

ARCHIVAL REPORT

Heritability of Subcortical and Limbic Brain Volume and Shape in Multiplex-Multigenerational Families with Schizophrenia

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Background: Brain abnormalities of subcortical and limbic nuclei are common in patients with schizophrenia, and variation in these structures is considered a putative endophenotype for the disorder. Multiplex-multigenerational families with schizophrenia provide an opportunity to investigate the impact of shared genetic ancestry, but these families have not been previously examined to study structural brain abnormalities. We estimate the heritability of subcortical and hippocampal brain volumes in multiplex-multigenerational families and the heritability of subregions using advanced shape analysis.

Methods: The study comprised 439 participants from two sites who underwent 3T structural magnetic resonance imaging. The participants included 190 European-Americans from 32 multiplex-multigenerational families with schizophrenia and 249 healthy comparison subjects. Subcortical and hippocampal volume and shape were measured in 14 brain structures. Heritability was estimated for volume and shape.

Results: Volume and shape were heritable in families. Estimates of heritability in subcortical and limbic volumes ranged from .45 in the right hippocampus to .84 in the left putamen. The shape of these structures was heritable (range, .40–.49), and specific subregional shape estimates of heritability tended to exceed heritability estimates of volume alone.

Conclusions: These results demonstrate that volume and shape of subcortical and limbic brain structures are potential endophenotypic markers in schizophrenia. The specificity obtained using shape analysis may improve selection of imaging phenotypes that better reflect the underlying neurobiology. Our findings can aid in the identification of specific genetic targets that affect brain structure and function in schizophrenia.

Key Words: Endophenotypes, heritability, hippocampus, neuroimaging-genetics, schizophrenia, structural MRI

The identification of genetic variants that influence brain structure (1–3) is an important step in elucidating the biological mechanisms underlying neuropsychiatric disorders. Structural brain abnormalities are common in patients with schizophrenia (4–6), and variation in regional brain volume is considered a putative endophenotype for the disorder (7–9). Reduced gray matter volume associated with schizophrenia is present before illness onset and is heritable (8,10). Consistent with the endophenotype concept (11), unaffected first-degree relatives of patients with schizophrenia also exhibit reduced regional brain volume compared with healthy control subjects, but of a lesser extent than that observed in patients (12–16).

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There is evidence that quantitative brain measurements, such as volume and cortical thickness, are heritable, as shown in healthy (17–22) and neuropsychiatric samples (8,10,23–25). However, little is known about specific genetic targets underlying structural brain variation in schizophrenia. The gap may relate to the heterogeneity of the disorder (26) or the morphometric abnormalities (4,7,27). Because heritability differs across brain structures (22), it is possible that particular regions or subregions would show greater heritability (28). Neuroimaging studies in healthy individuals (17,29–31) and in large extended pedigrees (22) show a substantial range of heritability estimates across brain structures (22); this pattern also extends to subcortical brain regions and hippocampus (17,32). These findings suggest that some brain structures and measures are more heritable than others and may serve as better endophenotypes. The few studies supporting heritability of brain volume in patients with schizophrenia employed twin pairs (33) or mostly nuclear families (24). No previous study has examined heritability of brain structures in large extended families affected with schizophrenia. In the present study, we evaluate the influence of shared genetic ancestry on brain structure within large, multiplex-multigenerational families with schizophrenia.

In this study, we focus on subcortical and limbic brain structures, which are heritable in healthy (17,29,31,34) and clinical populations (35,36). These regions show consistent volumetric reductions in patients with schizophrenia (4,37–43) and to some extent in family members (13,44–47). More recently, morphometric changes in schizophrenia have been scrutinized further using shape analysis (13,39,43,44,48,49), which allows for the estimation of disease-related regional deformation. This complex approach is a reliable (48,50–53) and sensitive measure (54) of subtle, localized morphologic changes in brain structure in patients with

Table 1. Sample Characteristics for Sample as a Whole and at Each Study Site

Group	Site	Sample Size	Sex (M/F)	Age (Years)	GAF ^a	SANS ^b	SAPS ^c
SZ	All	33	23/10	52 (11) ^d	50 (16) ^{d,e}	34 (21) ^{d,e}	31 (25) ^{d,e}
	Penn	20	14/6	54 (9)	46 (17)	41 (18)	36 (27)
	Pitt	13	9/4	49 (12)	56 (11)	18 (17)	18 (15)
FAM	All	153	74/79	43 (18)	82 (14) ^d	9 (13) ^d	1 (4) ^d
	Penn	75	41/34	41 (19)	78 (14)	12 (14)	2 (5)
	Pitt	78	33/45	45 (17)	88 (12)	3 (9)	1 (3)
HC	All	246	115/131	39 (16)	90 (11)	3 (7)	0 (1)
	Penn	125	57/68	40 (16)	87 (8)	6 (9)	0 (1)
	Pitt	121	58/63	39 (16)	93 (13)	1 (1)	0 (1)

Values are mean (SD).

F, females; FAM, multiplex family members; GAF, Global Assessment of Functioning; HC, healthy comparison subjects; M, males; SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Positive Symptoms; SZ, patients with schizophrenia.

^aTests based on sample of 222, HC; 30, SZ; 129, FAM.

^bTests based on sample of 222, HC; 29, SZ; 127, FAM.

^cTests based on sample of 222, HC; 29, SZ; 127, FAM.

^dSignificantly different from HC; permutation tests, 100,000 permutations, $p < .01$.

^eDifferent from SZ; permutation tests, 100,000 permutations, $p < .01$.

schizophrenia (27,39,43,49) and, to a lesser extent, in family members (13,44). Such localized alterations may be related to distinctive dimensions of psychopathology and may be determined by specific genetic risk factors that are unique to subsets of patients with schizophrenia or particular families (13,28,55). The specificity obtained using shape analysis may improve the selection of imaging phenotypes that are closer to schizophrenia pathophysiology and that may be affected by risk gene variants.

In this study, we estimate heritability of subcortical and limbic brain regions in multiplex-multigenerational families and healthy comparison subjects. We focus on estimating heritability of 1) volume of subcortical and limbic brain regions, including the amygdala, caudate, hippocampus, accumbens, pallidum, putamen, and thalamus, and 2) the local deformation patterns of these brain structures.

Methods and Materials

Participants

The sample consisted of 439 participants from two sites (223 from University of Pennsylvania, 216 from University of Pittsburgh),

including 190 European-Americans from 32 multiplex-multigenerational families with schizophrenia and 249 healthy volunteers (Table 1). This cohort is a subsample of a previously characterized cohort (56,57) with the addition of new family members. Patients had an extended multigenerational family and a consensus best-estimate DSM-IV diagnosis of schizophrenia or schizoaffective disorder. An example pedigree is shown in Figure 1. Participants were >15 years old at initial contact and provided signed informed consent. The institutional review boards of the University of Pennsylvania and University of Pittsburgh approved the study. For minors <18 years old, assent was obtained from the child, and consent was obtained from a parent. These data were collected as part of a larger project examining genetic mechanisms of schizophrenia. To reduce genetic heterogeneity, the sample was restricted to Caucasian individuals.

Patients with schizophrenia were competent to provide informed consent, capable to participate, and not exhibiting acute positive symptoms that required medication adjustment or hospitalization. Medications included second-generation antipsychotics in 23 patients, first-generation antipsychotics in 2 patients, and a combination of first-generation and second-generation antipsychotics in

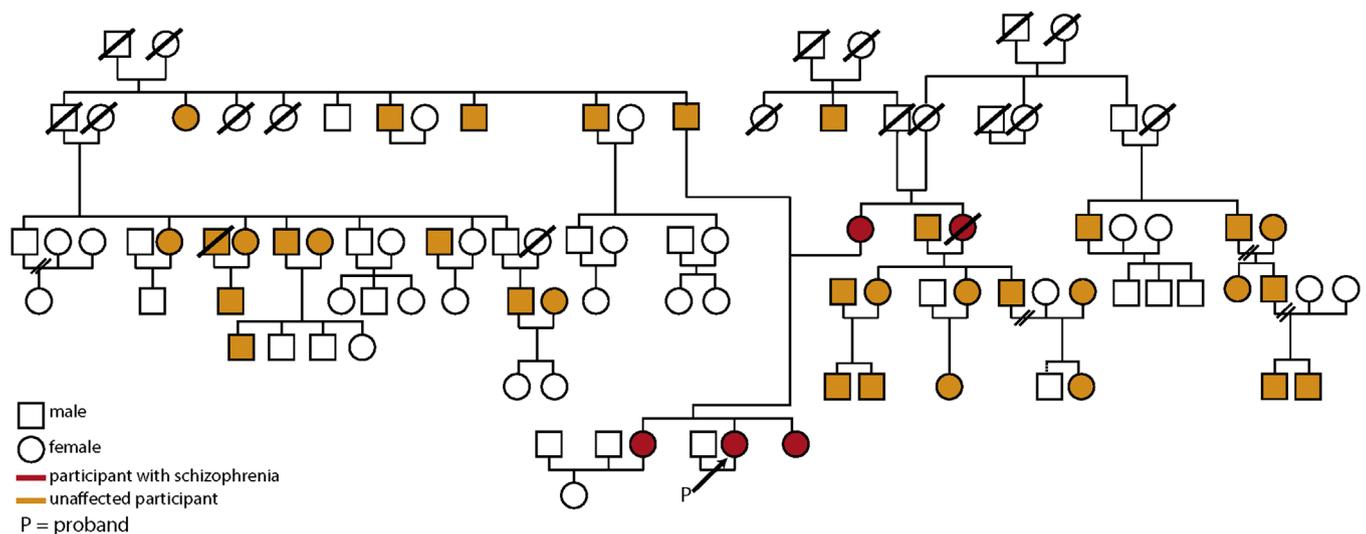


Figure 1. Example pedigree of a multiplex-multigenerational family with schizophrenia. This pedigree consists of 99 identified family members, 38 of whom were enrolled in this study, and 14 were eligible and completed structural magnetic resonance imaging.

4 patients. One individual was not medicated, and medication information was unavailable for one other patient. Family members were excluded if they had mental retardation (IQ <70), had a central nervous system disorder that could potentially affect brain function, or were not proficient in English. Global functioning was measured using the Global Assessment of Functioning (58) with higher scores indicating better functioning. The Scale for the Assessment of Negative Symptoms (59) was used to rate the presence and severity of negative symptoms, and the Scale for the Assessment of Positive Symptoms (60) was used to rate the presence and severity of positive symptoms. At least one patient with schizophrenia and at least one family member (135 individuals) were provided to the sample by 21 families, only patients (3 individuals) were provided by 2 families, and only family members (52 individuals) were provided by 9 families. Overall, the multiplex sample included 33 patients with schizophrenia and 156 family members. There is a higher prevalence of mood (~26% vs. ~10%) (61) and substance-related disorders (~15% vs. ~6%) compared with the general population (62) (Table 2).

The healthy comparison group included 249 psychiatrically, medically, and neurologically healthy European-Americans with no Axis I or Axis II cluster A disorders and no history of psychosis or mood disorder in first-degree relatives. Healthy comparison subjects were recruited from the same communities as patients and families and underwent urine drug testing to rule out current substance use. There were no related individuals in the comparison group; this randomly sampled group was included in analyses to estimate normal shape of subcortical brain structures and improve the accuracy of the statistical model used to estimate familial variance. Because of the lack of family inclusion within the controls, no comparisons of heritability between multiplex families and the comparison group were performed.

Demographic and clinical information for subjects who passed imaging quality control analysis (see later) is provided in Table 1. Permutation tests (100,000 permutations) were used to assess pairwise group differences in age and scores for Global Assessment of Functioning, Scale for the Assessment of Negative Symptoms, and Scale for the Assessment of Positive Symptoms. Permutation tests were used in place of *t* tests because the data were not normally distributed based on quantile-quantile plots and the Shapiro-Wilk test for normality.

Image Acquisition

A 5-minute magnetization-prepared rapid acquisition gradient echo T1-weighted image (repetition time = 1680 msec; echo time

Table 2. Prevalence Rates of Axis I and II Disorders in Multiplex-Multi-generational Family Members

Family Members	Current	Percent	
		Current	Past
Mood Disorders	40	26.14%	9
Substance-Related Disorders	24	15.68%	8
Other	12	7.84%	0
None	77	50.98%	0

Axis I and II diagnoses were grouped into the following subcategories: mood disorders, substance-related disorders, other, and none. Mood disorders were the most prevalent followed by substance-related disorders. Substance-related disorders included abuse of alcohol, cannabis, and opioids. Other disorders included attention-deficit/hyperactivity disorder, bereavement, intermittent explosive disorder, brief psychotic disorder, delusional disorder, and paranoia-delusional disorder.

= 4.67 msec; field of view = 180 mm × 240 mm; matrix size = 192 × 256; flip angle = 15 degrees; effective voxel resolution = .94 mm × .94 mm × 1 mm) was acquired as part of a larger imaging protocol. Data were acquired with 3.0T Siemens Tim Trio magnetic resonance imaging systems (Siemens Medical Solutions, Erlangen, Germany) at both sites. Radiofrequency transmission used a quadrature body coil, and reception used an eight-channel head coil. Every effort was made to minimize potential differences between sites by using identical scanners, head coils, and acquisition protocols. In addition, all data were checked for quality assurance, and site was accounted for in the analyses. The results of a pilot study demonstrated good comparability between the two imaging sites in both image quality and functional activation. Image signal-to-noise ratio varied more between subjects than between sites as in a previous study (63).

Image Analysis

Subcortical Volumetric Analysis. Structural images were segmented, and vertex meshes were created within FSL v4.1.7 (FMRIB Software Library; www.fmrib.ox.ac.uk/fsl) (64–66) using the FIRST subcortical segmentation procedure (53). FIRST segments 15 regions, including the brainstem, bilateral nucleus accumbens, amygdala, caudate, hippocampus, pallidum, putamen, and thalamus. The brainstem was not considered for analysis. The segmented subcortical regions of interest (ROIs) were corrected for intracranial volume (ICV) using FSL SIENA/X procedure. In addition, outlier detection (>2.5 SD) was performed for uncorrected brain volumes, ICV-corrected brain volumes, and laterality ($LAT = 2 * [L - R] / [L + R]$) in each FIRST region. These parameters were selected to flag observations that may have had poor subcortical segmentation or ICV estimation. An expert analyst (SNV) visually inspected flagged regions for final determination of inclusion or exclusion. Seven subjects (three healthy comparison subjects, one patient with schizophrenia, three multiplex family members) with poor imaging data quality failed inspection on >5 ROIs and were excluded from analysis. The final sample size for each ROI is detailed in Table S1 in Supplement 1. False discovery rate correction was used to control for multiple comparisons in all analyses of the 14 subcortical volumes.

To ensure comparability across the two acquisition sites, data from three human phantoms were acquired at the inception of the study. The same structural (magnetization-prepared rapid acquisition gradient echo) scan was collected at both sites, and these data were analyzed according to the description in the Methods section. Overall, we found moderate to high intraclass correlations across brain structure (Table 3) indicating high reliability of measurement and analysis within this study.

Subcortical Shape Analysis. Changes in local regional shape of all structures were measured using FSL vertex analysis utility in FSL v5.0.0. Briefly, during FIRST segmentation, a mesh was created for each subject that comprising a net of points (vertices) in three dimensions that described the shape of each ROI. For each ROI, averaging the location of each vertex across all subjects was performed to generate a mean shape mesh. To measure the overall shape difference in each ROI quantitatively, a distance (in millimeters) from the average shape mesh was calculated at each vertex using only data from healthy individuals. Distance was calculated in the direction perpendicular to the surface of the average shape mesh, which indicated whether a vertex was inward (e.g., smaller) or outward (e.g., larger) relative to the average. Measured distances for each subject were projected onto a mean shape image template for analysis. All vertex

Table 3. Intraclass Correlation Coefficients and 95% Confidence Intervals for Each Subcortical Region in Three Human Phantoms

Region	Hemisphere	ICC	Lower CI	Higher CI
Accumbens	L	.86	-.10	1.00
	R	.60	-.60	.99
Amygdala	L	.52	-.67	.98
	R	.60	-.60	.99
Caudate	L	.99	.84	1.00
	R	.99	.93	1.00
Hippocampus	L	.90	.09	1.00
	R	.83	-.20	1.00
Pallidum	L	.97	.63	1.00
	R	.95	.38	1.00
Putamen	L	.91	.15	1.00
	R	.94	.38	1.00
Thalamus	L	.96	.54	1.00
	R	.89	.02	1.00

The same structural scan was collected at each site for each participant.

CI, confidence interval; ICC, intraclass correlation coefficient; L, left; R, right.

analyses were conducted in standard space (Montreal Neurological Institute) to control for between-subject differences in ICV.

Heritability Analysis. Heritability estimates were generated for brain volumes and shapes similar to previous studies (22,67). Briefly, standard maximum likelihood variance component methods (68–71) were implemented in the Sequential Oligogenic Linkage Analysis Routines (Department of Genetics, Texas Biomedical Research Institute, San Antonio, Texas). Covariance among family members was modeled as a function of additive genetic effects, with this variance component structured by a kinship matrix, and heritability was estimated as the ratio of the additive genetic variance to the total phenotypic variance. Likelihood ratio tests were used to compare models in which the additive genetic component was estimated versus constrained to zero. Each individual's volume (or shape) was modeled as a function of measured covariates (specifically, age, sex, and site), additive genetic effects estimated from correlations among family members, and individual-specific residual environmental factors. For the shape analysis, heritability was estimated at each voxel, and corrected *p* values were generated using false discovery rate. Finally, all statistical images were projected onto the group average template using nearest neighbor interpolation in R statistical software (72) allowing for visualization in KVMeshvisu software (73).

Results

Volumetric Analysis

Significant heritability (h^2) of subcortical and limbic volumes among families was present (Figure 2). Heritability was significant in 12 of the 14 volumes (Figure 2, inset), including the bilateral accumbens, caudate, hippocampus, pallidum, putamen, and thalamus. Estimates of heritability did not reach significance in bilateral amygdala. We also estimated heritability of ICV. The heritability of normalized ICV is .36, whereas the heritability of raw ICV was .68. These data indicate that ICV is heritable and is not accounted for through normalization. However, all measures reported are corrected for ICV, and the estimates of heritability of these regions should not be unduly influenced by ICV.

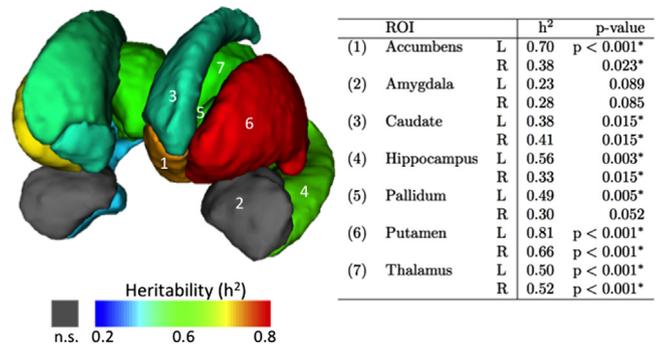


Figure 2. Three-dimensional reconstruction of subcortical brain structures. Most, but not all, subcortical volumes were heritable in multiplex-multigenerational families. Individual regions are color-coded according to heritability estimates presented in the accompanying table. Maps shown are false discovery rate corrected thresholded at $p < .05$. L, left; n.s., not significant; R, right; ROI, region of interest. * $p < .05$, FDR-corrected.

Comparison of subcortical and limbic volumes among patients, family members, and healthy comparison subjects is provided in Table 4. There were significant group differences for bilateral accumbens, caudate, hippocampus, putamen, and thalamus but not for amygdala or pallidum. In general, patients with schizophrenia had smaller volumes compared with family members and healthy comparison subjects. As a whole, multiplex family members were similar to healthy comparison subjects; however, when comparisons were limited to first-degree family members, an intermediate pattern emerged in several regions (putamen, caudate, and hippocampus) (Table 4). Overall, in subcortical and limbic regions, there is significant genetic contribution of volume among multiplex-multigenerational families with schizophrenia. Many of these regions with high familial heritability frequently have been found to be abnormal in patients with schizophrenia, including the bilateral caudate, hippocampus, and thalamus (4).

Vertex Analysis

Shape analyses were performed on the subcortical and limbic surfaces to identify specific loci that may be heritable. Subregions of bilateral accumbens, amygdala, caudate, putamen, and thalamus and portions of left hippocampus and left pallidum were found to be heritable (Figure 2). Specific subregional estimates of heritability tended to exceed heritability estimates of volume alone. For example, heritability of a subregion of right ventral amygdala was .76 (Figure 3B) compared with .28 (Figure 2, table) for the entirety of right amygdala volume. Cumulatively, significant heritability was found across the surface of each structure. These effects ranged from 3% of right amygdala to 97% of right thalamus (Table 5). These data indicate that distinct local shape patterns of subcortical brain structures are heritable in multiplex-multigenerational families.

Discussion

The current study estimated heritability in both volume and shape of subcortical and limbic brain structures in multiplex-multigenerational families affected with schizophrenia. Heritability estimates for most brain volumes were moderate to high. The largest heritability estimates of brain volume were observed in bilateral putamen and left nucleus accumbens. Estimates of heritability of shape were moderate, but substantial extents of surface shape of most subcortical and limbic regions were found

Table 4. Comparison of Subcortical and Hippocampal Volumes Across Diagnostic Groups

Region	Hemisphere	SZ	FAM	HC	F Test <i>p</i> Value	Comparisons (<i>p</i> Value)
Accumbens	L	537 (28)	631 (13)	641 (10)	1.4×10^{-5}	SZ < FAM (.006) ^a SZ < HC (.001) ^a FAM = HC (.80)
	R	437 (26)	550 (12)	544 (9)	2.1×10^{-7}	SZ < FAM (< .001) ^a SZ < HC (< .001) ^a FAM = HC (.93)
Amygdala	L	1554 (44)	1578 (20)	1588 (16)	.89	SZ < FAM (.87) SZ < HC (.74) FAM = HC (.92)
	R	1552 (55)	1587 (26)	1549 (20)	.54	SZ < FAM (.83) SZ < HC (.99) FAM = HC (.47)
Caudate	L	4316 (82)	4658 (37)	4709 (29)	2.4×10^{-10}	SZ < FAM (< .001) ^a SZ < HC (< .001) ^a FAM = HC (.52)
	R	4378 (84)	4678 (39)	4754 (30)	7.5×10^{-11}	SZ < FAM (.003) ^a SZ < HC (< .001) ^a FAM = HC (.27) ^b
Hippocampus	L	4272 (97)	4618 (43)	4658 (34)	1.2×10^{-5}	SZ < FAM (.003) ^a SZ < HC (< .001) ^a FAM = HC (.74)
	R	4297 (97)	4649 (45)	4757 (35)	2.7×10^{-6}	SZ < FAM (.003) ^a SZ < HC (< .001) ^a FAM = HC (.14) ^c
Pallidum	L	2244 (43)	2288 (19)	2263 (15)	.52	SZ < FAM (.62) SZ < HC (.90) FAM = HC (.58)
	R	2245 (43)	2299 (19)	2284 (15)	.51	SZ < FAM (.48) SZ < HC (.66) FAM = HC (.82)
Putamen	L	5898 (113)	6314 (52)	6335 (41)	2.1×10^{-7}	SZ < FAM (.002) ^a SZ < HC (< .001) ^a FAM = HC (.94)
	R	6028 (99)	6232 (46)	6352 (36)	2.1×10^{-7}	SZ < FAM (.15) SZ < HC (< .001) ^a FAM = HC (.09) ^b
Thalamus	L	9961 (133)	10,641 (60)	10,659 (47)	5.6×10^{-12}	SZ < FAM (< .001) ^a SZ < HC (< .001) ^a FAM = HC (.97)
	R	9731 (126)	10,391 (58)	10,410 (45)	1.0×10^{-12}	SZ < FAM (< .001) ^a SZ < HC (< .001) ^a FAM = HC (.96)

Number of individuals included for each regional analysis can be found in [Table S1](#) in [Supplement 1](#).

FAM, multiplex family members; HC, healthy comparison subjects; L, left; R, right; SZ, patients with schizophrenia.

^a*p* < .05.

^bFirst-degree family members significantly smaller than HC.

^cFirst-degree family members nominally smaller than HC (*p* = .06).

to be significantly heritable, with high heritability estimates in focal subfields of each region. Specific subregional estimates of heritability exceed heritability estimates of volume alone in some instances. Overall, these data confirm previous reports that subcortical regional volumes are heritable. Our results show, for the first time, heritability of localized subfields within these subcortical structures. These data add to more recent findings using healthy populations that subcortical brain volume and shape may aid in the selection of imaging endophenotypes associated with genetic variants underlying structural brain abnormalities in patients with schizophrenia.

Our volumetric findings confirm and extend previous work. Patients with schizophrenia had smaller volumes than family

members or healthy comparison subjects. Compared with other studies in healthy individuals (29,30,74), we report similar heritability, albeit slightly lower, in subcortical brain volumes using a cohort of affected patients with schizophrenia and their relatives. Specifically, our heritability estimates in most brain regions, including the hippocampus (23,34), caudate (17,30), putamen (29,31,34), pallidum (29,31), and thalamus (29,31) (but see Wright *et al.* (34)) align with previous findings. Our pattern of heritability estimates in the accumbens (higher h^2 in left accumbens compared with right accumbens) is consistent with one previous report (29). We also report no significant heritability in amygdala volume, which is at odds with a prior finding of significant heritability in this region (29). These discrepancies may be due to

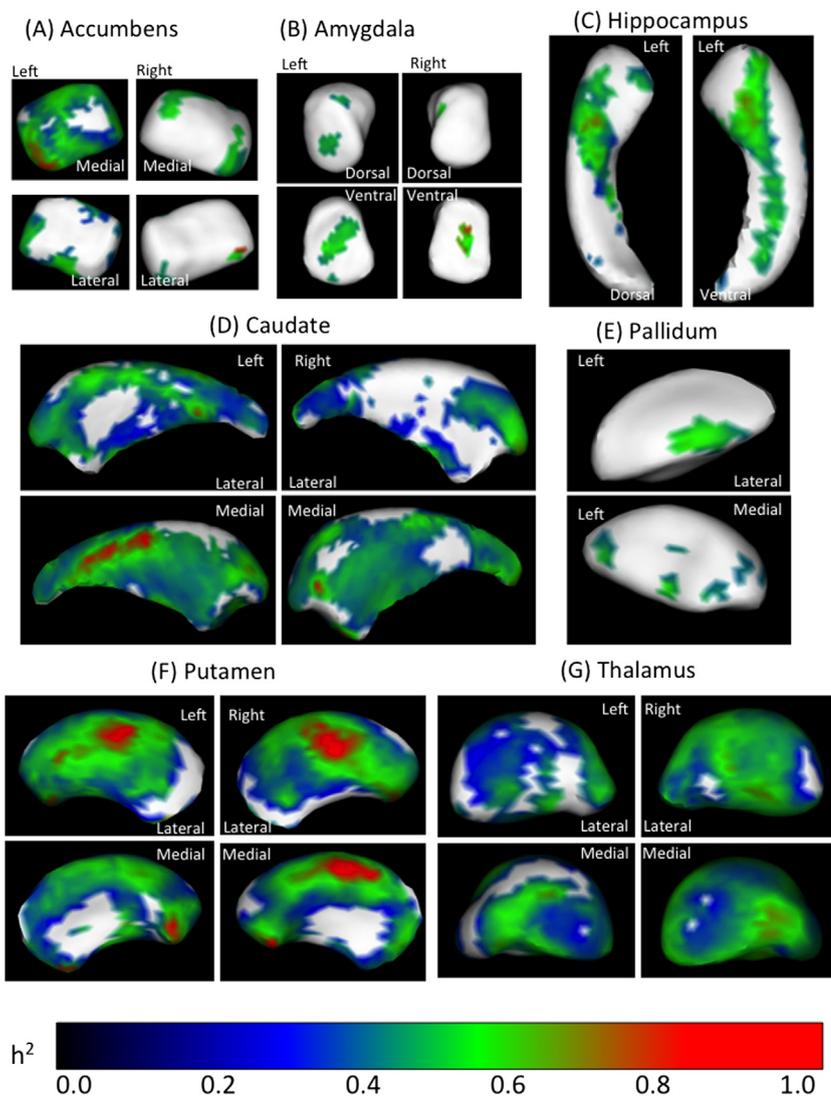


Figure 3. (A–G) Estimates of heritability in subcortical shape. Varying extents of the bilateral accumbens (A), amygdala (B), caudate (D), putamen (F), and thalamus (G) and portions of left hippocampus (C) and left pallidum (E) were found to be significantly heritable. Many of these subfields have high heritability estimates ($h^2 > .8$). Subcortical volumes that were not heritable (e.g., amygdala) do have focal subfields that are heritable based on shape analysis. Maps shown are false discovery rate corrected thresholded at $p < .05$

Table 5. Heritability of Subcortical Shape in Multiplex-Multigenerational Families with Schizophrenia

Region	Hemisphere	Heritability	% Surface Heritable
Accumbens	L	.48 (.27–.75)	61
	R	.44 (.31–.75)	12
Amygdala	L	.48 (.38–.59)	11
	R	.62 (.52–.76)	3
Caudate	L	.45 (.21–1.00)	78
	R	.43 (.20–.80)	61
Hippocampus	L	.49 (.28–.72)	28
	R	.42 (.22–.82)	64
Pallidum	L	.46 (.35–.66)	10
	R	NS	NS
Putamen	L	.47 (.20–1.00)	83
	R	.49 (.20–1.00)	82
Thalamus	L	.41 (.21–.69)	76
	R	.48 (.21–.70)	97

All results are false discovery rate corrected and thresholded at $p < .05$. Heritability indicates mean (minimum-maximum) heritability in the thresholded surfaces after controlling for age and site; % Surface Heritable indicates the percent of the subcortical surface that has significant heritability. L, left; NS, not significant; R, right.

differences in the technical approach, such as image acquisition parameters, bias in regional partitioning (17,32) or processing software (75), sample composition (e.g., age, family size), or the ascertainment strategy employed.

The few studies estimating heritability of brain volume in patients with schizophrenia were encouraging, yet most employed twin pairs (33) or were limited to nuclear families (24). Our findings in large extended pedigrees of families affected with schizophrenia corroborate previous work and further solidify subcortical volumes as meaningful endophenotypes. Our heritability estimates of brain volume in multiplex families with schizophrenia are lower compared with den Braber *et al.* (17), the only study to estimate heritability of subcortical and limbic structures in a healthy sample. However, our use of multiplex-multigenerational families is likely to represent a more homogeneous and targeted group of instrumental genes and pathways (76) compared with studies of unrelated individuals (76). As previously discussed (56), it is unlikely that heritability estimates in extended pedigrees are inflated by shared environment; if that were the case, each rung on the family tree would require a fixed proportional decrease in shared environment for it to mimic the genetic heritability estimated here, which is unlikely.

Significant heritability of subregional shape deformations in subcortical and limbic brain structures establishes for the first time the shape of these structures as promising endophenotypic markers of schizophrenia. Despite the absence of heritability in some subcortical volumes (e.g., amygdala), we found significant heritability subregionally. Measurement of subregional variation with shape analysis provides a more sensitive method to detect subtle abnormalities compared with traditional volumetric methods (13,54,77). Local shape may imply volumetric change within that specific area, but it also suggests differences deeper in a given structure. Systematic investigation of subcortical shape may provide insight into the underlying genetics of microscopic cytoarchitecture; for example, changes in shape may indicate neurodevelopmental changes in parenchymal volume or physiologic compensation secondary to variations in activity (55). Shape deformations may provide significant information about neurodevelopmental abnormalities seen in patients with schizophrenia and family members (13,44). Previous work suggests that physical tension during neurodevelopment may lead to specific, localized structural abnormalities (78,79). Additionally, the onset of schizophrenia typically occurs during a critical period of dynamic, progressive gray matter reductions (80); this may lead to localized changes within gray matter structures and possibly affect cognition. It is also possible that subregions with high heritability may help differentiate families with high burden of illness from families with a lower burden. Epidemiologic work suggests that schizophrenia is associated with numerous individually rare mutations that likely differ among families (81–83). If unique genes are responsible for illness among families, the phenotypes (e.g., brain volume or shape) may differ enough such that pooling results across families would preclude identification of “common” abnormalities. A previous study of patients and unaffected family members reported that family explained ~10% of the variance in hippocampus volume (23). It is possible that particular families contribute more than others to these markers, and ongoing work in these multiplex-multigenerational families is aimed at identifying endophenotypes that result from rare alleles with large effect, which is another specific advantage of using large extended pedigrees (82,83). In general, it appears that localized shape of subcortical structures in large families may be useful as endophenotypic markers of illness in frank schizophrenia. We have focused on subcortical and limbic structures; future work is required to assess heritability of cortical structures, including cortical thinning (22), which may represent another informative endophenotype in schizophrenia.

It is likely that the multiplex-multigenerational aspects (e.g., mixture of relationships within and across multiple generations and the unequal numbers of observations per family) of this study add to the specificity of calculated heritability (84). It may result in preferential selection of patients and families with less genetic loading for pathologic endophenotypic values, which may decrease the value, but not reduce the significance, of our heritability estimates. The inclusion of affected individuals likely affects our heritability results given known changes in brain structure in patients with schizophrenia (4). It is possible that lower heritability compared with the findings of den Braber *et al.* (17) reflects disease-specific differences in the variation of brain volumes in and across families affected with schizophrenia. In addition, the effect of environmental biases such as antipsychotic medication introduces nongenetic variation and reduces our estimate of heritability (4). Our sample is a specifically selected subpopulation that likely has lower genetic variability than the population at large, and this restricted range may affect estimates

of heritability. However, we find significant heritability in many, but not all, brain regions, which likely speaks to the heightened liability that schizophrenia has on regional brain volume in multiplex families. Other factors, such as sample size, age, degree of relationship, and even measurement type (1.5T vs. 3T data acquisition), may affect comparison across studies. Given the similar patterns observed in our data and in the data presented by den Braber *et al.* (17), we believe that our estimates of heritability are appropriate and useful. The heritability estimates presented may provide more specific, relevant targets for genomic studies of brain development in patients with schizophrenia.

The present study has some limitations. Our use of multiplex family members provides a unique perspective on subcortical volume and shape, but these data may not translate to simplex families because multiplex families may have higher incidences of other Axis I or Axis II disorders. Although we did not consider other psychiatric diagnoses, future studies are aimed at using a dimensional approach to assess phenotypic heritability. Our sample has a higher prevalence of mood and substance-related disorders compared with the general population, but we did not observe any effect on volumetric measures. However, the numbers here are small, particularly if family is considered as an additional factor, making it difficult to interpret the influence of Axis I and Axis II disorders on brain volume in multiplex families. The older age and inclusion of only Caucasian individuals makes broad generalizations complex. Compared with twin studies, which typically report higher heritability, the use of extended pedigree in the estimation of heritability is more susceptible to uncontrolled age-related influence. However, we have attempted to mitigate this by statistically correcting for age. One advantage over twin studies is that heritability estimates in extended pedigrees are less likely to be unduly inflated by shared environment (76). Relatively few genes are both known to be associated with brain structure and relevant in schizophrenia (85,86). This scarcity may reflect the genetic heterogeneity of schizophrenia and suggests that different alleles in different families may be responsible for morphologic abnormalities. Our use of an automated segmentation tool takes advantage of more recent developments in the neuroimaging community; however, this technique is not without its limitations. Regions that showed the lowest ICC were also the least heritable. This finding is in agreement with a more recent publication that assessed heritability of these same structures in healthy individuals (17). There may be higher measurement error in some regions compared with others, as has been previously suggested (32,75). Nonetheless, automated segmentation in most subcortical and limbic structures appears robust and repeatable. Finally, our approach can also reduce heterogeneity by allowing for the analysis of subgroups or subfamilies, and follow-up studies in large pedigrees should consider family-specific effects. In the present study, we are unable to compare volume or family heritability within specific regions with a comparable group of healthy subjects because of a lack of related individuals within the healthy sample. Future endeavors can incorporate large pedigrees of healthy comparison subjects to determine if there are subtypes of families with schizophrenia that show especially large abnormalities in brain structure.

In conclusion, subcortical brain volume and shape are heritable in multiplex-multigenerational families with schizophrenia. Such localized features may be related to distinct dimensions of psychopathology or may be determined by specific genetic risk factors unique to patients with schizophrenia or to particular families. The specificity obtained using shape analysis of brain structures may improve the selection of imaging phenotypes that better reflect the underlying neurobiology and aid in the

identification of specific genetic targets that affect brain structure and function in schizophrenia.

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