Original Investigation

Altered Functional Subnetwork During Emotional Face Processing
A Potential Intermediate Phenotype for Schizophrenia

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IMPORTANCE Although deficits in emotional processing are prominent in schizophrenia, it has been difficult to identify neural mechanisms related to the genetic risk for this highly heritable illness. Prior studies have not found consistent regional activation or connectivity alterations in first-degree relatives compared with healthy controls, suggesting that a more comprehensive search for connectomic biomarkers is warranted.

OBJECTIVES To identify a potential systems-level intermediate phenotype linked to emotion processing in schizophrenia and to examine the psychological association, task specificity, test-retest reliability, and clinical validity of the identified phenotype.

DESIGN, SETTING, AND PARTICIPATIONS The study was performed in university research hospitals from June 1, 2008, through December 31, 2013. We examined 58 unaffected first-degree relatives of patients with schizophrenia and 94 healthy controls with an emotional face-matching functional magnetic resonance imaging paradigm. Test-retest reliability was analyzed with an independent sample of 26 healthy participants. A clinical association study was performed in 31 patients with schizophrenia and 45 healthy controls. Data analysis was performed from January 1 to September 30, 2014.

MAIN OUTCOMES AND MEASURES Conventional amygdala activity and seeded connectivity measures, graph-based global and local network connectivity measures, Spearman rank correlation, intraclass correlation, and gray matter volumes.

RESULTS Among the 152 volunteers included in the relative-control sample, 58 were unaffected first-degree relatives of patients with schizophrenia (mean [SD] age, 33.29 [12.56]; 38 were women), and 94 were healthy controls without a first-degree relative with mental illness (mean [SD] age, 32.69 [10.09] years; 55 were women). A graph-theoretical connectivity approach identified significantly decreased connectivity in a subnetwork that primarily included the limbic cortex, visual cortex, and subcortex during emotional face processing (cluster-level *P* corrected for familywise error = .006) in relatives compared with controls. The connectivity of the same subnetwork was significantly decreased in patients with schizophrenia ($F = 6.29$, $P = .01$). Furthermore, we found that this subnetwork connectivity measure was negatively correlated with trait anxiety scores ($P = .04$), test-retest reliable (intraclass correlation coefficient = 0.57), specific to emotional face processing ($F = 17.97$, $P < .001$), and independent of gray matter volumes of the identified brain areas ($F = 1.84$, $P = .18$). Replicating previous results, no significant group differences were found in face-related amygdala activation and amygdala–anterior cingulate cortex connectivity (*P* corrected for familywise error =.37 and .11, respectively).

CONCLUSIONS AND RELEVANCE Our results indicate that altered connectivity in a visual-limbic subnetwork during emotional face processing may be a functional connectomic intermediate phenotype for schizophrenia. The phenotype is reliable, task specific, related to trait anxiety, and associated with manifest illness. These data encourage the further investigation of this phenotype in clinical and pharmacologic studies.

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Schizophrenia is a highly heritable mental disorder characterized by severe deficits in emotion processing.[1,2] Meta-analyses of patient data have pointed to a strong association between emotional deficits and dysfunctions of the limbic system.[3-5] However, whether and how these alterations relate to the genetic risk of schizophrenia remain unclear. A useful strategy to identify genetic mechanisms is the search for intermediate phenotypes, which are heritable traits related to the genetic predisposition to the disorder.[6,7] The study of unaffected first-degree relatives, who share an enriched set of schizophrenia risk genes but do not manifest clinical symptoms, provides important evidence for the establishment of a putative intermediate phenotype.[7-10] Although this strategy has been successful in cognitive domains, such as working memory,[11] declarative memory,[12,13] and reward processing,[14] findings in emotional face processing have been inconclusive, with decreased,[15,16] increased,[17] or unchanged[18] amygdala reactivity and/or connectivity in relatives compared with controls.

This incongruence suggests that the search for an emotion-related intermediate phenotype may have to look beyond regional activation and connectivity analyses, which focus on a restricted set of regions and possibly overlook potential multidimensional network changes. Previous work[19-22] using graph theory–based methods has found significant alterations in the brain connectome of patients with schizophrenia during active tasks and resting state. The connectomic measures are heritable,[23,24] implying their utility for identifying intermediate phenotypes related to emotional face processing.

Our study aimed to identify potential connectomic intermediate phenotypes related to emotional face processing in schizophrenia. First, we used graph theory–based characterization of the connectome to identify potential functional network changes in unaffected first-degree relatives (58 relatives, 94 controls) using functional magnetic resonance imaging (fMRI) and a well-established emotional face-matching task.[25] In accordance with prior findings,[26] we posited alterations in connectivity between the visual cortex and the limbic system in relatives compared with controls. We further performed several follow-up analyses to investigate the utility of this potential intermediate phenotype by testing for psychological association, task specificity, test-retest reliability, and potential structural confounds. Second, we tested in an independent sample for the presence of the identified phenotype in schizophrenia (31 patients, 45 controls) for clinical validation.

Key Points

Question Do unaffected first-degree relatives of patients with schizophrenia have functional brain abnormalities during emotion processing?

Findings This study identified significantly decreased connectivity in a network, primarily including the limbic cortex and the visual cortex, during emotional face processing in unaffected first-degree relatives of patients with schizophrenia compared with controls. The connectivity of the same network was also significantly decreased in patients with schizophrenia.

Meaning Altered connectivity in a visual-limbic network during emotional face processing may be an intermediate phenotype for schizophrenia.

Methods

Participants

One hundred fifty-two healthy volunteers from 3 sites in Germany (Mannheim, Bonn, and Berlin) were included in our relative-control sample from June 1, 2008, through December 31, 2013. Among these, 58 were unaffected first-degree relatives of patients with schizophrenia (mean [SD] age, 33.29 [12.56] years; 38 women), and 94 were healthy controls without a first-degree relative with mental illness (mean [SD] age, 32.69 [10.09] years; 55 women). The groups were balanced for a broad range of demographic, psychological, task performance, and image quality parameters ($P > .10$ for all) (**Table 1** and eMethods in the Supplement). All participants provided written informed consent for the protocols approved by the institutional review boards of the University of Heidelberg, University of Bonn, and Universitätsmedizin Charité, Berlin.

MRI Modalities and Paradigms

Participants completed a well-established emotional face-matching fMRI task.[25,27,28] The face-matching task is an implicit emotional processing paradigm designed to challenge the amygdala.[25,27,28] The block-designed task consists of 2 conditions: an emotional condition (matching faces) and a control condition (matching forms). In the emotional condition, participants are presented with trios of faces from a standard set of pictures of facial affect[29] that depict fearful or angry expressions. Participants are instructed to match the 2 corresponding stimuli that illustrate the same individual. In the control condition, participants are presented with trios of simple geometric shapes (circles, vertical and horizontal ellipses) and are asked to match the 2 corresponding geometric shapes. In addition, we acquired resting-state fMRI images and high-resolution structural images for the participants (eMethods in the Supplement).

Data Acquisition and Quality Control

The MRI data were acquired from 3 Siemens 3-T scanners (Siemens Trio) with identical protocols. For fMRI data quality assurance, we quantified several head motion parameters and signal-to-noise ratio. Head motion parameters were quantified as previously detailed[14,30-32] and included the sum of volume to volume translational excursions across the time series, the sum of volume to volume rotational excursions across the time series, and the mean voxel-based framewise displacement. The signal to noise ratio of images was calculated using the New York University Center for Brain Imaging dataQuality toolbox (http://cbi.nyu.edu/software/dataQuality.php).[33-35] Statistical comparisons of data quality parameters between relatives and controls were performed with SPSS statistical software, version 20 (SPSS Inc), using independent *t* tests. As detailed in Table 1, groups were balanced for data quality assurance measures.

Table 1. Sample Characteristics of Relatives of Patients With Schizophrenia and Healthy Controls for the Identification of an Intermediate Phenotype[a]

Characteristic	Relatives (n = 58)	Controls (n = 94)	t or χ^2 Value	P Value
Demographic information				
Age, y	33.29 (12.56)	32.69 (10.09)	−0.31	.76
Male/female sex, No.	20/38	39/55	0.74[b]	.39
Site, Mannheim/Berlin/Bonn, No.	24/16/18	35/24/35	0.61[b]	.74
Educational level, y	15.34 (2.43)	15.52 (2.72)	−0.40	.69
Handedness, right/left/both, No.	51/4/3	90/3/1	3.61[b]	.17
Psychological assessments[c]				
NEO-FFI-neuroticism	18.13 (10.09)	16.22 (7.32)	NA	.48
NEO-FFI-extraversion	30.13 (6.45)	30.30 (6.53)	NA	.95
NEO-FFI-openness	31.05 (6.96)	30.03 (7.49)	NA	.29
NEO-FFI-agreeableness	33.36 (5.56)	33.08 (6.11)	NA	.91
NEO-FFI-conscientiousness	32.93 (7.38)	34.78 (6.25)	NA	.16
STAI-trait	38.36 (11.94)	36.01 (10.03)	NA	.36
ERQ-suppression	13.21 (5.33)	14.08 (4.89)	NA	.25
ERQ-reappraisal	28.54 (7.11)	28.19 (5.84)	NA	.30
SRRS	289.37 (141.35)	266.27 (200.63)	NA	.10
SPQ sum	11.12 (10.93)	10.30 (9.63)	NA	.72
SCL-90-R mean	0.26 (0.27)	0.21 (0.21)	NA	.60
BDI-I	4.71 (6.08)	3.09 (3.31)	NA	.25
WAIS-MR-III	21.07 (3.44)	20.27 (3.74)	NA	.24
fMRI task performances[c]				
Correct ratio face matching, %	98.64 (2.52)	98.45 (3.91)	NA	.61
Correct ratio form matching, %	95.83 (5.57)	96.41 (4.95)	NA	.61
Head motion parameters				
Sum translational excursions, mm	0.48 (0.56)	0.43 (0.36)	0.62	.54
Sum rotational, °	0.70 (0.68)	0.69 (0.58)	0.11	.91
Mean framewise displacement, mm	0.10 (0.05)	0.09 (0.03)	1.44	.15
fMRI image quality				
Signal to noise ratio	88.93 (20.33)	92.97 (18.03)	−1.26	.21

Abbreviations: BDI-I, Beck Depression Inventory (version I); ERQ, Emotion Regulation Questionnaire; fMRI, functional magnetic resonance imaging; NA, data not applicable; NEO-FFI, NEO Five Factor Inventory; SCL-90-R, Symptom Checklist-90–Revised; SPQ, Schizotypal Personality Questionnaire; SRRS, Social Readjustment Rating Scale; STAI-trait, trait subscale of the State-Trait Anxiety Inventory; WAIS-MR-III, matrix reasoning subscale of Wechsler Adult Intelligence Scale (version III).

[a] Data are presented as mean (SD) unless otherwise indicated.

[b] χ^2 Value.

[c] For psychological assessments and task performances, Mann-Whitney nonparametric tests were used because of the nonnormal distribution of the data.

Activity and Seed-Based Connectivity Analyses

We first aimed to replicate the negative amygdala activation and seed-based connectivity results reported by Rasetti et al.[18] We followed the methods outlined in that study and used standard procedures implemented in Statistical Parametric Mapping, version 8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). Briefly, for the activity analysis, functional images were realigned, slice time corrected, normalized to the Montreal Neurological Institute brain template, spatially smoothed, and subjected to first-level general linear model estimation. For the analysis of group differences, first-level contrast images (face matching greater than form matching) were entered into a second-level random-effects model. Results were reported after familywise error (FWE) correction across an a priori defined anatomical mask of the bilateral amygdala from the Automated Anatomical Labeling (AAL) atlas.[36] For the connectivity analysis, the mean time series of the bilateral amygdala from the AAL atlas were extracted after noise correction and entered into the first-level model. These first-level connectivity maps were then entered into a second-level random-effects model. Results for between-group effects were reported after FWE correction across a bilateral anterior cingulate cortex mask from the AAL atlas (eMethods in the Supplement).

Brain Network Analyses

Brain network analyses followed our previously published procedures.[31] The mean time series during the face-processing task were extracted from each of the 90 anatomical nodes defined by the AAL atlas and corrected for noise. Whole-brain connectivity matrices were then calculated by pairwise Pearson correlations between nodes. The derived connectivity matrices were analyzed at 2 levels. At the global level, we further thresholded the matrices with multiple densities and computed 4 commonly used global network properties (smallworldness, modularity, global efficiency, and characteristic path length) for each density. Repeated-measures analysis of covariance (ANCOVA) models were used for group comparison with density as the within-subject factor and group (relatives vs controls) as the between-subject factor, covarying for age, sex, site, and mean framewise displacement. At the local level, the network-based statistic[37,38] was used for link-

Table 2. Sample Characteristics of the Patient and Control Groups for the Clinical Validation Study[a]

Characteristic	Patients With Schizophrenia (n = 31)	Healthy Controls (n = 45)	*t* or χ^2 Value	*P* Value
Demographic information				
Age, y	30.58 (7.05)	32.00 (9.50)	−0.75	.46
Male/female sex, No.	24/7	26/19	3.15[b]	.08
Site, Mannheim/Bari, No.	12/19	19/26	0.09[b]	.76
fMRI task performances[c]				
Face matching, % correct	90.99 (16.88)	96.37 (4.51)	NA	.15
Form matching, % correct	92.21 (9.52)	97.78 (4.40)	NA	.16
Head motion parameters				
Sum translational excursions, mm	0.57 (0.66)	0.63 (0.56)	−0.44	.67
Sum rotational excursions, º	0.86 (0.79)	0.71 (0.56)	0.98	.36
Mean framewise displacement, mm	0.13 (0.11)	0.08 (0.05)	2.64	.03
fMRI image quality				
Signal to noise ratio[d]	203.73 (104.37)	220.43 (113.35)	0.65	.52

Abbreviations: fMRI, functional magnetic resonance imaging; NA, data not applicable.

[a] Data are presented as mean (SD) unless otherwise indicated.

[b] χ^2 Value.

[c] For task performances, Mann-Whitney nonparametric tests were used because of the nonnormal distribution of the data.

[d] Note that the data was partly derived from a different fMRI scanner compared with the relatives study.

wise comparisons of the connectivity matrices between groups. This was done as previously described[37,38] by 2 steps. First, we computed initial *t* test statistics for all pairwise connections (contrasting relatives and controls and controlling for age, sex, site, and mean framewise displacement), which generated a set of suprathreshold links. Second, permutation testing was used to derive corrected *P* values for the generated link clusters (eMethods in the Supplement).

Follow-up Analyses

We performed several follow-up analyses to substantiate our connectomic finding as a potential intermediate phenotype for schizophrenia. In follow-up analyses 1 to 4, we reduced data dimensionality by averaging the connectivity estimates of all links in the identified subnetwork for each participant. See eMethods in the Supplement for details on the rationale and methods of the follow-up analyses.

Association With Trait Anxiety

Trait anxiety is an important assessment of emotion stability and may plausibly relate to the identified subnetwork connectivity phenotype. We probed for the presence of this association by calculating the Spearman rank correlation between the mean subnetwork connectivity estimates and trait anxiety scores derived from the State-Trait Anxiety Inventory across the participants in the control group. Significance was set at $P < .05$.

Clinical Association

We further aimed to verify the presence of alterations in the identified subnetwork connectivity phenotype in schizophrenia. For this, we collected fMRI data from independent samples of 31 patients with schizophrenia (mean [SD] age, 30.58 [7.05] years; 7 women) and 45 healthy controls (mean [SD] age, 32.00 [9.50] years; 19 women). Participants were recruited from psychiatric hospitals and communities in and around Mannheim, Germany, and Bari, Italy. All patients were diagnosed as having schizophrenia by psychiatrists according to the Structured Clinical Interview for *DSM-IV* and were taking antipsychotic medication. Participants from Mannheim completed the same face-matching fMRI task used in the relatives study, and participants from Bari underwent scanning with a slightly modified version of the task[39] and a different scanner (Signa 3-T scanner, GE Healthcare) (**Table 2** and eMethods in the Supplement). Data processing followed the same procedures described in the first study. Mean subnetwork connectivity was calculated for each participant and group difference in the derived metric was tested using an ANCOVA model, covarying for age, sex, site and mean framewise displacement.

Test-Retest Reliability

Test-retest reliability of the identified subnetwork connectivity measure was analyzed using an independent sample of 26 healthy controls scanned twice with the same face-matching task (mean [SD] age, 24.4 [2.8] years; 15 women; mean [SD] scan interval, 14.6 [2.1] days; data previously reported by Cao et al[31]). Intraclass correlation coefficient (ICC) was used as an index of robustness. Consistent with established criteria,[30,31,40] an ICC greater than 0.40 was interpreted to be indicative of fair to good reliability.

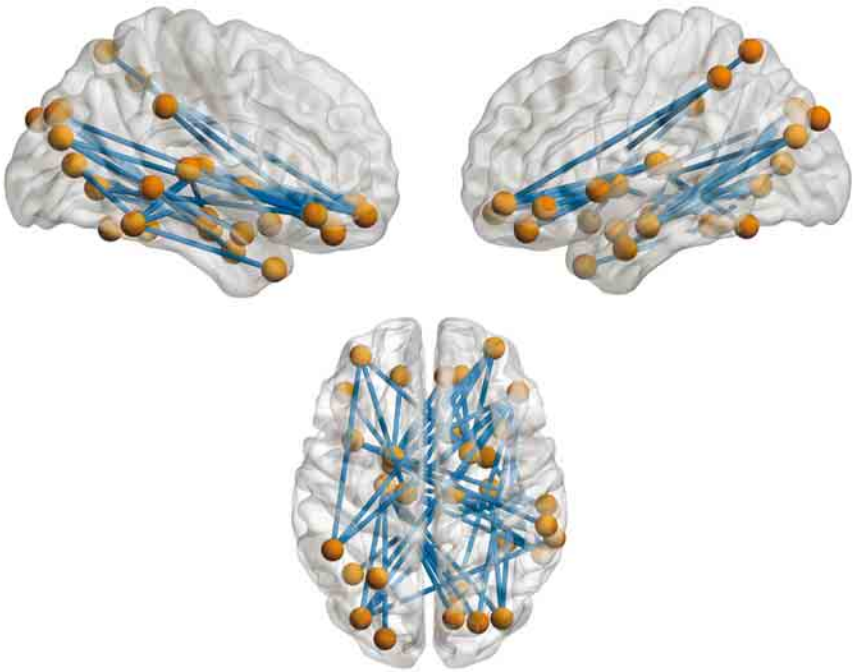
Task Specificity

To investigate whether the identified subnetwork finding is specific to emotional face processing or represents a task-independent functional connectomic alteration, we compared the subnetwork connectivity measure during face processing with that during the resting state in the same individuals. Group differences during 2 fMRI task conditions (ie, face matching, resting state) and group by task interaction effects were examined using a repeated-measures ANCOVA model.

Structural Analysis

We analyzed high-resolution structural data of relatives and controls to test whether the identified functional alterations may be influenced by structural differences. We compared mean gray matter volumes of the identified subnetwork nodes

Figure 1. Altered Visual-Limbic Subnetwork in Relatives of Patients With Schizophrenia During Emotional Face Processing



All links in this subnetwork show decreased connectivity in relatives compared with controls. Cluster-level *P* corrected for familywise error = .006.

derived from high-resolution structural MRI and voxel-based morphometry. We also computed structural covariance matrices of cross-participant correlations between gray matter volumes and investigated linkwise group differences in the structural matrices. Significance was set at $P < .05$ after FWE correction.

Results

Activity and Seed-Based Connectivity Analyses in Relatives and Controls

Consistent with a previous study,[18] significant differences in amygdala activity (small-volume *P* corrected for FWE = .37) and amygdala-anterior cingulate cortex seeded connectivity (small-volume *P* corrected for FWE = .11) between healthy first-degree relatives and controls during the face-matching task were not detected.

Brain Network Analysis in Relatives and Controls

At the global level, no significant group differences were found for the measured network properties ($P = .82$ for smallworldness, $P = .59$ for modularity, $P = .46$ for global efficiency, $P = .43$ for characteristic path length) (eFigure 1 in the Supplement). At the subnetwork level, the network-based statistic identified a cluster that showed significantly decreased coupling in relatives compared with controls (*P* corrected for FWE = .006), which consisted of 49 links between pairs of 33 nodes chiefly in the limbic system (amygdala, hippocampus, parahippocampal gyrus, insula, orbitofrontal cortex), the visual cortex (calcarine sulcus, fusiform gyrus, superior and middle occipital gyrus, cuneus, lingual gyrus), and the subcortex (pallidum and thalamus) (**Figure 1** and eTable 1 in the Supplement). The mean connectivity across all subnetwork links was significantly decreased in relatives of patients with schizophrenia.

Follow-up Analyses

Psychological Association

The Spearman correlation analysis revealed a significantly negative correlation between mean subnetwork connectivity and State-Trait Anxiety Inventory trait scores ($\rho = -0.22$, $P = .04$) (**Figure 2**), suggesting that lower subnetwork connectivity is associated with higher anxiety in healthy individuals.

Clinical Association

We observed a significant decrease of mean subnetwork connectivity in patients with schizophrenia compared with healthy controls ($P = .01$, partial $\eta^2 = 0.08$, indicating a medium effect size[41]) (**Figure 3**). This finding indicates that the identified subnetwork alteration is also present in patients with the same directionality detected in first-degree relatives.

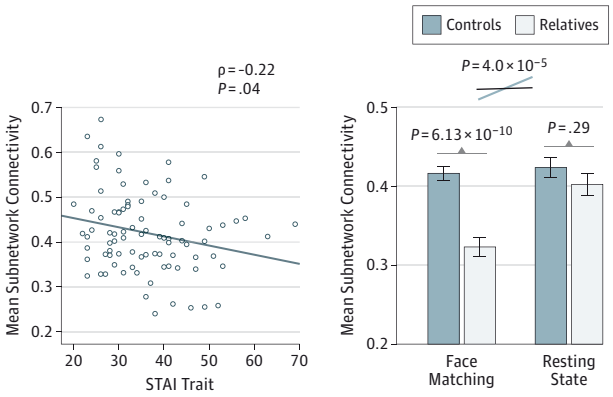
Test-Retest Reliability

The ICC values for mean subnetwork connectivity were $ICC_{2,1} = 0.57$ and $ICC_{3,1} = 0.57$. These values indicate a fair to good reliability of the identified intermediate phenotype.

Task Specificity

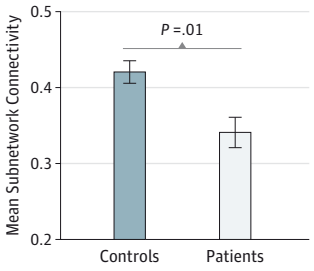
In contrast to a significant group difference detected for the emotional task ($P = 6.13 \times 10^{-10}$), analysis of participants' rest-

Figure 2. Follow-up Analyses for the Identified Subnetwork



The mean subnetwork connectivity is negatively correlated with trait anxiety scores derived from the State-Trait Anxiety Inventory (STAI) in the subsample of healthy controls. The mean subnetwork connectivity reveals a significant difference between relatives of patients with schizophrenia and healthy controls during the emotional face-matching task but not in the resting state. The group by task interaction is significant. Bars indicate mean values. Error bars indicate SEs.

Figure 3. Alteration of the Identified Subnetwork in Patients



Similar to first-degree relatives of patients with schizophrenia, patients with schizophrenia have significantly decreased connectivity estimates in the identified visual-limbic subnetwork compared with healthy controls. Bars indicate mean values. Error bars indicate SEs.

ing state data revealed no significant between-group differences in mean subnetwork connectivity (P = .29). In addition, a significant group by task interaction was found for the subnetwork measure ($P = 4.0 \times 10^{-5}$) (Figure 2).

Structural Analysis

There were no significant differences in mean gray matter volumes of the identified subnetwork nodes (P = .18) (eFigure 2 in the Supplement) and in the constructed structural covariance matrices (P corrected for FWE > .99) between relatives and controls.

Discussion

In this study, we identified decreased functional connectivity in a visual-limbic subnetwork in unaffected first-degree relatives during emotional face processing, verified comparable abnormalities in patients with schizophrenia, and provided evidence of the utility of this potential intermediate phenotype by analyses of its psychological association, reliability, task dependence, and potential structural confounds. In humans, emotion processing depends on at least 2 multinodal cooperative brain systems[42-44]: a ventral system for the identification of emotional significance and the initiation of corresponding responses, including fusiform gyrus, amygdala, insula, basal ganglia, thalamus, ventral anterior cingulate cortex, and orbitofrontal and ventrolateral prefrontal cortex, and a dorsal system for the regulation of affective states, including dorsolateral and dorsomedial prefrontal cortex, dorsal anterior cingulate cortex, and hippocampus. In schizophrenia, meta-analytical evidence points toward functional alterations in multiple brain nodes during emotional face processing, including fusiform gyrus,[3,5] amygdala,[3-5] hippocampus,[5] medial and dorsolateral prefrontal cortex,[3,5] and subcortex.[3,5] Because these deficits range across more than 1 aforementioned system, they may plausibly reflect a failure in the coordination of interacting functional subunits of the emotional network. A previous study[26] further found decreased effective connectivity among several of these brain regions in unaffected siblings of patients with schizophrenia. This finding may imply that the proposed multisystem coordination failure relates to the genetic risk of the illness. In line with this proposal, our study identified a connectivity deficit in a large-scale emotional face-processing network in unaffected first-degree relatives of patients. The deficit spanned the ventral (eg, fusiform gyrus, amygdala, insula, basal ganglia, thalamus, orbitofrontal cortex) and dorsal (eg, hippocampus) emotion-processing systems. Because we verified consistent abnormalities in patients with schizophrenia, these findings highlight a potential connectomic intermediate phenotype for schizophrenia.

We performed several follow-up analyses to sustain our subnetwork finding as a potential intermediate phenotype related to emotional face processing. First, we found that the subnetwork connectivity was significantly correlated with trait anxiety scores, suggesting a link between the identified subnetwork and emotion-related personality. Second, we found that the subnetwork connectivity alteration in first-degree relatives was evident in the face-matching task but not in resting state, suggesting a degree of functional specificity of this potential intermediate phenotype. Third, consistent with our prior work,[31] we found that the subnetwork measure was test-retest reliable, suggesting that the proposed connectivity feature is a promising target intermediate phenotype for longitudinal studies, such as drug interventions.

We also addressed several important confounding factors in this study. First, in accordance with common practice,[6,8,14] we controlled for demographic differences by balancing our sample for age, sex, site, educational level, and handedness. Second, similar to a prior study,[14] we controlled for group differences in psychological characteristics and fMRI task performances, thereby ensuring that the detected subnetwork anomalies are not confounded by these behavioral measures. Third, considering that head motion may cause spurious effects on connectivity measures,[45,46] we balanced our sample for several head motion and signal-quality parameters and adjusted for head motion in our analyses. Fourth,

given that morphometric abnormalities have been reported for some subnetwork nodes in patients with schizophrenia,[47-49] we probed for potential group differences in gray matter volumes and structural covariance patterns of the nodes. The results suggest that the functional subnetwork alterations are not explained by preexisting structural anomalies.

Our data have provided empirical evidence of several conceptual requirements outlined for putative intermediate phenotypes.[6-10] Specifically, this subnetwork is altered in unaffected individuals with increased genetic risk for schizophrenia, is associated with the illness, and is quantitatively reliable. Although establishing a neuroimaging intermediate phenotype that satisfies all requirements is extremely challenging and rarely accomplished in practice,[10] and some other measures for this subnetwork finding require further investigation (eg, heritability), our study points to a promising intermediate phenotype for schizophrenia during emotional face processing.

There were some negative findings of this study. First, consistent with the findings of Rasetti et al,[18] we did not find significant differences in face-related amygdala activation and seed-based connectivity measures, suggesting a greater sensitivity of the connectomic approach in search for intermediate phenotypes during emotional face processing. Second, there were no significant group differences in measured global network properties. Global properties are, by nature, less sensitive to focal changes at the subnetwork level yet require stringent correction for multiple comparisons.[50] Furthermore, global network properties derived from the face-matching task have been found less reliable than connectivity properties on which the network-based statistic method is based.[31] These points may have plausibly contributed to the negative results in global properties.

Our study has several limitations. First, despite the relatively large sample size, first-degree relatives in our study were from several generations, with a small number of individuals younger than 20 years. However, most previous studies[15,17,18] only focus on healthy siblings older than 20 years to minimize potential confounding effects, such as cohort-specific life experiences and prodromal effects.[7,8] Second, our findings are based on an emotional face-matching task that allows the assessment of perceptual functions related to emotional face processing but not the differentiation of specific emotional functions in the amygdala and other limbic regions. Third, the exclusion of psychiatric disorders in relatives and controls in our study were based on self-report assessments rather than standardized interviews. Fourth, future work should replicate and extend these results by examining test-retest properties of the identified subnetwork pattern and investigating the ability of the subnetwork to distinguish relatives and controls in independent samples.

Conclusions

Our study provides initial evidence of a visual-limbic subnetwork alteration during emotional face processing that may be a promising intermediate phenotype for schizophrenia. The data presented in the study support the further exploration of this potential connectomic intermediate phenotype in clinical and pharmacologic studies.

ARTICLE INFORMATION

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Study concept and design: Cao, Walter, Tost, Meyer-Lindenberg.
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Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods

Identification of a potential connectomic intermediate phenotype

Sample characteristics and psychological assessments

Functional network alterations related to the genetic risk for schizophrenia were examined in 58 unaffected first-degree relatives of schizophrenia patients and 94 healthy controls without a first-degree relative with mental illness. All individuals were of European ancestry. Exclusion criteria included a lifetime history of significant general medical, psychiatric or neurological illness, prior drug or alcohol abuse, and head trauma. The psychiatric diagnoses of the index patients were established by psychiatrists according to the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders. The recruited relatives were asked to provide official documents signed by psychiatrists which confirmed that they had a first-degree relative with schizophrenia. The first-degree relatives consisted of different generations including siblings, parents and children of the index patients (22 siblings, 10 parents, 24 children, with 4 subjects age < 20 years). All subjects were evaluated by a battery of clinical and psychological assessments for the exclusion of overt mental illness. Clinical assessments included the Symptom Checklist-90 Revised (SCL-90-R) [1], the Schizotypal Personality Questionnaire (SPQ) [2] and Beck Depression Inventory (BDI) [3]. Intelligence was tested by the Matrix Reasoning subscale of Wechsler Adult Intelligence Scale (WAIS-MR) [4]. Personality and emotion regulation were tested by using the NEO Five Factor Inventory (NEO-FFI) [5], the State-trait Anxiety Inventory (STAI) [6] and the Emotion Regulation Questionnaire (ERQ) [7]. Stressful life events were quantified using the modified Social Readjustment Rating Scale (SRRS) [8], with the assessment for the past two years instead of the original one year. Both groups were well balanced for a broad range of variables including demographics, head motion, image quality, task performances, and psychological measures (all *P* values > 0.10, see Table 1 in the main manuscript for details).

MRI data acquisition

Magnetic resonance imaging data were acquired in Mannheim, Bonn and Berlin using three identical 3-Tesla MR scanners (Siemens Trio, Erlangen, Germany) and identical scanner protocols. Functional data were acquired with gradient-recalled echo-planar imaging (GRE-EPI) sequences with the parameters TR 2000 ms, TE 30 ms, 28 oblique slices (descending acquisition) per volume, 4 mm slice thickness, 1 mm slice distance, 80° flip angle, 192 mm FOV, and 64 × 64 matrix. High-resolution structural data were acquired by using 3D magnetization-prepared rapid gradient-echo (MP-RAGE) sequences with the parameters TR 1570 ms, TE 2.75 ms, TI 800 ms, 176 sagittal slices, 1 mm slice thickness, 256 mm FOV, and 15° flip angle.

Image quality control

Head motion parameters were quantified as previously detailed [9-12] and included the sum of volume-to-volume translational excursions across the time series, the sum of volume-to-volume rotational excursions across the time series, and the mean voxel-based frame-wise displacement. The first measures calculate the sum of the root mean square of the three translational and rotational motion vectors from the x, y, and z axes. The third measure is a nonlinear combination of the volume-wise translations and rotations and reflects the mean voxel-specific distance relative to the previous image. The signal-to-noise ratio of images was calculated using the NYU CBI dataQuality toolbox (http://cbi.nyu.edu/software/dataQuality.php). Here, signal is defined as the mean voxel intensity of a given ROI in the brain, and noise is defined as the standard deviation of the voxel intensities of a given ROI in the background. For more details, please refer to previous studies [13-15].

Face processing and resting state tasks

The face-matching task used in this study is presented in eight blocks of six trials (or 30 s) each with alternating epochs of face- and form-matching conditions (task duration: 4.3 min or 130 whole-brain scans). During the five minutes (or 150 whole-brain scans) resting state task, participants are instructed to close their eyes, relax, and refrain from specific mental activities. Afterwards, investigators verified with the participants that they had not fallen asleep during the scan.

Emotion processing: activation and seeded connectivity analyses

Data preprocessing

For image preprocessing we followed standard routines of the Statistical Parametric Mapping software (SPM8, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). Briefly, this included the realignment to the first image of the scan, slice time correction, spatial normalization to the Montreal Neurological Institute (MNI) space with resampling to $3 \times 3 \times 3$ mm^3 voxels, and smoothing with a 9 mm full-width at half-maximum (FWHM) Gaussian kernel.

Amygdala activation analysis

Since we aimed for a replication of two previously reported negative findings with this task, the amygdala activation and seeded connectivity analyses of the face-matching task closely followed the procedures described in Rasetti *et al* [16]. For the activation analysis, the preprocessed images were analyzed in a two-level procedure. At the first level, general linear models (GLM) were defined for each subject that included the boxcar reference vectors for the task conditions (convolved with the standard SPM hemodynamic response function) and the six head motion parameters from the realignment step (as covariates of non-interest). During model estimation the data were high-pass filtered (cut-off 128 seconds) and individual maps of the "*face-matching* > *form-matching*" contrast were computed. The contrast images were used for a second-level

random effects analysis with group (relatives vs. controls) as variable of interest and age, sex, and site as nuisance covariates. Significance was measured at $P < 0.05$ family-wise error (FWE) corrected across an *a priori* defined anatomical mask of the bilateral amygdala from the Automated Anatomical Labeling (AAL) atlas [17].

Amygdala seeded functional connectivity analysis

Individual first-level GLMs of the seed-based connectivity analysis included the mean time series of the amygdala as regressor of interest (extracted using the bilateral AAL amygdala mask), and the following nuisance regressors: the six head motion parameters from the realignment step, the mean time series derived from cerebrospinal fluid (CSF) and white matter (WM) masks, and two regressors encoding for the task conditions. During model estimation the data were high-pass filtered (cut-off 128 seconds). The resulting statistical parametric connectivity maps were used for a second-level random effects group analysis as described above. Significance was measured at $P < 0.05$ FWE corrected across the bilateral ACC mask from the AAL atlas.

Emotion processing: brain functional network analyses

Construction of connectivity matrices

Following the procedures of our previous study (Method M2 in the reference paper) [11], we extracted the mean task time series for each of the 90 nodes defined by the AAL atlas. The time series were corrected for task conditions, WM and CSF signals, and the six head motion parameters, and were high-pass filtered (cut-off 128 seconds). For the construction of brain networks, we computed pairwise Pearson correlation coefficients between the processed time series of each node, which resulted in a 90 × 90 two-dimensional correlation matrix for each subject.

Calculation and analysis of global graph properties

We used two different methodological approaches for the analyses of the functional connectivity matrices. At the global level, the matrices were thresholded in 1% intervals over a range of 31 densities from 10% to 40% where, for example, in the 10% thresholded matrix the top 10% of the highest positive correlations are represented as internode connections. We selected this density range based on our previous study with the same task and analysis method which shows that small-world networks are present in this range [11]. We calculated four commonly used global properties for each of the densities: smallworldness (a measure of combination of large clustering coefficient (i.e. high segregation) and short path length (i.e. high integration)), modularity (the degree to which the network can be divided into non-overlapping modules), global efficiency (a measure of the efficiency of the whole network as quantified by the average inverse shortest path length) and characteristic path length (the average path length between all pairs of nodes in the network). For the detailed definition and calculation of these properties, please refer to past reviews [18,19]. The computation for smallworldness and modularity were based on 100 times

network randomizations. We chose these specific properties since prior evidence suggest that they are heritable [20,21], and abnormalities in these measures have been repeatedly observed in schizophrenia [22-25]. For statistical inference, repeated measures analysis of covariates (ANCOVA) models were used with densities as within-subject factor and group (relatives vs. controls) as between-subject factor, and age, sex, site, and mean head motion frame-wise displacement (FD) as covariates of non-interest.

Network-based statistic (NBS)

At the local level, network-based statistic (NBS) was used to identify clusters of functional links that are altered in the relatives group compared to the controls. NBS is a method to effectively control cluster-level family-wise error (FWE) for link-wise matrical comparisons and offers a larger power than mass-univariate tests on independent links. For details on this method, please refer to Zalesky *et al* [26,27]. Consistent with prior studies [23,26-28], we applied initial t-tests (contrasting relatives and controls) to each of the *N(N-1)/2 = 4005 (N = 90)* links in the connectivity matrices, while controlling for age, sex, site and FD. This generated a *P*-value matrix representing the probability of accepting the null hypothesis for each link. All links with *P* values < 0.0005 were then thresholded into a set of suprathreshold links [28], and connected clusters within this set were identified by breadth first search [29]. The significance of the identified clusters was tested by permutation testing, where the subjects were randomly reallocated into one of the two groups and the maximal size of the identified cluster was recalculated during each of the 5000 permutations. The corrected *P* value for the cluster was determined by the proportion of the derived cluster sizes in the permutation distribution that were larger than the observed group difference.

Follow-up analyses

Rationale for the follow-up supplemental analyses

Subsequent to the identification of the visual-limbic subnetwork abnormalities during emotion processing in the first-degree relatives of schizophrenia patients, we performed four post-hoc supplemental analyses to further validate the identified connectomic phenotype: 1) An analysis to investigate potential association between the subnetwork connectivity measures and trait anxiety scores, 2) An analysis to establish the test-retest reliability of the connectivity estimates during emotion processing in the identified visual-limbic subnetwork, 3) An analysis probing the specificity of the visual-limbic alterations by examining the same connectivity measure during resting state, and 4) An analysis probing the influence of potential structural confounds by quantifying the grey matter volumes and structural covariance of the identified subnetwork nodes in the first-degree relatives compared to the controls. In analyses 1-3 we reduced data dimensionality by averaging the connectivity estimates of all links in the identified visual-limbic

cluster and condensed them into subject-specific univariate metrics that were then subjected to between-group comparisons. We chose this strategy to increase the sensitivity of these analyses and to stay consistent with the NBS method per se, which treats the whole cluster as an entity for which the null hypothesis can only be rejected at the cluster level [26]. For the very same reason, in analysis 4), the mean grey matter volumes of the nodes in the identified visual-limbic cluster were condensed into subject-specific univariate metric (i.e., mean grey matter volume of the cluster nodes) for subsequent between-group volumetric analysis.
Since for methodological reasons, NBS findings can only be interpreted at the cluster level, we chose the cluster outcome of the comparison of relatives and controls as a starting point for subsequent analyses since the reverse strategy (i.e., the comparison of controls and patients as a starting point) would plausibly yield link-wise differences that relate to medication influences rather genetic predisposition, which would complicate our validation efforts and contradict the basic goal of this study.

Correlation between subnetwork connectivity and trait anxiety

Trait anxiety is an important assessment of emotion stability and may plausibly relate to the identified subnetwork connectivity phenotype. Here, we calculated the Spearman rank correlation between the mean subnetwork connectivity estimates and trait anxiety scores derived from the State-trait Anxiety Inventory (STAI). This correlation was only tested in the control group. Additional analyses showed that no significant correlation was found in relatives or in a combined sample, suggesting an independent replication is necessary to guard a type I error.

Test-retest reliability analysis of visual-limbic connectivity estimates

We have previously reported fair to good reliabilities for whole-brain connectivity estimates during emotional processing [11]. However, it is unclear whether this observation generalizes to the specific visual-limbic subnetwork identified in this study. To probe this question, we reanalyzed the test-retest data from our prior study in which 26 healthy subjects were scanned twice with the same emotional face-matching task within two consecutive weeks (mean time interval: 14.6 ± 2.1 days, mean age: 24.4 ± 2.8 years, 15 females, see Cao *et al.* [11] for further details). The preprocessing and network constructions followed the procedures described above, and the mean connectivity estimates of the identified visual-limbic subnetwork were extracted for each subject and measurement time point. Consistent with our previous studies [9,11], intra-class correlation coefficients (ICCs) were calculated to quantify reliability, namely, ICC(2,1) and ICC(3,1) according to the formula

$$ICC(2,1) = (BMS-EMS) / (BMS + (k-1)EMS + k(JMS-EMS)/n)$$
$$ICC(3,1) = (BMS-EMS) / (BMS + (k-1)EMS)$$

where BMS is the between-subject mean square, EMS is the residual mean square, JMS is the between-session mean square, n is the number of subjects, and k is the number of sessions. These coefficients reflect the absolute agreement and relative consistency of measurements between sessions [30,31].

Visual-limbic resting state analysis

The vast majority of the relatives and controls (150 out of 152, 58 relatives and 92 controls) also participated in a five minutes resting-state fMRI experiment. This allowed us to examine the second question relevant to the utility of the observed visual-limbic subnetwork alterations: Whether the alterations in the relatives group are specific to emotion processing or a fundamental feature of the functional organization of brain networks in general. With the exception of a different band-pass setting (0.008 - 0.1Hz) the preprocessing of the resting state data, node definition, and the construction of connectivity matrices followed the same procedures as those described for the emotional face-matching task. To test for group-dependent differences and group by task interactions, we extracted the mean connectivity estimates of the identified visual-limbic subnetwork links from the resting-state connectivity matrices and entered those as the dependent variable into a repeated-measures ANCOVA model. In this model, the tasks (i.e., emotional task, resting state) were included as within-subject factor and group (i.e., relatives, controls) were included as between-subject factor. Age, sex, site, and FD were included as covariates of non-interest. Significance was measured at $P < 0.05$.

Visual-limbic morphometry and structural covariance analysis

We acquired high-resolution structural images from the vast majority of the participants (58 relatives and 93 controls). The investigation of potential structural confounds followed a two-step procedure. First, we subjected the T1-weighted images to voxel-based morphometry (VBM8, http://dbm.neuro.uni-jena.de/vbm8/) [32] to calculate voxel-wise grey matter volumes for each subject. Briefly, this included tissue classification, spatial normalization to MNI space with a diffeomorphic image registration algorithm (DARTEL), correction for image intensity non-uniformity, correction for global brain grey matter volume, and smoothing with a 10 mm FWHM Gaussian kernel. We then extracted the grey matter volumes for each of the identified subnetwork nodes, averaged the volumes for each subject, and entered the values as dependent variable into an ANCOVA model that included group (relatives vs. controls) as variable of interest, and age, sex, site and FD as nuisance covariates. In addition, we constructed group-specific structural covariance matrices by computing pair-wise Pearson correlations of the grey matter volumes of the identified nodes across all subjects in that group. Following previously published procedures [33], significant differences of the structural covariance matrices between relatives and controls were calculated by permutation testing (5000 times), where in each permutation, the subjects were randomly reallocated into one of the two groups and the deviations of each entry in the

covariance matrices were recalculated. *P* values for each entry were derived as the proportion of the deviations of each entry in the permutation distribution that were larger than the observed group difference. Afterwards, FWE correction was used to control for the false positive rate of the multiple comparisons of entries in the matrices.

Association of the identified phenotype with schizophrenia

Sample characteristics

While the examination of patient populations often bears the limitation of a potential medication-related confounding of fMRI data, the demonstration of comparable abnormalities in schizophrenia is an important piece of evidence for the clinical validation of the proposed intermediate phenotype. We tested this question in an independent sample with 31 schizophrenia patients (age 30.58 ± 7.05 years, 7 females) and 45 healthy controls (age 32.00 ± 9.50 years, 19 females) recruited from psychiatric hospitals and communities in and around Mannheim, Germany and Bari, Italy. The two groups were balanced for age, sex, site, and task performances (all *P* values > 0.08, see Table 2). The schizophrenia patients were diagnosed according to the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and were under stable anti-psychotic medications. The healthy controls were recruited after the exclusion of a lifetime history of significant general medical, psychiatric or neurological illness, prior drug or alcohol abuse, head trauma, and the presence of a first-degree relative with a history of psychiatric illness. All the subjects provided written informed consent for a protocol approved by the institutional review board of the Universities of Heidelberg and Bari.

MRI data acquisition

fMRI data of the subjects from Mannheim used the same sequence as reported above in the relatives study, while the data of the subjects from Bari were acquired on a GE Signa 3 Tesla scanner (Milwaukee, Wisconsin) with GRE-EPI sequence and the following sequence parameters: TR 3000 ms, TE 30 ms, 24 oblique slices per volume, 5 mm slice thickness, no distance factor, 240 mm FOV, 64 × 64 matrix.

Emotion processing task

The subjects from Mannheim participated in the same face-matching task as described above. The blocked-design emotion processing task acquired in Bari is a similar but extended version of the emotion processing task that has been used for the relatives- and imaging genetics studies in this work. Briefly, in addition to the face-matching and form-matching conditions described above, the task includes a third experimental condition challenging explicit emotion processing (face-labelling). In this condition, subjects were asked to label emotional target faces depicting fearful

or angry expressions by selecting the adequate expression out from two word presentations (angry, fear) that were simultaneously presented at the bottom of the screen. The task was presented in nine blocks of six trials (or 32 seconds) each, with alternating epochs of task conditions (task duration: 4.8 min or 99 whole-brain scans). Please refer to Blasi *et al* [34] for further details.

Data processing and analysis

Data preprocessing, node definition, and construction of connectivity matrices followed the procedures described above for the relatives study. To test for group differences we extracted the mean connectivity estimates of the identified visual-limbic subnetwork links from the connectivity matrices of each subject and entered them as dependent variable into an ANCOVA model with group (i.e., patients, controls) as variable of interest and age, sex, site and FD as covariates of non-interest. Significance was measured at $P < 0.05$, and effect size of the group difference was quantified by computing partial eta-squared values in SPSS20. Following Cohen [35], eta-squared values > 0.06 indicate a medium effect size while eta-squared values > 0.14 indicates a large effect size.

Additional analysis

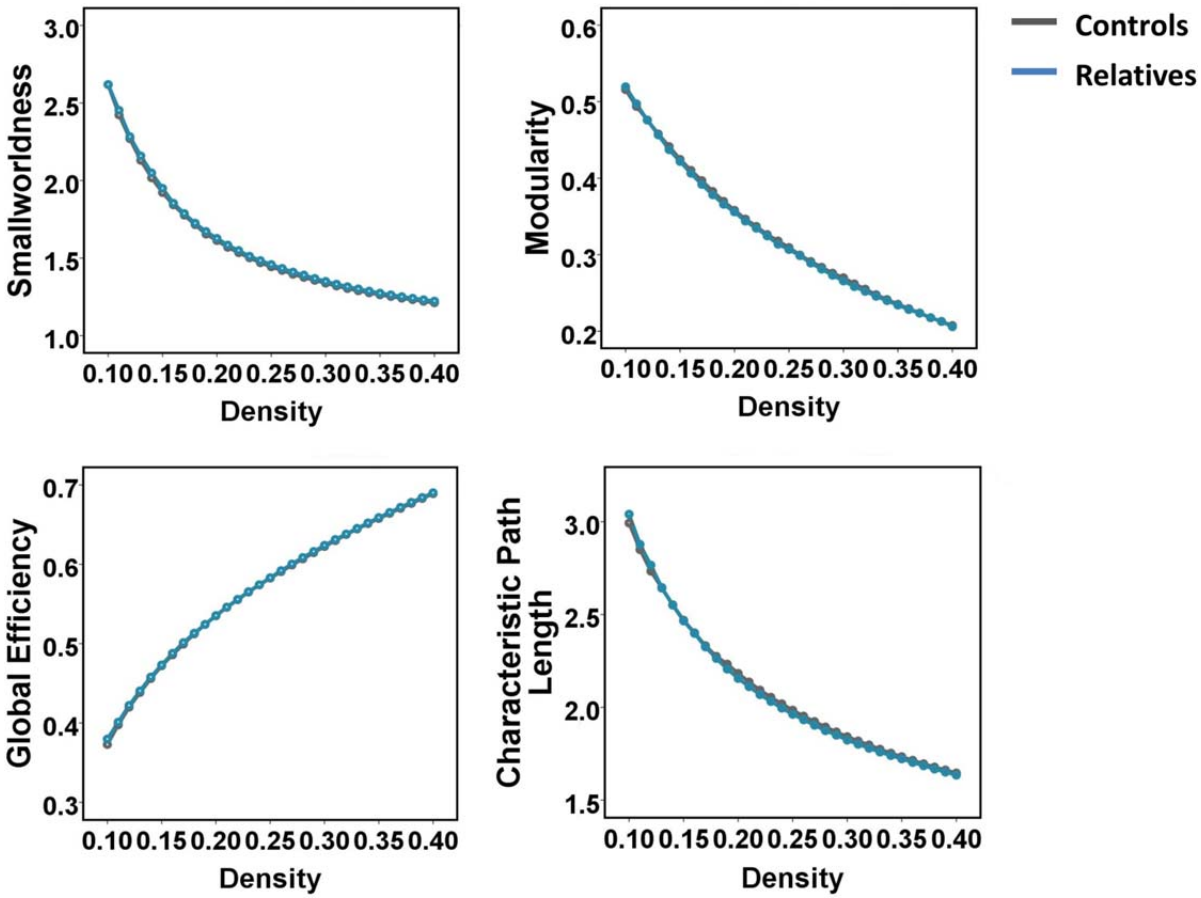
Global property analysis with the patient data

Considering that significant differences in global properties have been reported for schizophrenia in previous studies [22,24], it is interesting to examine whether such global effects are only seen in the patient group but not in the relatives group, which might have potential implications for the selection of global vs. local graph properties for schizophrenia intermediate phenotype studies. To address this question, we performed an additional analysis to test whether the results for global properties could be replicated in our patient-control sample. The graph analysis followed exactly the same procedure as described in the relatives study, and group differences of the 4 measured global properties were examined using the repeated measures ANCOVA model, with densities (0.1-0.4) as within-subject factor, groups (patients vs. controls) as between-subject factor, covarying for age, sex, site and frame-wise displacement. Our analysis identified significant group differences in 3 out of the 4 examined global properties: smallworldness ($P = 0.001$), modularity ($P = 0.001$), and global efficiency ($P = 0.028$). These findings are consistent with prior results suggesting that the brain networks of patients with schizophrenia are characterized by altered balance between network integration and segregation. Together with the negative findings in relatives data, these results suggest that altered global network properties are more related to clinical state rather than genetic risk of schizophrenia, and local properties (e.g. connectivity strength on which the NBS methods is based) rather than global properties reflect genetically associated intermediate phenotypes for schizophrenia.

Reanalysis of the relative-control sample with band-pass filtering

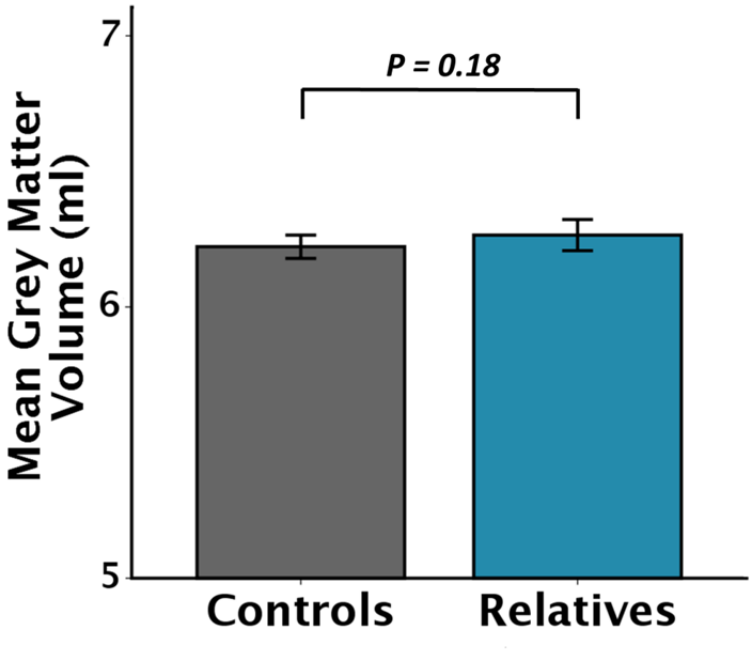
For the task specificity analysis, we used different temporal filtering for the processing of task-related and resting-state data. The choice of these filters was based on the literature as well as our previous studies [11,36-38]. However, in theory, it is possible that different filters may influence our results, rendering direct comparisons between the two paradigms difficult. To address this question, we performed an additional analysis, in which both the task and resting-state data of the relative-control sample were reprocessed with the same band-pass filter (cut-off frequency: 0.008-0.1 Hz). Following exactly the same procedures described above (except for a different filtering for the face-matching task), we extracted the mean connectivity measures of the identified subnetwork for each individual and then subjected them into a repeated-measures ANCOVA model. Similar to our previous findings, we detected a significant decrease of mean subnetwork connectivity in relatives during the face-matching task ($P = 3.69 \times 10^{-9}$) and a significant group by task interaction for the subnetwork connectivity measure ($P = 1.1 \times 10^{-5}$). These results suggest that the reported task specificity is unlikely to be driven by differences in the temporal filtering of data.

eFigure 1. Mean Global Network Properties for Relatives and Controls Across All Densities



No between-group differences have been found.

eFigure 2. Comparison of Mean Gray Matter Volumes for the Brain Nodes of the Identified Subnetwork



No significant difference between schizophrenia relatives and healthy controls was found. Error bars indicate standard errors.

eTable. Details of the Nodes and Links in the Identified Subnetwork During Emotion Processing

Connections between		Abbreviations		Initial uncorrected p values
Node A	Node B	Node A	Node B	
Left fusiform gyrus	Right thalamus	L.FUS	R.THA	0.0002
Left hippocampus	Left middle occipital gyrus	L.HIP	L.MOG	0.0001
	Left superior occipial gyrus		L.SOG	<0.0001
	Right calcarine sulcus		R.CAL	<0.0001
	Right cuneus		R.CUN	0.0002
	Right lingual gyrus		R.LIN	0.0001
	Right middle occipital gyrus		R.MOG	0.0002
	Right middle temporal gyrus		R.MTG	<0.0001
	Right superior occipital gyrus		R.SOG	<0.0001
	Right superior temporal gyrus		R.STG	0.0002
Left inferior parietal lobe	Left middle orbitofrontal gyrus	L.IPL	L.MOFG	0.0003
	Right inferior orbitofrontal gyrus		R.IOFG	0.0002
	Right middle orbitofrontal gyrus		R.MOFG	0.0003
Left insula	Right superior temporal gyrus	L.INS	R.STG	0.0004
	Right supramarginal gyrus		R.SMG	0.0003
Left middle occipital gyrus	Right hippocampus	L.MOG	R.HIP	0.0004
	Right middle temporal gyrus		R.MTG	0.0004
	Right inferior orbitofrontal gyrus		R.IOFG	0.0002
Left inferior orbitofrontal gyrus	Left pallidum	L.IOFG	L.PAL	0.0001
Left middle orbitofrontal gyrus	Left pallidum	L.MOFG	L.PAL	<0.0001
	Left thalamus		L.THA	0.0001
	Right pallidum		R.PAL	<0.0001
Left superior orbitofrontal gyrus	Left pallidum	L.SOFG	L.PAL	0.0003
	Right pallidum		R.PAL	0.0005
Left pallidum	Right inferior temporal	L.PAL	R.ITG	0.0002

	gyrus			
	Right olfactory cortex		R.OLF	0.0001
	Right inferior orbitofrontal gyrus		R.IOFG	<0.0001
	Right middle orbitofrontal gyrus		R.MOFG	<0.0001
	Right superior orbitofrontal gyrus		R.SOFG	<0.0001
	Right rectus gyrus		R.REC	0.0003
Left parahippocampal gyrus	Right mioddle temporal gyrus	L.PHP	R.MTG	0.0003
Left superior parietal lobe	Right inferior orbitofrontal gyrus	L.SPL	R.IOFG	0.0002
Left thalamus	Right inferior temporal gyrus	L.THA	R.ITG	0.0001
	Right inferior orbitofrontal gyrus		R.IOFG	0.0003
	Right middle orbitofrontal gyrus		R.MOFG	0.0004
Right amygdala	Right middle temporal gyrus	R.AMY	R.MTG	0.0003
Right calcarine sulcus	Right fusiform gyrus	R.CAL	R.FUS	0.0003
Right hippocampus	Right middle occipital gyrus	R.HIP	R.MOG	0.0002
	Right middle temporal gyrus		R.MTG	<0.0001
Right inferior temporal gyrus	Right middle temporal pole	R.ITG	R.MidTP	0.0003
	Right thalamus		R.THA	0.0005
Right lingual gyrus	Right inferior orbitofrontal gyrus	R.LIN	R.IOFG	0.0002
Right middle occipital gyrus	Right inferior orbitofrontal gyrus	R.MOG	R.IOFG	0.0002
Right olfactory cortex	Right pallidum	R.OLF	R.PAL	0.0001
Right inferior orbitofrontal gyrus	Right pallidum	R.IOFG	R.PAL	<0.0001
	Right superior occipital gyrus		R.SOG	0.0002
Right middle orbitofrontal gyrus	Right pallidum	R.MOFG	R.PAL	0.0003
Right superior orbitofrontal gyrus	Right pallidum	R.SOFG	R.PAL	0.0001
Right pallidum	Right rectus gyrus	R.PAL	R.REC	0.0001

All links show decreased connectivity in schizophrenia relatives compared to controls

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