Unaffected Family Members and Schizophrenia Patients Share Brain Structure Patterns: A High-Dimensional Pattern Classification Study

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Background: A number of studies have provided evidence for genetic modulation of brain structure in unaffected family members (FM) of schizophrenia patients using conventional volumetric analysis. High-dimensional pattern classification methods have been reported to have the capacity to determine subtle and spatially complex structural patterns that distinguish schizophrenia patients from healthy control subjects using standard magnetic resonance imaging. This study investigates whether such endophenotypic patterns are found in FM via similar image analysis approaches.

Methods: A high-dimensional pattern classifier was constructed from a group of 69 patients and 79 control subjects, via an analysis that identified a subtle and spatially complex pattern of reduced brain volumes. The constructed classifier was applied to examine brain structure of 30 FM.

Results: The classifier indicated that FM had highly overlapping structural profiles with those of patients. Moreover, an orbitofrontal region of relatively increased white matter was found to contribute significantly to the classification, indicating that white matter alterations, along with reductions of gray matter volumes, might be present in patients and unaffected FM.

Conclusions: These findings provide evidence that high-dimensional pattern classification can identify complex and subtle structural endophenotypes that are shared by probands and their unaffected FM.

Key Words: Classification, high-dimensional pattern, schizophrenia, structural MRI, unaffected family member

Magnetic resonance imaging (MRI) studies in unaffected family members (FM) of schizophrenia patients (SCZ) suggest that genetic factors affect brain structure (1–5), with FM generally showing characteristics of brain structure midway between patients and healthy control subjects (HC). Likewise, high-risk individuals such as patient offspring with no clinical history of psychosis, have similar structural abnormalities, most notably in hippocampus and amygdala, with some contradictory results (6–9) having been reported in the literature.

Although previous studies have provided evidence for genetic modulation of brain structure in FM, they have been limited in two ways. First, only measurements of a priori sets of selected brain regions of interest (ROI) were obtained, thereby potentially biasing the results toward a priori hypotheses. The ROI approach might also miss effects in brain regions where the preselected ROIs are not optimally defined to capture phenotypic characteristics of FM. Second, although most studies have reported group differences in some brain structures, there has been substantial overlap of brain volumes among FM, HC, and patients. This overlap prohibits identification of structural phenotypes in individuals. These features, in addition to the labor-intensive processing, limit the use of structural MRI to generate robust endophenotypic markers for use in large-scale genetic studies.

This study was aimed to overcome these limitations by employing an automated whole brain morphometric analysis approach using high-dimensional image warping methods. These methods have increasingly been applied to brain disorders to provide unbiased measures that probe the entire brain, rather than predefined ROIs (10–12). We added a high-dimensional pattern classification technique, which identifies subtle and spatially complex patterns of brain structure that are able to classify individuals with established specificity and sensitivity (10,13–15).

These methodologies were applied in our earlier studies (10,13–15) to a group of 69 SCZ, recruited from families without any other incidence of schizophrenia, and 79 HC. A pattern classifier was first trained to recognize distinct structural phenotypes of SCZ contrasted with HC. The classifier had sensitivity of 92.8% and specificity of 89.9% (14), determined via leave-one-out cross-validation. In our study, the classifier derived previously from SCZ and HC is applied to 30 unaffected FM, probing the presence of the schizophrenia-specific structural phenotype in FM. We hypothesized that FM would partially display the subtle and spatially complex pattern of structural brain abnormality that was characteristic of the patients.

Methods and Materials

Participants

The sample of SCZ and HC has been described previously (10). Briefly, 69 (46 men, 23 women) and 79 (41 men, 38 women) HC participated in the study. All were right-handed, and women were premenopausal. The groups did not differ sociodemographically in age (Mean ± SD, SCZ 29.9 ± 8.4, HC 28.2 ± 7.5 years) or parental education (SCZ 13.9 ± 3.8, HC 14.5 ± 3.5 years, both p values > .17). Participants were recruited and assessed by the Schizophrenia Research Center and underwent...
medical, neurologic, and psychiatric evaluations to exclude for history of illness affecting brain function such as substance abuse, hypertension, metabolic disorders, neurologic disorders, and head trauma with loss of consciousness (16). Of the 69 SCZ patients, 32 were first-episode neuroleptic-naïve and 37 were treated with antipsychotics; 13 patients were on first-generation neuroleptics, and 24 were taking second-generation medications at the time of study, of whom 11 were previously treated with first-generation agents.

Family members included 30 unaffected relatives (14 men, 16 women) of schizophrenia patients. Their average age was 36.8 ± 15.0 years and parental education was 15.1 ± 2.1 years. They consisted of 8 parents, 14 siblings and 8 uncles, aunts or cousins. FM were an entirely separate group from HC.

Healthy participants underwent the SCID-NP (17) and re-reported having no first-degree relative with schizophrenia or affective illness. The sample overlaps with samples reported in earlier publications (18–20). The current data set includes all SCZ patients, their family members, and demographically balanced HC who have been studied on the same scanner with the same protocol and who had suitable quality of scan for automated analysis of the entire supratentorial brain.

| Table 1. List of Brain Regions and Clusters with Volumes Jointly Comprising a Spatial Pattern of Brain Structure Found to Be Highly Specific to Schizophrenia Patients |
|---------------------------------------------------------------|---------------------------------------------------------------|
| **Females** | **Males** |
| Gray Matter | Medial aspect of left amygdala*  |
| Left lateral hippocampus* | Left anterior caudate*  |
| Left posterior hippocampus* | Right posterior and inferior occipital  |
| Right posterior occipitopetal temporal | Left and right posterior part of inferior temporal gyrus  |
| Left superior, middle, and inferior temporal gyri | Left insula  |
| Left insula | Left and right parietocipital fissure  |
| Left parietocipital fissure | Right parasagittal posterior cingulate  |
| Left parasagittal posterior cingulated | Inferior to right hippocampus (parahippocampal gyrus)  |
| White Matter | Right superior temporal gyrus, posterior part*  |
| Lateral to the right hippocampus | Right prefrontal  |
| Vicinity of the right amygdala and anterior hippocampus* | Inferior aspect of right occipital  |
| Right orbitofrontal* | Lateral aspect of corpus callosum and adjacent left frontal*  |
| Left prefrontal | Adjacent to left insula*  |
| Inferior aspect of the left occipital |  |
| Left postcentral gyrus* |  |
| Left superior parietal gyrus* |  |

The regions found in male subjects were roughly matched to respective anatomic structures found in female subjects, except regions denoted by *, which were unique to the respective groups.

**Imaging Protocol**

T1-weighted images were obtained on a GE Signa 1.5-Tesla scanner using spoiled gradient recoil pulse sequence (flip angle 35°, repetition time = 35 msec, echo time = 6 msec, field of view 24 cm, number of excitations = 1, 1-mm slice thickness, and no interslice gaps). In-plane resolution was .9375 × .9375 mm. No parenchymal lesions or skull abnormalities were evident neuro-radiologically.

**Image Analysis**

Images were preprocessed using the methods described in Goldszal et al. (21), which resulted in segmentations into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF). To compare structural patterns across individuals, we spatially transformed each segmented image into a common coordinate system (stereotaxic space). A mass-preserving framework was adopted (22) to ensure that the volumes of brain tissue were preserved during the transformation and to provide tissue density maps of GM, WM, and CSF for each individual that reflected the spatial distribution of these tissue volumes. For example, relatively lower GM tissue density in a brain region would indicate reduced tissue volume in that region.

| Table 2. t Tests Applied to the Abnormality Scores Obtained from Male and Female Schizophrenia Patients (SCZ), Family Members (FM), and Healthy Control Subjects (HC) |
|---------------------------------------------------------------|---------------------------------------------------------------|
| **A** | **B** | **C** |
| Female Subjects |  |  |
| FM vs. SCZ | .4477 | .77615 | .22385 |
| HC vs. FM | 8.4957e-009 | 1 | 4.2479e-009 |
| HC vs. SCZ | 3.7729e-009 | 1 | 1.8864e-009 |
| Male Subjects |  |  |
| FM vs. SCZ | .013857 | .0069287 | .99307 |
| HC vs. FM | 3.1414e-010 | 1 | 1.5707e-010 |
| HC vs. SCZ | 2.1316e-014 | 1 | 1.0658e-014 |

Significant differences were found between FM and HC and between SCZ and HC. A marginally significant difference was found between SCZ and FM, which disappeared after correction for multiple comparisons.

A, mean (X) ≠ mean (Y); B, mean (X) > mean (Y); C, mean (X) < mean (Y).
In this section, we summarize the methodology for constructing the SCZ-HC classifier, which was presented in detail in Fan et al. (14). We do this because this classifier yields a phenotypic “score” that reflects how similar an individual brain morphology is to either SCZ or HC, when applied to the FM of the current study. Patterns of the spatial distribution of GM, WM, and CSF volumes derived in the previous section were then examined with a pattern classification technique (14), and patterns specific to SCZ were determined. A feature extraction procedure (14) was first applied, which identified the minimal set of regional clusters that provided the best separation between SCZ and HC. A feature selection mechanism (14) was used to extract the most pertinent local clusters of GM, WM, and CSF volumes contributing to the discrimination between SCZ and HC groups. These were used to train a nonlinear support vector machine classifier, using a Gaussian kernel (13,23). The classifier produced an abnormality score: positive values indicated a structural pattern resembling SCZ, whereas negative values indicated brain structure in HC. A jackknife (leave-one-out) cross-validation was used to test the predictive power of this analysis on new data sets not involved in the selection of optimal brain clusters and training of the classifier. In this analysis, the scan of one participant was put aside, and the classifier was constructed from the scans of all other individuals. Thus the individual being classified was not included in the training data set for development of the classifier. This classifier was then applied to the left-out individual.

To form an image of the brain regions that constitute a pattern of brain tissue distribution characteristic of SCZ, we created a spatial map of brain regions for which volumes change fastest as one follows the path of the abnormality score from positive (SCZ-like) to negative (HC-like). These regions jointly form a pattern that optimally characterizes the differences between SCZ and HC from the perspective of classification. A value from 0 to 1 is determined for each region, reflecting its relative importance in classification. The classifier integrates volumetric measurements from a number of brain regions in a multivariate nonlinear statistical model that optimally separates SCZ from HC. Thus, it is possible to determine which brain regions contribute the most to the separation of the two groups, that is, which dimensions in the high-dimensional space of regional volumetric measurements have the highest discriminatory power. These brain regions collectively form a structural pattern (13,14).

**Testing the Presence of SCZ-like Phenotypes in FM**

The classifiers that optimally separated SCZ and HC were applied to the MRI scans of FM to test the hypothesis that SCZ-like structural phenotypes would be found in FM. Because we previously observed differences between the structural phenotypes of men and women (10), we performed this analysis separately for males and females, that is, we con-

![Figure 2](image2.png)  
**Figure 2.** Histograms of the MRI-based classification scores for the normal control subjects (HC), the schizophrenia patients (SCZ), and the family members (FM), for female and male participants separately. Family members display mostly positive scores, that is, their MRI scans indicate that they possess the structural phenotype characteristic of schizophrenia.

![Figure 3](image3.png)  
**Figure 3.** Histograms of the volume of the cluster indicated by the center of the crosshair: orbitofrontal white matter was relatively increased in both female patients and family members (FM), indicating that for this specific brain region, the schizophrenia (SCZ)-like structural phenotype involved increased brain volumes relative to healthy control subjects (HC). Images are in radiology convention.
structured two classifiers, one for males and one for females, then applied these classifiers to the corresponding subgroups of FM. The image analysis and classification process is summarized in Figure 1.

Whole-Brain Voxel-Based Volumetric Analysis

Regional volumetric differences between FM, HC, and SCZ were examined using voxel-based morphometric analysis (24), and after correction for multiple comparisons using the false discovery rate (FDR) method in the SPM software (25). This approach is “mass univariate” in that it tests GM and WM tissue volumes in many regions separately, in contrast to pattern classification, which forms a single multivariate nonlinear model out of the entire data set.

Results

Figure 2 shows the histograms of the scores obtained for the FM females and males, respectively, including the scores of SCZ and HC. Positive scores reflect presence of the SZC-specific structural pattern, and vice versa. Figure 2 indicates that the structural pattern that distinguishes SCZ from HC was found in most FM, especially males. The regions that were determined to form jointly the pattern that distinguished SCZ from HC included several clusters, summarized in Table 1. It is worth noting that one of the regions listed in Table 1, the right orbitofrontal WM, displayed relatively larger volumes in SCZ and FM, compared with HC (see Figure 3).

Lilliefors tests at the 5% level confirmed the normality of the distribution of these scores. Two sample $t$ tests on the abnormality scores of FM, SCZ, and HC yielded the $p$ values shown in Table 2. For the hypothesis testing of samples X versus Y, three alternative hypotheses are specified as follows: A, mean (X) $>$ mean (Y); B, mean (X) $<$ mean (Y); and C, mean (X) $\neq$ mean (Y). Significant differences were found between FM and HC and between SCZ and HC. A significant difference was found between SCZ and FM, which became marginal after correction for multiple (6) comparisons at the $p = .05$ level, and insignificant at the $p = .01$ level.

The results of the voxel-based evaluation of brain volumes is shown in Figures 4–6 and contrasted with the pattern classification results in Table 1. They indicate that the abnormal pattern detected by the classifier must have been subtle, because brain structure of FM appeared to have generally similar regional volumes as HC. In particular, Figure 4 shows the results of voxel-by-voxel evaluation of the tissue density GM and WM maps of male participants, without correction for multiple comparisons, and Figure 5 shows the FDR corrected maps for males. Although some trends were seen in Figure 4 (top row), indicating that HC $>$ FM, none of them was significant after FDR correction. In contrast, several regions of significant differences, with FM $>$ SCZ, survived FDR correction for multiple comparisons, as
Family members displayed classification scores that were mostly in the patient range, thereby indicating that the structural phenotype that distinguishes between SCZ and HC was present in most family members. Male FM displayed relatively higher abnormality scores, which might indicate a sex-specific relationship between genotype and phenotype in schizophrenia. To the extent that brain structural phenotypes portend risk for SCZ, the results also indicate that male FM might be at higher risk for developing the disease. These results support evidence for moderating effects of gender in SCZ (19,26). In future studies, however, potential sex-specific patterns must be examined in greater depth using larger samples.

The structural pattern characteristic of schizophrenia was subtle and spatially complex. It was distributed over several brain regions, as evaluated by the classifier (Table 1); had relatively small magnitude; and was identified by jointly considering volumetric measurements from all brain regions. Indeed, when evaluated by voxel-based analysis on a region-by-region basis, FM and HC had very similar brain structure, whereas FM differed from SCZ patients. Thus, the overall structural phenotype of FM would seem to be much closer to that of HC than of SCZ. However, the SCZ-specific structural pattern identified with high-dimensional nonlinear pattern classification was highly prevalent among FM, especially male subjects. This suggests that FM possess subtle structural characteristics of SCZ patients; however, the patients have additional abnormalities, which were highlighted by regional volumetric analysis. Future prospective studies are needed to examine whether the more extended structural abnormalities of the patients are found in early years, especially before disease onset, or whether they are a secondary effect of disease progression.

Notably, although SCZ patients had lower orbitofrontal GM than HC (10), when evaluated via voxel-based analysis of the tissue density maps, patients and FM had larger orbitofrontal WM compared with HC (male subjects showed only a trend in this direction at the $p = .08$ level). The orbitofrontal WM was the only brain region forming the SCZ-like structural pattern, in which increased volume was found in patients and FM compared with HC. Because there is some evidence that brain connectivity might be affected in schizophrenia (27), this finding is novel and merits further investigation. The lack of diffusion tensor images for these participants did not allow us to localize the WM changes on any specific fiber pathway. Our study is also unable to determine whether this WM abnormality is developmental and genetically determined or whether it relates to a compensatory biological response of increased axonal formation triggered by the reduced GM in the same region. Studies of younger populations at risk are necessary to further elucidate this finding.

The brain regions identified by region-by-region analysis (Figures 4–6) were somewhat different from the regions that formed the structural pattern of SCZ patients (Table 1) because the underlying methods have fundamental differences (28). Specifically, pattern classification determines the collection of regions that jointly achieves the optimal separation between patients and HC, whereas voxel-based analysis examines each region individually and determines whether the region differs among groups of interest. Therefore, it is possible that a brain region with a relatively small difference between two groups is important for classification. Indeed, a brain region could display no group differences and still be an important normalization factor and therefore valuable for classification and optimal group separation. Conversely, regions that might display significant group differences might not be important for classification if they correlate strongly with other regions that are already included in the classifier. Therefore, from a number of correlated measurements, it might be sufficient to sample only a subset to achieve optimal classification accuracy. The results of these two types of analysis should be interpreted with these caveats in mind. Overall, these results indicate that brain structure of FM was generally much more similar to HC than to SCZ when evaluated according to conventional voxel-based analysis, that is, region-by-region evaluation of brain volumes. With respect to the subtle and spatially distributed pattern that best and most consistently differentiated between HC and SCZ, however, FM resembled the patients rather than the HC. This has potentially important implications because it would argue in favor of the existence of subtle endophenotypic structural patterns in both SCZ and FM, which were identified by pattern classification. On the other hand, the relatively more extensive structural differences between SCZ and FM and between SCZ and HC may reflect changes that relate to the course of the disease. Future longitudinal studies are necessary to elucidate whether this endophenotypic pattern is present before disease onset, whether it is different in people who eventually develop the
disease, and to what extent it progresses throughout the course of illness.

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Figure 6. Voxel-based analysis of the tissue density maps for the female participants (left column show gray matter comparisons; right column shows white matter results). Uncorrected $p$ values are shown. Images are in radiology convention. Top row: group difference between healthy controls (HC) and family members (FM). The yellow-red scale indicates that HC > FM, and the blue-green scale indicates HC < FM. Bottom row: The yellow-red scale indicates that FM > schizophrenia patients (SCZ), and the blue-green scale indicates that FM < SCZ.