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Altered Cortical Network Dynamics

A Potential Intermediate Phenotype for Schizophrenia and Association With ZNF804A

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Context: Studies have shown patterns of abnormal dorsolateral prefrontal cortex (DLPFC) functional connectivity with other brain areas in schizophrenia and association of these patterns with a putative susceptibility gene (ZNF804A). However, whether these patterns are trait phenomena linked to genetic risk for illness is unclear.

Objective: To test the hypotheses that altered DLPFC connectivity is (1) a familial, likely heritable feature of genetic risk for schizophrenia, (2) a novel intermediate phenotype independent of altered DLPFC engagement, and (3) selectively modulated by a polymorphism in ZNF804A.

Design: Cross-sectional case-control study using blood oxygen level–dependent functional magnetic resonance imaging during a working memory task and genotyping of rs1344706 in ZNF804A.

Setting: Research center.

Participants: A total of 402 subjects (153 cognitively normal controls, 171 healthy siblings of patients with schizophrenia, and 78 patients).

Main Outcome Measures: Task-independent and task-dependent physiologic coupling between the DLPFC and other brain “target” regions investigated with (1) seeded connectivity and (2) psychophysiological interaction analysis.

Results: Siblings and patients showed greater DLPFC “inefficiency” than controls. Abnormal DLPFC functional coupling with the hippocampus and, to a lesser degree, the rest of the prefrontal cortex, was observed in patients and siblings when compared with controls using both connectivity analyses. Prefrontal activation and connectivity measures within siblings did not correlate, implying that they were independent phenomena. The ZNF804A genotype significantly modulated DLPFC coupling with the hippocampus and prefrontal cortex but not DLPFC activity in the control group. Similarly, ZNF804A genotype modulated right DLPFC–hippocampal formation coupling in siblings and patients.

Conclusions: Coupling between the DLPFC and hippocampus is compromised in siblings of patients with schizophrenia and is independent of DLPFC engagement. The selective association with a single-nucleotide polymorphism in ZNF804A suggests that this intermediate phenotype proxies a distinct neural system mechanism related to genetic risk for schizophrenia and the biology of this gene.

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Imaging techniques have been increasingly used to elucidate the role and functions of genes associated with genetic risk for psychiatric illnesses. Imaging genetics studies suggest that genes associated with risk of schizophrenia not only modulate brain structure and activity in specific regions implicated in the neurobiology of schizophrenia but also the functional coupling of different brain areas. The latter findings are particularly intriguing because brain processing of even simple information engages networks of interacting neural systems and schizophrenia, like most behavioral disorders, may be better characterized as a syndrome of altered functional coupling within cortical circuits. In fact, the growing literature of imaging genetic studies in samples of cognitively normal subjects confirms that many genes associated with risk of schizophrenia modulate normal variation in brain activation, tuning, and functional coupling within circuits. A recent example of this trend is a high-profile study of cognitively normal subjects that described a strong association of a single-nucleotide polymorphism (SNP) (rs1344706) in ZNF804A (OMIM 602282), a gene showing statistically significant clinical association in a genome-wide as-
association study of psychosis,\textsuperscript{24} with altered connectivity of the dorsolateral prefrontal cortex (DLPFC) with other cortical regions.\textsuperscript{20} The patterns of altered connectivity associated with ZNF804A genotype were similar to those previously reported to be characteristic of patients with schizophrenia when compared with control subjects.\textsuperscript{25,27} The interpretation of this finding was that the imaging association identified a neural system mechanism that could underlie the clinical association of ZNF804A with schizophrenia, at least partially. However, this conclusion may not be justified by those data alone. Patterns of altered cortical function in patients with schizophrenia may reflect genetic, nongenetic, and/or illness-related factors (eg, treatment, symptoms, smoking, general health issues).\textsuperscript{28} Moreover, many genes will impact on brain function by mechanisms not necessarily related to those by which they relate to clinical risk. To link the imaging association to a potential mechanism of genetic risk for the clinical diagnosis requires demonstration at the least that the neural association represents a heritable, susceptibility-related phenotype (ie, an intermediate or endophenotype).\textsuperscript{29}

One strategy to identify neuroimaging intermediate phenotypes, ie, abnormal imaging responses that resemble those observed in patients and associated with increased genetic risk for illness, is to study individuals who carry risk-associated genes but not confounding factors.\textsuperscript{30,32} The healthy siblings of patients with schizophrenia are one such population. This approach has been widely and successfully used by ourselves and others to define discrete abnormal brain activities as imaging intermediate phenotypes (for example, prefrontal cortex activity during the N-Back task),\textsuperscript{33,36} but there have been a limited number of studies examining the heritability of coupling between different brain regions in healthy individuals who carry the genes that convey risk.\textsuperscript{35-38} These few studies are characterized by small samples that cross generations, differing methods used to measure the functional coupling, and performance differences between groups that can weaken the interpretation of results. Therefore, the nature of functional coupling abnormalities in schizophrenia as inherited vulnerability is unclear.

The first aim of this study was to test whether coupling of the DLPFC with other brain areas, mainly the hippocampus, consistently described as altered in schizophrenia, fulfilled the characteristics of a potential intermediate phenotype related to genetic risk for schizophrenia. We explored the functional coupling between fronto-frontal, fronto-hippocampal, fronto-parietal, and fronto-striatal regions because these networks have received the most attention in the neuroimaging literature related to schizophrenia and putative susceptibility genes.\textsuperscript{30,39} We used both task-dependent and task-independent approaches to explore the functional coupling during the execution of a working memory task\textsuperscript{31} in a relatively large sample of cognitively normal controls, patients with schizophrenia, and nonpsychotic siblings of patients with schizophrenia with similar performance to the control group. The second aim of the study was to test whether the potential intermediate phenotype of abnormal DLPFC coupling is independent of an earlier established intermediate phenotype related to abnormal DLPFC activity (inefficiency),\textsuperscript{33} and thus whether these 2 physiological measures are not redundant and might represent independent neural risk mechanisms. For this aim, we tested the correlation of these phenotypes within individuals. Finally, to validate the biological independence of the 2 intermediate phenotypes (DLPFC activation and coupling) and replicate a prior association of ZNF804A rs1344706 with DLPFC coupling, we tested for a selective modulation of DLPFC-hippocampus coupling by this SNP.\textsuperscript{30}

### METHODS

#### PARTICIPANTS

Patients with schizophrenia and their unaffected siblings were recruited as part of the Clinical Brain Disorders Branch Sibling Study (National Institutes of Health Protocol 93-M-6130), a study of neurobiological aspects of the illness related to genetics. Cognitively normal controls were recruited from the National Institutes of Health Clinical Research Volunteer Program. Inclusion and exclusion criteria are described in the supplementary “Methods” section (eAppendix; http://www.archgenpsychiatry.com).

### EXPERIMENTAL PARADIGM AND IMAGING DATA

Subjects performed a 2-Back working memory paradigm during whole-brain 3-T blood oxygen level functional magnetic resonance imaging. A measure of seeded connectivity was estimated to assess for brain connectivity between right DLPFC and other brain regions after adjusting for task-related activity. Using Statistical Parametric Mapping 5 (SPM5; Wellcome Trust Centre for Neuroimaging, London, England), the time series were temporally filtered to remove low-frequency signals and adjusted for global signal, movement parameters, white matter, and cerebrospinal fluid. The resulting B images were then analyzed in random effects models in SPM5. For Psychophysiological Interaction Analysis (PPI), a general linear model was constructed at the first level using 3 regressors: (1) the deconvolved blood oxygen level–dependent signal from the right DLPFC seed region (the same seed defined in the seeded connectivity analysis), (2) the task-related predictor (2-Back and 0-Back), and (3) the interaction term between the first and the second regressor. The contrasts were taken to a second-level random-effects analysis to examine differences across groups in cortical and subcortical regions functionally coupled with the right DLPFC, specifically because of the change in the working memory load. Details on task, analysis of imaging data, and statistical inference are described in the supplementary “Methods” section (eAppendix).

To control for nonindependence of data obtained from siblings and patients, cluster correction analysis was applied (STATA 9.2; StataCorp LP, College Station, Texas) and only results that were still significant with cluster correction are reported. In addition, we repeated all of the analyses on the intermediate phenotype in the sample of controls, siblings, and patients, after excluding 41 unaffected siblings who had an affected family member included in the analysis.

### COMPARISON BETWEEN ABNORMAL ACTIVATION AND ABNORMAL COUPLING IN SIBLINGS DURING WORKING MEMORY

To evaluate whether the significant difference obtained in DLPFC activation in siblings when compared with controls could ex-
plain the significant difference in DLPFC coupling observed in siblings when compared with controls, an intraclass correlation coefficient was calculated between DLPFC activation and each of the connectivity measurements in the sibling group.41

ANALYSIS OF 2NF804A POLYMORPHISM EFFECT

We genotyped the original 2NF804A SNP rs1344706, previously described (1) as the region with strongest evidence of association with risk for psychosis in the genome-wide association study (A allele, major allele)34 and (2) to modulate prefrontal cortex (PFC) coupling.20 The effect of rs1344706 on DLPFC activation and functional coupling using seeded connectivity and PPI approaches was explored in each group (controls, siblings, and patients). Details of genotyping methods and neuroimaging analysis are in the supplementary “Methods” section (eAppendix).

RESULTS

DEMOGRAPHIC AND BEHAVIORAL DATA

Demographic, clinical, and performance data for the sample (n=402) are in the Table. Controls and siblings did not differ in age, sex, intelligence quotient (measured by Wechsler Adult Intelligence Scale and Wide Range Achievement Test), or performance, while patients differed in these variables. Notably, because controls and siblings did not differ in performance data (accuracy and reaction time), differences in the functional magnetic resonance imaging data between these 2 groups reflected differences in how the information is processed in the brain and not task performance.

DIFFERENCE IN WORKING MEMORY–RELATED DLPFC ACTIVITY BETWEEN GROUPS

As previously reported,33,34 siblings showed a significant increase in DLPFC activation when compared with controls during working memory (2-Back–0-Back task; x, y, z = 33, 39, 39; z = 2.96; P = .04, familywise error [FWE]–corrected within ROI) for the same level of performance (ie, greater inefficiency). Patients also showed greater activation of the right DLPFC compared with controls (x, y, z = +5, 48, 3; z = +1.8; P = .02, false discovery rate–corrected for whole brain; P = .10, FWE-corrected for whole brain).

SEEDED CONNECTIVITY ANALYSIS

Main Effect

Seeded connectivity in each group (1-sample t test) is shown in Figure 1. Each group (controls, siblings, and patients) showed significant coupling between right DLPFC and ipsilateral and contralateral PFC, including the anterior cingulate cortex, bilateral inferior temporal gyri, bilateral superior and inferior parietal lobules (IPL), and striatum. The opposite contrast showed significant coupling between right DLPFC and medial frontal gyrus, bilateral superior and middle temporal gyrus, bilateral hippocampal formation (HF), bilateral posterior central gyrus, and posterior cingulate/precuneus (eTable 1).

Group Comparison Analyses

Right DLPFC–Bilateral HF Coupling. A significant difference in coupling between the right DLPFC and right HF was found across groups (x, y, z = 15, −6, −15; z = 3.40; P = .05, FWE-corrected within bilateral HF ROI). This difference was driven by an abnormal coupling both in siblings and patients compared with controls (controls vs patients P < .001; controls vs siblings P = .04; siblings vs patients P = .02) (Figure 2A). The results were similar, even when using performance as a covariate (controls vs patients P < .001; controls vs siblings P = .04; siblings vs patients P = .02). The coupling between right DLPFC and left HF also showed a similar pattern; however, the difference across groups did not survive correction for multiple comparisons (x, y, z = −30, −39, 3; z = 2.42; P = .008,
Siblings show a significant reduction in task-related modulation of right DLPFC–right HF coupling, while patients show minimal task-related modulation of this.

Controls showed stronger coupling compared with siblings and patients (Fisher least significant difference post hoc 1-tail test: controls vs siblings F = .04; controls vs patients F = .02; siblings vs patients F = .001). Controls showed stronger coupling compared with siblings and patients (Fisher least significant difference post hoc 2-tail t test: controls vs siblings F = .04; controls vs patients F = .001; siblings vs patients F = .001). C and D. Psychophysiological Interaction Analysis (PPI) group comparison analyses. C, A significant effect of task load on right DLPFC–HF coupling (planned comparison 1-tail t test: controls vs siblings F = .04; controls vs patients F = .001; siblings vs patients F = .001). D, A significant effect of task-load modulation on right DLPFC–left ventrolateral PFC coupling (planned comparison 1-tail t test: controls vs siblings F = .04; controls vs patients F = .001; siblings vs patients F = .001). Images are thresholded at P < .05 within ROIs only for illustrative purpose. Bars are mean ± standard error; AU indicates arbitrary units.

uncorrected) (refer to eFigure 1A.1 for the plot of first eigenvalues).

Right DLPFC–Bilateral PFC Coupling. A cross-group analysis showed a significant difference in the coupling within right DLPFC (ipsilateral x, y, z = 42, 18, 15; z = 3.98; P = .03, FWE-corrected within bilateral PFC ROI) (controls > siblings > patients). However, planned comparisons showed that decrease in coupling among patients drove the results while siblings showed a trend (controls vs siblings P = .07; controls vs patients P < .001; siblings vs patients P = .005). Similar results were observed when performance was used as a covariate of no interest (controls vs siblings P = .07; controls vs patients P < .001; siblings vs patients P = .005).

Figure 1. Functional coupling between right dorsolateral prefrontal cortex (DLPFC) and the rest of the brain (seeded connectivity). Significant coupling between right DLPFC and the rest of the brain during the 2-Back task in each group (1-sample t test). Rendered images are thresholded at P < .001, familywise error–corrected for the whole brain. The hippocampus is shown inside the bilateral hippocampus region of interest (z = –18; P = .05, uncorrected).

Figure 2. A and B. Seeded connectivity group comparison analyses. A, A significant difference is seen in coupling between the right dorsolateral prefrontal cortex (DLPFC) and right hippocampal formation (HF) (contrast controls > siblings > patients x, y, z = 15, –6, –15; z = 3.40; P = .05, familywise error [FWE]–corrected within bilateral HF region of interest [ROI]). Patients and siblings showed significantly altered coupling compared with controls (post hoc 1-tail test: controls vs patients P < .001; controls vs siblings P = .04; siblings vs patients P = .02). B, A significant difference is seen in the coupling between the right DLPFC and right inferior parietal lobules (IPL) (F test x, y, z = 42, –33, 30; F = 13.37; z = 4.57; P = .002, FWE-corrected within bilateral IPL ROI). Controls showed stronger coupling compared with siblings and patients (Fisher least significant difference post hoc 2-tail t test: controls vs siblings P = .04; controls vs patients P < .001; siblings vs patients P = .001). C and D. Psychophysiological Interaction Analysis (PPI) group comparison analyses. C, A significant effect of task load on right DLPFC–HF coupling (planned comparison 1-tail t test: controls vs siblings F = .04; controls vs patients P = .001; siblings vs patients P = .001). B. A significant difference is seen in the coupling between the right DLPFC and right inferior parietal lobules (IPL) (F test x, y, z = 42, –33, 30; F = 13.37; z = 4.57; P = .002, FWE-corrected within bilateral IPL ROI). Controls showed stronger coupling compared with siblings and patients (Fisher least significant difference post hoc 2-tail t test: controls vs siblings P = .04; controls vs patients P < .001; siblings vs patients P = .001). C and D. Psychophysiological Interaction Analysis (PPI) group comparison analyses. C, A significant effect of task load on right DLPFC–HF coupling (planned comparison 1-tail t test: controls vs siblings F = .04; controls vs patients P = .001; siblings vs patients P = .001). D, A significant effect of task-load modulation on right DLPFC–left ventrolateral PFC coupling (contrast controls vs siblings > patients x, y, z = 42, 18, 15; z = 3.98; P = .03, FWE-corrected within bilateral PFC ROI) (controls > siblings > patients). However, planned comparisons showed that decrease in coupling among patients drove the results while siblings showed a trend (controls vs siblings P = .07; controls vs patients P < .001; siblings vs patients P = .005). Similar results were observed when performance was used as a covariate of no interest (controls vs siblings P = .07; controls vs patients P < .001; siblings vs patients P = .005).
siblings vs patients \( P = .005 \) (refer to eFigure, A.2 for the plot of first eigenvalues).

**Right DLPFC–Bilateral IPL.** A significant difference across groups was found in the coupling of right DLPFC and bilateral IPL (left IPL \( x, y, z = -36, -39, 30; F = 17.56; z = 5.33; P < .001 \), FWE-corrected and \( x, y, z = -33, -27, 30; F = 12.89; z = 4.48; P = .003 \), FWE-corrected; right IPL \( x, y, z = 36, -24, 27; F = 14.12; z = 4.72; P = .001 \), FWE-corrected and \( x, y, z = 42, -33, 30; F = 13.37; z = 4.57; P = .002 \), FWE-corrected within bilateral IPL ROI). Post hoc analyses showed that controls had stronger coupling compared with siblings and patients between the right DLPFC and right IPL in the following voxel: \( x, y, z = 42, -33, 30; \) controls vs siblings \( P = .04 \); controls vs patients \( P < .001 \); siblings vs patients \( P = .001 \) (Figure 2B). This difference remained statistically significant when performance was used as a covariate of no interest (controls vs siblings \( P = .04 \); controls vs patients \( P < .001 \); siblings vs patients \( P = .001 \) (refer to eFigure 1A.3 for the plot of first eigenvalues). The differences observed across groups in the other significant voxels were driven by the patients group (controls vs siblings \( x, y, z = -36, -39, 30; P = .81 \); controls vs patients \( P = .56 \); siblings vs patients \( P = .51 \)).

**Right DLPFC–Striatum.** F test in SPM showed a main effect of group in the right DLPFC–striatum coupling (eTable 2), but post hoc analyses revealed that these differences were due to a reduction in the coupling between right DLPFC and striatum in patients, while the controls and siblings were not significantly different (controls vs siblings \( x, y, z = 18, 15, 15; P = .64 \); controls vs patients \( P = .29 \); patients vs siblings \( P = .98 \)).

**Right DLPFC–Rest of Brain.** To complete our observations, an exploratory analysis on right DLPFC coupling with the rest of the brain was performed. Only peak voxels from the \( F \) test that survived correction for multiple comparisons across the whole brain are reported (eTable 2). None of the voxels that showed altered coupling with the right DLPFC (mostly in cingulate gyrus) across groups was different between siblings and controls (controls vs siblings \( x, y, z = 15, 24, 36; P = .17 \); controls vs patients \( P = .69 \); siblings vs patients \( P = .36 \)), but the abnormal coupling in all of these areas was due to a reduction in coupling in patients group, except in the precuneus, where patients showed an increase in coupling compared with the other 2 groups (eTable 2).

**PPI ANALYSIS**

**Main Effect**

Psychophysiological Interaction Analysis (Figure 3) showed task load–related modulation (from 0-Back to 2-Back) between the right DLPFC and contralateral ventrolateral PFC in all 3 groups (1-sample \( t \) test). Interestingly, the task load positively modulated the right DLPFC-bilateral hippocampus coupling in controls and siblings, while in patients this modulation was negative. In addition, each group (controls, siblings, and patients) showed task load–related modulation between the right DLPFC and bilateral angular gyrus (Brodmann area 39), bilateral superior frontal gyrus (Brodmann area 8, Brodmann area 9), medial frontal gyrus (Brodmann area 8, Brodmann area 9), and left postcentral gyrus. The opposite contrast showed significant task load modulation of coupling between right DLPFC and posterior cingulate, cuneus, and precuneus in all 3 groups (eTable 1).

**Group Comparison Analyses**

**Task Load–Related Modulation of Right DLPFC–Bilateral HF Coupling.** Across-group analysis showed a significant difference in the task load modulation of coupling between right DLPFC and bilateral HF (left
The intraclass correlation coefficient between the various intermediate phenotype measures within the siblings was not significant, regardless of the functional connectivity analysis used (ie, seeded connectivity or PPI approach), suggesting that these physiological measures are not redundant.

**RELATIONSHIP BETWEEN MEASURES OF ABNORMAL DLPFC ACTIVATION AND COUPLING IN SIBLINGS**

The coupling between right DLPFC and right IPL (x, y, z = 42, −33, 30) in the whole sample (controls, siblings, and patients) correlated with the percentage of correct responses on the 2-Back (r = 0.1; P = 0.5). No other significant correlations were found.

**EFFECT OF ZNF804A RS1344706 POLYMORPHISM**

**Controls**

Genotype groups (N=96; nAA=37; nAC=45; nCC=14) did not differ in age, sex, intelligence quotient, handedness, or performance (eTable 4). Figure 4 illustrates genotype-based DLPFC coupling differences. There was no significant difference in DLPFC activation across ZNF804A genotype groups, but subjects homozygous for the risk-associated allele (major allele, AA) showed a disruption in task-related modulation of right DLPFC–left HF coupling in the PPI analysis (x, y, z = −15, 30; z = 3.60; P = 0.05; FWE-corrected within bilateral HF ROI; Fisher least significant difference [LSD] post hoc 2-tail t test: CC vs CA P = 0.04; CC vs AA P < .001; CA vs AA P = 0.04) (Figure 4A). A similar trend was observed in the coupling between the right DLPFC and right HF, although it did not survive correction for familywise error (x, y, z = −27, 24, −9; z = 3.63; P = 0.02, FWE-corrected within bilateral HF ROI and x, y, z = −33, 30, 0; z = 3.73; P = 0.02, FWE-corrected within bilateral HF ROI). Post hoc analyses revealed that siblings had a significant reduction in the modulation of coupling between the right DLPFC and right HF, while patients showed almost no modulation (x, y, z = 33, −33, 0; controls vs siblings P = 0.04; controls vs patients P < .001; siblings vs patients P = 0.008; x, y, z = 24, −36, 9; controls vs siblings P = 0.03; controls vs patients P < .001; siblings vs patients P = 0.02; similar results were observed when performance was used as a covariate of no interest) (Figure 2C) (refer to eFigure, B.1 for the plot of first eigenvalues). Although siblings showed reduced modulation of right DLPFC–left HF coupling, this difference was not significant (P = 0.08), while patients significantly differed from controls and siblings in this modulation (patients vs siblings P = 0.002; patients vs controls P < .001).

**Task Load–Related Modulation of Right DLPFC–Bilateral PFC Coupling**

Across-group comparison showed a reduction in the modulation of the coupling between right DLPFC and left ventrolateral PFC (x, y, z = −36, 15, −9; z = 3.83; P = 0.04, FWE-corrected within bilateral PFC ROI). This decrease was owing to both siblings and patients compared with controls (controls vs siblings P = 0.01; controls vs patients P < .001; siblings vs patients P = 0.02) (Figure 2D). Similar results were observed when performance was used as a covariate of no interest (controls vs siblings P = 0.01; controls vs patients P < .001; siblings vs patients P = 0.02; siblings vs patients P = 0.03; patients vs controls P < .001; siblings vs patients P = 0.02). (refer to eFigure, B.2 for the plot of first eigenvalues).

**Task Load–Related Modulation of Right DLPFC–Bilateral IPL, Right DLPFC–Striatum, and Right DLPFC–Rest of Brain Coupling**

The differences in these analyses did not survive correction for multiple comparisons. Analyses of the intermediate phenotype in the controls, siblings, and patients (after excluding 41 unaffected siblings who had an affected family member) included in the analysis showed significant results similar to those obtained with the whole sample, although only at a trend level in the seeded connectivity analysis (eAppendix; eTable 3).
ity analysis showed similar results, although they did not survive correction for multiple comparisons (x, y, z = −18, −39, −12; \(P = .004\), uncorrected; Fisher LSD post hoc 2-tail t test: CC vs CA \(P = .20\); CC vs AA \(P = .01\); CA vs AA \(P = .07\); Figure 4B). Moreover, as previously reported, there was a reduction in functional coupling between right DLPCF and contralateral PFC (x, y, z = −48, 33, 30; \(z = 3.52\); \(P < .001\), uncorrected; \(P = .10\), FWE-corrected within bilateral PFC ROI; Fisher LSD post hoc 2-tail t test: CC vs CA \(P = .02\); CC vs AA \(P < .001\); CA vs AA \(P = .07\)) and within the right DLPCF (right PFC x, y, z = 33, 54, 30; \(z = 3.12\); \(P = .001\); Fisher LSD post hoc 2-tail t test: CC vs CA \(P < .001\); CC vs AA \(P < .001\); CA vs AA \(P = .87\)) in risk allele–homozygous subjects (AA) when compared with subjects homozygous for the C allele (heterozygous subjects with the AC allele showed an intermediate pattern), although not significant with correction for multiple comparisons. The connectivity of DLPCF with other brain regions did not show a significant difference across ZNF804A genotype groups.

**Siblings**

Within the sample of siblings with available genotype data (N = 83; \(n_{AA} = 26\); \(n_{AC} = 47\); \(n_{CC} = 10\)), genotype groups did not differ in age, sex, intelligence quotient, handedness, or performance (eTable 5). Although sex distribution was not significantly different across genotype groups, because the number of women in the AA group (risk group) was more than in the other 2 groups, we included sex as a covariate of no interest in the neuroimaging analyses. Seeded connectivity in the sample of siblings confirmed the results observed in controls, with subjects homozygous for the risk allele (AA) showing reduced coupling between right DLPCF and left PFC (x, y, z = −36, 9, 39; \(z = 2.69\); \(P = .004\), uncorrected; Fisher LSD post hoc 2-tail t test: CC vs CA \(P = .11\); CC vs AA \(P = .02\); CA vs AA \(P = .16\)) and abnormal coupling between right DLPCF and HF (x, y, z = −18, −36, 9; \(z = 2.47\); \(P = .007\), uncorrected; Fisher LSD post hoc 2-tail t test: CC vs CA \(P = .07\); CC vs AA \(P = .02\); CA vs AA \(P = .27\)). Although these results did not survive correction for multiple comparison. Psychophysiological Interaction Analysis, however, failed to show any significant difference in task-related modulation of right DLPCF–HF coupling in this relatively small sample. Surprisingly, there was an increase of task-related modulation of coupling within the right DLPCF in siblings homozygous for the risk allele (AA) (x, y, z = 42, 30, −15; \(z = 4.12\); \(P = .02\), FWE-corrected within bilateral PFC ROI; Fisher LSD post hoc 2-tail t test: CC vs CA \(P = .001\); CC vs AA \(P < .001\); CA vs AA \(P = .04\)).

**Patients**

Genotype groups in the sample of patients with schizophrenia (genotype information was available for 33 patients [AA = 11; AC = 17; CC = 5]) did not differ in age, sex, intelligence quotient, handedness, or performance (eTable 6). Subjects homozygous for the minor allele (CC) were few (n = 5); therefore, a 2-sample t test was performed comparing homozygotes for the high-frequency risk-associated allele (AA) against the other 2 genotypes combined (AC + CC).

Psychophysiological Interaction Analysis in this small sample of patients confirmed the results observed in controls, with a significant effect of genotype (AA < C carriers) on coupling between the right DLPCF and left HF (x, y, z = −27, −9, −12; \(z = 3.32\); \(P = .001\), uncorrected; \(P = .09\), FWE-corrected within bilateral HF ROI), between the right DLPCF and contralateral bilateral HF ROI, and within the right DLPCF (ipsilateral DLPCF) (x, y, z = −42, 45, 15; \(z = 2.65\); \(P = .004\), uncorrected), and within the right DLPCF (ipsilateral DLPCF) (x, y, z = 57, 3, 30; \(z = .11\); \(P < .001\), uncorrected).

No effect of genotype was found in right DLPCF–bilateral HF coupling using the seeded connectivity approach. Additionally, contrary to the observations in controls and siblings, increased coupling within the right DLPCF (x, y, z = 57, 18, 30; \(z = 3.97\); \(P = .05\), FWE-corrected within ROI) was observed in patients homozygous for the high-risk allele (AA).

### ABNORMAL PFC COUPLING

Although there are many studies suggesting a disconnectivity hypothesis of schizophrenia,\(^2^{44}\) and the heritability of DLPCF abnormal reactivity,\(^45\) the heritability of connectivity disruptions has not been extensively explored in a single data set. Having established the existence of functional connectivity abnormalities in schizophrenia, it is important to ask if these abnormalities are genetically determined and related to genetic risk for schizophrenia, since having an affected family member remains the strongest risk factor for schizophrenia. This study investigated the familiality of abnormal functional coupling within brain regions described in schizophrenia. To this end, we examined the functional coupling of DLPCF during a working memory task in a large sample of controls, nonpsychotic siblings of patients with schizophrenia, and patients with schizophrenia. Two different approaches to investigate the functional coupling were applied. First, we used seeded connectivity analysis to investigate differences in task-independent coupling within multiple networks across groups. Second, with PPI we estimated the differences across groups in the changes in connectivity using different experimental conditions, ie, we estimated the stimulus-induced change of the modulatory effect of DLPCF on other regions. Our results demonstrate that functional interregional coupling abnormalities are familial and likely
heritable vulnerabilities, present to an intermediate degree in healthy siblings of patients with schizophrenia. Trait-related aberrant coupling was shown for the fronto-hippocampal and the fronto-frontal circuits, and we observed abnormal connectivity also in the fronto-parietal circuit, although this was not replicated in PPI analysis. Seeded connectivity, interestingly, showed that fronto-striatal and fronto-cingulate coupling was compromised only in patients with schizophrenia, suggesting a state-related dysfunction for these circuits at least during our working memory task.

COUPLING BETWEEN DLPFC AND HIPPOCAMPUS

Our results showed that DLPFC-HF coupling is compromised in siblings as well as patients. Psychophysiological Interaction Analysis indicates that the pattern of coupling observed in controls is reduced in siblings and disrupted and inverted in patients. Moreover, these data are consistent with the seeded connectivity analysis that showed patients having a stronger correlation during all of the time courses when compared with controls; siblings also show a pattern of connectivity intermediate between the 2 groups. Although the coupling of PFC with the highlighted hippocampal voxel was positive in controls, almost absent in siblings, and strongly negative in patients, as evident in Figure 2A, a further exploration of the whole cluster showed an increasingly negative pattern across all 3 groups (eAppendix). Nevertheless, while our results clearly show a difference between groups, the directionality (more positive or more negative) of this measurement needs to be interpreted cautiously.

These findings extend results from a previous study conducted with the same task paradigm, albeit a positron emission tomography study with oxygen 15–labeled water, showing that during the 2-Back task, DLPFC-HF coupling was altered in patients during the switch from 0-Back to 2-Back in contrast to controls. However, we now show that this coupling is also impaired in siblings, suggesting that DLPFC-HF coupling, regardless of task-dependence, is potentially a robust imaging-related intermediate phenotype, and the investigation of this coupling may be a good strategy for future genetic research. Indeed, a recent study in knockout mice used prefrontal hippocampal connectivity as a physiologic phenotype to validate their genetic construct, though the mice did not display the pattern of abnormal coupling found in patients with schizophrenia and their siblings.

The role of the hippocampus in working memory is not clear. One possibility is that the abnormalities observed in hippocampal-prefrontal coupling in siblings, which are more accentuated in patients with schizophrenia, may contribute to the deficits in working memory that characterize these populations. The lack of correlation between our coupling measures and DLPFC activation suggests that the 2 risk-associated neural function phenotypes are independent neural components of these cognitive problems.

COUPLING BETWEEN THE DLPFC AND IPL

Siblings as well as patients showed a reduction in the coupling between the right DLPFC and ipsilateral IPL in the seeded connectivity analysis. This result extends previous findings of disrupted prefrontal-parietal cortex connectivity during working memory in patients compared with controls. The abnormal coupling in siblings suggests that the functional interaction between DLPFC and IPL is compromised in healthy subjects at increased genetic risk for schizophrenia, though performance is still intact. In contrast to the PPI results for the fronto-hippocampal coupling, the PPI analysis of intracortical coupling did not show significant results, suggesting that task load does not modulate DLPFC interactions with IPL during this task and that the association between the 2 areas is compromised independent of the working memory load component of the task. This finding is intriguing because it may indicate a more intrinsic deficit in this coupling, a deficit consistent with recent studies showing a dysfunction of IPL in multiple neurocognitive processes in schizophrenia. Moreover, because the result was observed only at a trend level in the sample, excluding 1 unaffected siblings who had an affected family member, further replications are necessary.

COUPLING WITHIN AND BETWEEN BILATERAL PFC

Psychophysiological Interaction Analysis also revealed a reduction in the modulation between right DLPFC and left ventrolateral PFC with increasing task load in siblings when compared with controls, while seeded connectivity analysis that was task independent showed a trend of significance for reduced coupling within the right DLPFC. Interestingly, a previous study using a response selection paradigm and involving cognitive processes likely also engaged in our N-Back task, reported a trend similar to our results, with siblings showing reduced right DLPFC connectivity with the contralateral PFC area (Brodman area 10) when compared with controls. Impairment in the connectivity strength between areas within the PFC across hemispheres suggests abnormalities in interhemispheric connections within frontal regions, a finding previously reported with diffusion tensor imaging studies in schizophrenia.

STATE-RELATED ABNORMAL COUPLING

Many aberrant couplings observed in patients in this study did not appear to be genetically determined, presumably because they relate to the state of illness or treatment and not to the trait of risk. In our data, this was found for coupling of the right DLPFC with the anterior cingulate cortex and striatum. Specifically, patients with schizophrenia, but not their siblings, had reduced connectivity between the right DLPFC and anterior cingulate cortex. The anterior cingulate cortex is implicated in many cognitive functions, and during the 2-Back task is likely related to attention and response selection. Regarding its connectivity, a previous positron emission tomography study, although with a much smaller sample...
and a different task exploring verbal fluency, showed abnormal connectivity between these 2 regions in patients but failed to find any difference in carriers of genetic risk.\textsuperscript{33}

Similar results were found in coupling between the DLPFC and striatum. The striatum has been extensively studied in schizophrenia, and consistent findings link dopamine activity in the striatum to the severity of clinical symptoms, indicating strong influences of state factors on this area.\textsuperscript{31} Although we covaried for performance variables, we cannot exclude that, given the differences in reaction time obtained in patients compared with their siblings and controls, the observed abnormal coupling can simply reflect a difference in task performance.

MODULATORY EFFECT OF ZNF804A ON DLPFC ACTIVITY AND COUPLING

We observed that a SNP (rs1344706) in the gene ZNF804A associated with risk for psychosis\textsuperscript{24,32-35} modulated DLPFC-HF\textsuperscript{2} coupling in controls, siblings, and patients. Alternately, we found no effect of this SNP on abnormal DLPFC activation, a measure that appears to represent an independent intermediate phenotype. The effect of the SNP on coupling of the right DLPFC with other prefrontal regions (ipsilateral and contralateral) was less consistent across diagnostic groups. The small sample size of subjects available for neuroimaging genetic analyses is a potentially limiting factor and may underlie the inconsistent results of coupling within the prefrontal regions across diagnostic groups. The genetic results, particularly in patients and the siblings, therefore require careful interpretation and necessitate further exploration in larger samples. Nevertheless, our results with ZNF804A on DLPFC-HF coupling are robust and consistent with the recent observations by Esslinger et al.\textsuperscript{20}

In all, an effect of this SNP on functional magnetic resonance imaging measures of prefrontal-hippocampal coupled activity has now been observed in 4 clinical samples (ie, 3 in this study and 1 in the earlier article\textsuperscript{20}). These findings support the notion that the modulation of biologic intermediate phenotypes and potential neural mechanisms of risk (abnormal DLPFC activation vs connectivity) may be differentially dependent on the biology of the gene implicated. We believe this should come as no surprise. It would be unlikely that all genetic risk factors for schizophrenia would map to the same neural functions.

This study has important limitations that need to be acknowledged. Many different analyses were conducted, and the reported difference between siblings and controls would not survive a rigorous application of agnostic correction for the number of tests performed. Nevertheless, our study was strictly guided by prior literature and prior associations. We explored a limited number of hypotheses based on findings from earlier studies and applied the same methods. We believe the strength of our prior assumptions and our explicit hypothesis-testing strategy supports our approach to statistical inference. Moreover, replication is a more compelling approach to validation than is statistical correction. While our samples are relatively small and thus potentially inconclusive, the imaging data are large by imaging genetics standards. The fact that relationships between genotype and connectivity measures tended in the same direction in each of the 3 groups supports the validity of the findings. We have assumed that failure to observe a within-subject correlation of our connectivity phenotypes with the previously well-characterized inefficiency phenotype implies no relationship, but this interpretation must be viewed as preliminary. These caveats notwithstanding, the data suggest that the abnormal coupling between DLPFC and other brain regions is a neural phenomenon independent of the previously described abnormalities in DLPFC activation.\textsuperscript{33} This is also supported by the observation that some genes can modulate only one of these intermediate phenotypes (ie, ZNF804A).

Our findings support the idea that distributed network-based neurointegrative deficits reflect genetic risk mechanisms for schizophrenia and that these deficits are indeed present, although to a lesser degree, in nonpsychotic siblings of patients with schizophrenia and in cognitively normal subjects who carry risk-associated genotypes. Our findings also suggest that altered PFC activation and PFC coupling are 2 independent intermediate phenotypes and that susceptibility genes can modulate one but not the other. A SNP within ZNF804A appears to modulate PFC-HF coupling but not PFC activation. The way in which DLPFC communicates with HF, IPL, and ventrolateral PFC during working memory is probably susceptible to complex genetic modulation, and our data suggest that genes related to increased schizophrenia susceptibility participate in this modulation.

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