizophrenia. Our data clearly indicate that the risk allele is associated with relatively intact cortical thickness in patients with schizophrenia.

Furthermore, at a whole-brain level, we could demonstrate that the effect of the genotype on cortical thickness

significantly differs between patients and healthy controls, ie, in healthy controls, the AA genotype is associated with reduced cortical thickness compared with AC/CC carriers. This opposite effect in patients has been uncovered for bihemispherical temporal brain regions and right ventrolateral prefrontal regions.

(2) Our study revealed new evidence that *ZNF804A* risk allele carrier status influences local cortical folding of the superior temporal cortex in patients.

Furthermore, differential effects on cortical folding of the left precuneus were found for patients and healthy controls.

It is of note that direct comparisons of cortical thickness and folding of the affected prefrontotemporal cortical clusters between patients and healthy controls revealed significant prefrontotemporal cortical thinning and disturbed superior temporal cortical folding in patients, which is in line with prior studies.[14,15,17,18] However, for both neuroanatomical measures, excluding nonrisk patients and comparing risk patients and healthy controls only, the differences of thickness and folding were not longer significant, implicating a protective role of *ZNF804A* risk status in patients.

(3) Along with this, our data show that *ZNF804A* gene expression is significantly lower with an increasing number of risk alleles in the prefrontal cortex of patients, which might constitute a molecular clue for the observed protective effects on cortical structure in patients. In healthy controls, the opposite effect has recently been described,[33] ie, *ZNF804A* gene expression is significantly higher with an increasing number of risk alleles.

Thus, the expression profile of *ZNF804A* appears to be distinctive between patients and healthy controls. It seems plausible that distinctive gene expression profile constitutes one molecular clue for the observed distinctive effect of *ZNF804A* on the cortical thickness and folding.

Cortical Thickness and ZNF804A

It is important to note that case-control studies investigating the influence of *ZNF804A* on cortical structure are rare but mandatory for the exploration of potential nonuniform effects of a risk genotype on brain structure between diagnostic groups. To date, only the study of Donohoe et al[9] has demonstrated an impact of *ZNF804A* on gray matter structure in a case-control design. They showed relatively intact gray matter volume in lateral superior temporal and medial temporal regions in patients with the AA genotype. Our data showing extensive effects of the risk genotype on prefrontotemporal regions in patients and a divergent effect of the genotype between the diagnostic groups on a whole-brain level extend and corroborate these initial findings. Voineskos et al[8] found significantly cortical thinning for the AA group in left superior temporal gyrus, left posterior cingulate cortex, and right anterior cingulate cortex in healthy controls.

In our genotype × diagnosis interaction, we found cortical thinning in controls in a cortical region very similarly to the superior temporal cortex finding of Voineskos et al. Thus, our data at least partly confirm the findings of Voineskos et al.

In a recent study of Bergmann et al,[49] the authors did not find an effect of *ZNF804A* on cortical thickness in patients with schizophrenia and healthy controls, which is in contrast to previous findings in healthy controls[8] and for cortical volume in patients with schizophrenia.[9] This discrepancy might mostly be due to the fact that Bergmann et al performed a regression analysis evaluating the effect of heterozygote, and major and minor homozygote alleles instead of grouping and comparing the genotype groups as done in the previous studies and our study. Because the effect of *ZNF804A* on cortical structure is mainly driven by the impact of the AA risk genotype, it is well explainable that this regressor model is, thus, not sensitive for detecting the effects of *ZNF804A* on cortical structure.

Cortical Folding and ZNF804A

Our study provides novel evidence that *ZNF804A* influences local cortical folding in schizophrenia. The affected superior temporal region is relevant for important pathophysiological processes in schizophrenia such as auditory processing[50,51] and working memory function.[25] Furthermore, the comparisons of cortical folding of this region between patients and healthy controls revealed local hypergyria in patients. Our finding of hypergyria is consistent with our and other previous results of increased cortical folding in schizophrenia.[44,45,52–54] However, in vivo local cortical folding has less intensively been explored compared with cortical thickness in schizophrenia and findings are heterogeneous showing hypo- and hypergyria,[55–57] which makes the interpretation of results more complex. Mechanical models of brain development propose a close linkage of forming of corticocortical connections and cortical folding,[58] and animal experiments demonstrated that early disruption of fiber pathways leads to local hypergyria.[59] Recently, Dauvermann et al[60] proposed that increased cortical folding may be related to increased local short range connectivity and reduced long range connectivity in subjects at high risk for schizophrenia using magnetic resonance imaging functional connectivity and cortical folding analysis. Taken together, disturbed cortical folding in schizophrenia might indicate an early disruption of brain development. Thus, based on our presented data, it can be argued that that *ZNF804A* risk status might attenuate neurodevelopmental cortical folding disturbance in schizophrenia.

Influence of ZNF804A on Cortical Thickness and Cognitive Functioning

We found a correlation of the premorbid intelligence level and cortical thickness in regions where the *ZNF804A* risk variant show significantly differing effects on cortical

thickness: ie, AA carriers in controls show a reduction of cortical thickness compared with AC/CC carriers; AA carriers in patients show a thicker cortex compared with AC/CC carriers (see also bar diagram in figure 3). Thus, this finding implicates that thicker cortex associated with the *ZNF804A* risk variant in patients is indeed associated with higher premorbid intelligence. Hence, our data might be interpreted in terms of a "protective" effect of the *ZNF804A* risk variant in patients on both premorbid intelligence and cortical thickness.

However, we are aware that it is only one cognitive measure showing this association, and further studies using more comprehensive test batteries are necessary to confirm this finding.

Potential Molecular Mechanism for Protective Effects of the ZNF804A on Neuroimaging Phenotypes in Schizophrenia

Strong evidence for protective effects of the *ZNF804A* risk genotype was provided by the study of Walters et al,[10] showing in a large cohort that the risk allele A is associated with better cognitive performance in memory domains of patients. Hitherto, the molecular mechanism underlying the observed protective effect of *ZNF804A* has remained unclear. In the present study, we demonstrate that in patients, an increasing number of risk alleles is associated with lower gene expression, which might constitute a molecular clue for the protective effects of the schizophrenia-associated risk allele A on cortical structure in patients. Together with the prior findings in healthy controls[33]—ie, higher gene expression is associated with an increasing number of risk alleles—the present finding indicates that the *ZNF804A* gene expression profile differs between patients and healthy individuals in terms of an opposite effect of the risk allele. Our findings give reason to assume that differing *ZNF804A* gene expression profiles between patients and healthy controls might be one molecular clue for distinctive *ZNF804A* genotype-phenotype relationships.

Interestingly, a recent study exploring the postmortem *ZNF804A* brain expression of nonpsychiatric individuals demonstrated an effect of the *ZNF804A* SNP in the second trimester fetal brain but not in the adult brain. Here, an increasing number of risk alleles was associated with decreasing *ZNF804A* gene expression.[34] These data suggest that *ZNF804A* exerts its effects on disease susceptibility in prenatal stages, which might be an explanation for our observation that imaging phenotypes with a strong linkage to brain development such as cortical thickness and folding[14,35,36] constitute sensitive markers for deviations of the cortical structure mediated by *ZNF804A*. However, the study of Williams et al[61] did not find a per se effect of rs1344706 on gene expression in the adult brain. The existing evidence for *ZNF804A* allelic cortical gene expression in the adult human brain is limited and conflicting. This is possibly related to methodological differences and sample composition.

Thus, to overcome these heterogeneities, further explorations on *ZNF804A* allelic gene expression including larger cohorts are needed.

Furthermore, it is of note that our gene expression analysis is restricted to the prefrontal cortex. Thus, it cannot been generalized that *ZNF804A* gene expression acts in the same way in other cortical regions underlining the need for further gene expression studies covering other cortical regions.

The *ZNF804A* risk variant appears to result in different phenotypes among patients and controls. This has been demonstrated for cognitive performance,[10] cortical volume,[9] and, by our data, for cortical thickness and folding. Furthermore, we showed that an increasing number of risk alleles is associated with a reduction of *ZNF804A* gene expression, whereas it is vice versa in controls.[33] Interestingly, Prata et al[62] found an opposite effect of the COMT Val158Met polymorphism on neuronal activity among patients with schizophrenia and controls. In a follow-up study, they demonstrated that COMT and DAT genes polymorphism interact nonadditively to modulate cortical function during executive processing, and also, that this effect significantly differed in schizophrenia, which might explain the opposite effects of the COMT SNP on neuronal activation among patients and controls. Thus, opposite effects of the *ZNF804A* risk allele on cortical structure might be explainable by such gene × gene interactions. Hence, it seems plausible that the *ZNF804A* SNP is in conjunction with interacting upstream genetic polymorphism functional.

Moreover, the phenomenon of different phenotypes associated with a genetic polymorphism—genetic pleiotropism—has been shown in human complex diseases and traits[63] including psychiatric diseases (eg, COMT SNP Val158Met[62]).[64] However, the molecular mechanisms underlying pleiotropic effects remain unclear. Differences in the genetic background and differing epistatic interactions might be one explanation. Differing epistatic interactions might result in differing gene expression profiles. Our data suggest that the *ZNF804A* gene expression differs between patients and controls, which might be due to differences of epistatic interactions based on differing genetic backgrounds among patients and healthy controls.

However, this interpretation remains speculative, and further studies, particularly molecular studies, on this issue are warranted.

We found reduced cortical area in association with the AA genotype in patients as well as in healthy controls. Point-by-point estimations of cortical area have rarely been done in psychosis research. Available studies showed cortical surface contraction in patients with schizophrenia.[65,66] Thus, our finding of *ZNF804A* associated cortical surface contraction implicates a role of *ZNF804A* in this neurobiological feature of schizophrenia. Together with our results regarding *ZNF804A* associated changes of

cortical thickness and folding, this finding gives further support for the hypothesis that *ZNF804A* might have pleiotropic effects on cortex architecture.

Conclusions

In conclusion, our analyses provide convergent support for the hypothesis that the schizophrenia-associated *ZNF804A* risk variant mediates protective effects on cortex structure in patients. In particular, the allele-specific expression profile in patients might constitute a molecular mechanism for the observed protective influence of *ZNF804A* on cortical thickness and folding and potentially other intermediate phenotypes.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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References

1. O'Donovan MC, Craddock N, Norton N, et al. Identification of loci associated with schizophrenia by genome-wide association and follow-up. *Nat Genet*. 2008;40:1053–1055.
2. Hill MJ, Jeffries AR, Dobson RJ, Price J, Bray NJ. Knockdown of the psychosis susceptibility gene ZNF804A alters expression of genes involved in cell adhesion. *Hum Mol Genet*. 2012;21:1018–1024.
3. Paulus FM, Krach S, Bedenbender J, et al.Partial support for ZNF804A genotype-dependent alterations in prefrontal connectivity. *Hum Brain Mapp*. 2013;34:304–313.
4. Esslinger C, Walter H, Kirsch P, et al.Neural mechanisms of a genome-wide supported psychosis variant. *Science*. 2009;324:605.
5. Esslinger C, Kirsch P, Haddad L, et al.Cognitive state and connectivity effects of the genome-wide significant psychosis variant in ZNF804A. *Neuroimage*. 2011;54:2514–2523.
6. Meyer-Lindenberg AS, Olsen RK, Kohn PD, et al.Regionally specific disturbance of dorsolateral prefrontal-hippocampal functional connectivity in schizophrenia. *Arch Gen Psychiatry*. 2005;62:379–386.
7. Lencz T, Szeszko PR, DeRosse P, et al. A schizophrenia risk gene, ZNF804A, influences neuroanatomical and neurocognitive phenotypes. *Neuropsychopharmacology*. 2010;35:2284–2291.
8. Voineskos AN, Lerch JP, Felsky D, et al. The ZNF804A gene: characterization of a novel neural risk mechanism for the major psychoses. *Neuropsychopharmacology*. 2011;36:1871–1878.
9. Donohoe G, Rose E, Frodl T, et al. ZNF804A risk allele is associated with relatively intact gray matter volume in patients with schizophrenia. *Neuroimage*. 2011;54:2132–2137.
10. Walters JT, Corvin A, Owen MJ, et al. Psychosis susceptibility gene ZNF804A and cognitive performance in schizophrenia. *Arch Gen Psychiatry*. 2010;67:692–700.
11. Wassink TH, Epping EA, Rudd D, et al. Influence of ZNF804a on brain structure volumes and symptom severity in individuals with schizophrenia. *Arch Gen Psychiatry*. 2012;69:885–892.
12. Winkler AM, Sabuncu MR, Yeo BT, et al. Measuring and comparing brain cortical surface area and other areal quantities. *Neuroimage*. 2010;61:1428–1443.
13. Fornito A, Yücel M, Wood SJ, et al. Surface-based morphometry of the anterior cingulate cortex in first episode schizophrenia. *Hum Brain Mapp*. 2008;29:478–489.
14. Kuperberg GR, Broome MR, McGuire PK, et al. Regionally localized thinning of the cerebral cortex in schizophrenia. *Arch Gen Psychiatry*. 2003;60:878–888.
15. Narr KL, Bilder RM, Toga AW, et al. Mapping cortical thickness and gray matter concentration in first episode schizophrenia. *Cereb Cortex*. 2005;15:708–719.
16. Narr KL, Toga AW, Szeszko P, et al. Cortical thinning in cingulate and occipital cortices in first episode schizophrenia. *Biol Psychiatry*. 2005;58:32–40.
17. Nesvåg R, Lawyer G, Varnäs K, et al. Regional thinning of the cerebral cortex in schizophrenia: effects of diagnosis, age and antipsychotic medication. *Schizophr Res*. 2008;98:16–28.
18. Schultz CC, Koch K, Wagner G, et al. Complex pattern of cortical thinning in schizophrenia: results from an automated surface based analysis of cortical thickness. *Psychiatry Res*. 2010;182:134–140.
19. Schultz CC, Koch K, Wagner G, et al. Reduced cortical thickness in first episode schizophrenia. *Schizophr Res*. 2010;116:204–209.
20. Venkatasubramanian G, Jayakumar PN, Gangadhar BN, Keshavan MS. Automated MRI parcellation study of regional volume and thickness of prefrontal cortex (PFC) in antipsychotic-naïve schizophrenia. *Acta Psychiatr Scand*. 2008;117:420–431.
21. Goldman AL, Pezawas L, Mattay VS, et al. Widespread reductions of cortical thickness in schizophrenia and spectrum disorders and evidence of heritability. *Arch Gen Psychiatry*. 2009;66:467–477.
22. Panizzon MS, Fennema-Notestine C, Eyler LT, et al. Distinct genetic influences on cortical surface area and cortical thickness. *Cereb Cortex*. 2009;19:2728–2735.
23. Cobia DJ, Csernansky JG, Wang L. Cortical thickness in neuropsychologically near-normal schizophrenia. *Schizophr Res*. 2011;133:68–76.
24. Crespo-Facorro B, Roiz-Santiáñez R, Pérez-Iglesias R, et al. Global and regional cortical thinning in first-episode psychosis patients: relationships with clinical and cognitive features. *Psychol Med*. 2011;41:1449–1460.
25. Ehrlich S, Brauns S, Yendiki A, et al. Associations of cortical thickness and cognition in patients with schizophrenia and healthy controls. *Schizophr Bull*. 2012;38:1050–1062.
26. Gutiérrez-Galve L, Wheeler-Kingshott CA, Altmann DR, et al. Changes in the frontotemporal cortex and cognitive correlates in first-episode psychosis. *Biol Psychiatry*. 2010;68:51–60.

27. Hartberg CB, Lawyer G, Nyman H, et al. Investigating relationships between cortical thickness and cognitive performance in patients with schizophrenia and healthy adults. *Psychiatry Res*. 2010;182:123–133.
28. Schultz CC, Koch K, Wagner G, et al. Reduced anterior cingulate cognitive activation is associated with prefrontal-temporal cortical thinning in schizophrenia. *Biol Psychiatry*. 2012;71:146–153.
29. Collarini EJ, Kuhn R, Marshall CJ, Monuki ES, Lemke G, Richardson WD. Down-regulation of the POU transcription factor SCIP is an early event in oligodendrocyte differentiation in vitro. *Development*. 1992;116:193–200.
30. Nielsen JA, Berndt JA, Hudson LD, Armstrong RC. Myelin transcription factor 1 (Myt1) modulates the proliferation and differentiation of oligodendrocyte lineage cells. *Mol Cell Neurosci*. 2004;25:111–123.
31. Chung HJ, Lee JY, Deocaris CC, Min H, Kim SH, Kim MH. Mouse Homologue of the Schizophrenia Susceptibility Gene ZNF804A as a Target of Hoxc8. *J Biomed Biotechnol*. 2010;2010:231708.
32. Lei H, Wang H, Juan AH, Ruddle FH. The identification of Hoxc8 target genes. *Proc Natl Acad Sci USA*. 2005;102:2420–2424.
33. Riley B, Thiselton D, Maher BS, et al. Replication of association between schizophrenia and ZNF804A in the Irish Case-Control Study of Schizophrenia sample. *Mol Psychiatry*. 2010;15:29–37.
34. Hill MJ, Bray NJ. Evidence that schizophrenia risk variation in the ZNF804A gene exerts its effects during fetal brain development. *Am J Psychiatry*. 2012;169:1301–1308.
35. Hilgetag CC, Barbas H. Role of mechanical factors in the morphology of the primate cerebral cortex. *PLoS Comput Biol*. 2006;2:e22.
36. White T, Hilgetag CC. Gyrification and neural connectivity in schizophrenia. *Dev Psychopathol*. 2011;23:339–352.
37. Annett M. The binomial distribution of right, mixed and left handedness. *Q J Exp Psychol*. 1967;19:327–333.
38. Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage*. 1999;9:179–194.
39. Fischl B, Sereno MI, Dale AM. Cortical surface-based analysis. II: inflation, flattening, and a surface-based coordinate system. *Neuroimage*. 1999;9:195–207.
40. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull*. 1987;13(2):261–276.
41. Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci USA*. 2000;97:11050–11055.
42. Gaser C, Luders E, Thompson PM, et al. Increased local gyrification mapped in Williams syndrome. *Neuroimage*. 2006;33:46–54.
43. Luders E, Thompson PM, Narr KL, Toga AW, Jancke L, Gaser C. A curvature-based approach to estimate local gyrification on the cortical surface. *Neuroimage*. 2006;29:1224–1230.
44. Schultz CC, Koch K, Wagner G, et al. Increased parahippocampal and lingual gyrification in first-episode schizophrenia. *Schizophr Res*. 2010;123:137–144.
45. Schultz CC, Wagner G, Koch K, et al. The visual cortex in schizophrenia: alterations of gyrification rather than cortical thickness-a combined cortical shape analysis. *Brain Struct Funct*. 2013;218:51–58.
46. White T, Andreasen NC, Nopoulos P, Magnotta V. Gyrification abnormalities in childhood- and adolescent-onset schizophrenia. *Biol Psychiatry*. 2003;54:418–426.
47. Lehrl S, Triebig G, Fischer B. Multiple choice vocabulary test MWT as a valid and short test to estimate premorbid intelligence. *Acta Neurol Scand*. 1995;91:335–345.
48. Schultz CC, Koch K, Wagner G, et al. Psychopathological correlates of the entorhinal cortical shape in schizophrenia. *Eur Arch Psychiatry Clin Neurosci*. 2010;260:351–358.
49. Bergmann O, Haukvik UK, Brown AA, et al. ZNF804A and cortical thickness in schizophrenia and bipolar disorder. *Psychiatry Res*. 2013;212:154–157.
50. Nenadic I, Smesny S, Schlosser RG, Sauer H, Gaser C. Auditory hallucinations and brain structure in schizophrenia: voxel-based morphometric study. *Br J Psychiatry*. 2011;196:412–413.
51. Palaniyappan L, Balain V, Radua J, Liddle PF. Structural correlates of auditory hallucinations in schizophrenia: a meta-analysis. *Schizophr Res*. 2012;137:169–173.
52. Harris JM, Moorhead TW, Miller P, et al. Increased prefrontal gyrification in a large high-risk cohort characterizes those who develop schizophrenia and reflects abnormal prefrontal development. *Biol Psychiatry*. 2007;62:722–729.
53. Narr KL, Bilder RM, Kim S, et al. Abnormal gyral complexity in first-episode schizophrenia. *Biol Psychiatry*. 2004;55:859–867.
54. Wisco JJ, Kuperberg G, Manoach D, et al. Abnormal cortical folding patterns within Broca's area in schizophrenia: evidence from structural MRI. *Schizophr Res*. 2007;94:317–327.
55. Cachia A, Paillère-Martinot ML, Galinowski A, et al. Cortical folding abnormalities in schizophrenia patients with resistant auditory hallucinations. *Neuroimage*. 2008;39:927–935.
56. Palaniyappan L, Mallikarjun P, Joseph V, White TP, Liddle PF. Folding of the prefrontal cortex in schizophrenia: regional differences in gyrification. *Biol Psychiatry*. 2011;69:974–979.
57. Sallet PC, Elkis H, Alves TM, et al. Reduced cortical folding in schizophrenia: an MRI morphometric study. *Am J Psychiatry*. 2003;160:1606–1613.
58. Hilgetag CC, Barbas H. Developmental mechanics of the primate cerebral cortex. *Anat Embryol (Berl)*. 2005;210:411–417.
59. Goldman-Rakic PS. Morphological consequences of prenatal injury to the primate brain. *Prog Brain Res*. 1980;53:1–19.
60. Dauvermann MR, Mukherjee P, Moorhead WT, et al. Relationship between gyrification and functional connectivity of the prefrontal cortex in subjects at high genetic risk of schizophrenia. *Curr Pharm Des*. 2012;18:434–442.
61. Williams HJ, Norton N, Dwyer S, et al. Fine mapping of ZNF804A and genome-wide significant evidence for its involvement in schizophrenia and bipolar disorder. *Mol Psychiatry*. 2011;16:429–441.
62. Prata DP, Mechelli A, Fu CH, et al. Opposite effects of catechol-O-methyltransferase Val158Met on cortical function in healthy subjects and patients with schizophrenia. *Biol Psychiatry*. 2009;65:473–480.
63. Sivakumaran S, Agakov F, Theodoratou E, et al. Abundant pleiotropy in human complex diseases and traits. *Am J Hum Genet*. 2011;89:607–618.
64. Smoller JW, Craddock N, Kendler K, et al. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet* 2013;381:1371–1379.
65. Palaniyappan L, Mallikarjun P, Joseph V, White TP, Liddle PF. Regional contraction of brain surface area involves three large-scale networks in schizophrenia. *Schizophr Res*. 2011;129:163–168.
66. Rimol LM, Hartberg CB, Nesvåg R, et al. Cortical thickness and subcortical volumes in schizophrenia and bipolar disorder. *Biol Psychiatry*. 2010;68:41–50.