

Heritability of Changes in Brain Volume Over Time in Twin Pairs Discordant for Schizophrenia

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Context: Structural brain abnormalities have consistently been found in schizophrenia, with increased familial risk for the disease associated with these abnormalities. Some brain volume changes are progressive over the course of the illness. Whether these progressive brain volume changes are mediated by genetic or disease-related factors is unknown.

Objective: To investigate whether genetic and/or environmental factors are associated with progressive brain volume changes in schizophrenia.

Design: Longitudinal 5-year follow-up in monozygotic (MZ) and dizygotic (DZ) twin pairs discordant for schizophrenia and healthy comparison twin pairs using brain magnetic resonance imaging.

Setting: Participants were recruited from the twin pair cohort at the University Medical Center Utrecht.

Participants: A total of 92 participants completed the study: 9 MZ and 10 DZ twin pairs discordant for schizophrenia and 14 MZ and 13 DZ healthy twin pairs.

Main Outcome Measures: Percentage volume changes of the whole brain; cerebral gray and white matter of the frontal, temporal, parietal, and occipital lobes; cerebellum; and lateral and third ventricles over time between and within twin pairs were compared using repeated measures analysis of covariance. Structural equation modeling was applied to estimate contributions of additive genetic and common and unique environmental factors.

Results: Significant decreases over time in whole brain and frontal and temporal lobe volumes were found in patients with schizophrenia and their unaffected co-twins compared with control twins. Bivariate structural equation modeling using cross-trait/cross-twin correlations revealed significant additive genetic influences on the correlations between schizophrenia liability and progressive whole brain (66%; 95% confidence interval [CI], 51%-100%), frontal lobe (76%; 95% CI, 54%-100%), and temporal lobe (79%; CI, 56%-100%) volume change.

Conclusion: The progressive brain volume loss found in patients with schizophrenia and their unaffected co-twins is at least partly attributable to genetic factors related to the illness.

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STRUCTURAL BRAIN ABNORMALITIES have consistently been found in patients with schizophrenia.^{1,2} Interestingly, at least some of these brain volume changes are progressive across the course of the illness.^{3,4} More specifically, schizophrenic patients show aberrant trajectories of brain volume change during adolescence⁵ and adulthood.^{6,7}

The cause of these progressive brain volume changes in schizophrenia is still unclear. Recently, more pronounced progressive brain volume changes over time have been associated with poor outcomes.^{6,8-10} Moreover, differential effects of typical vs atypical antipsychotic medication on progressive gray matter decreases were demonstrated.¹¹ Thus, both severity of the illness and medication use influence the

progressive brain volume changes in schizophrenia. However, genes involved with schizophrenia might also contribute to the progressive brain changes in this illness; the heritability (the percentage of variance explained by genetic factors) of developing schizophrenia is estimated to be around 80%.¹² Indeed, twin and family studies strongly suggest that genetic factors play a role in the decreased brain volumes found in schizophrenia.^{13,14} A familial influence on the progressive brain changes in schizophrenia was suggested in a study of patients with childhood-onset schizophrenia and their siblings.¹⁵ However, sibling studies cannot disentangle the extent of genetic and common environmental contributions to familial influences.

In this study, we set out to investigate the relative contributions of genetic and en-

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Table 1. Demographics of Monozygotic (MZ) and Dizygotic (DZ) Twin Pairs Discordant for Schizophrenia and Healthy Control (HC) Twin Pairs

Characteristic	Mean (SD)							
	MZ Twins				DZ Twins			
	Patient (n=9)	Co-twin (n=9)	HC 1 (n=14)	HC 2 (n=14)	Patient (n=10)	Co-twin (n=10)	HC 1 (n=13)	HC 2 (n=13)
Sex, M/F, No.	4/5	4/5	9/5	9/5	6/4	6/4	8/5	8/5
Age at first MRI, y	40.2 (12.2)	40.2 (12.1)	35.5 (11.8)	35.5 (11.8)	37.1 (11.9)	37.2 (11.9)	35.4 (10.6)	35.4 (10.6)
Height, cm	177.9 (11.0)	178.1 (11.7)	175.0 (6.6)	175.6 (6.3)	177.0 (12.1)	178.6 (9.9)	174.7 (11.5)	174.9 (8.9)
Handedness, R/L/A, No.	8/1/0	8/0/1	9/3/2	12/1/1	9/1/0	9/0/1	11/2/0	9/1/3
Level of education, y	11.8 (3.2)	11.9 (3.0)	12.7 (3.1)	12.6 (2.5)	10.4 (2.3)	13.2 (3.0)	12.8 (2.4)	13.0 (2.9)
Parental level of education, y	12.4 (2.7)	12.4 (2.7)	10.9 (2.4)	10.9 (2.4)	11.9 (2.5)	11.9 (2.5)	10.9 (2.5)	10.9 (2.5)
Follow-up duration, y	4.9 (1.0)	4.8 (1.0)	4.8 (0.2)	4.8 (0.6)	5.0 (0.6)	4.9 (0.7)	4.8 (0.4)	4.9 (0.5)
Firstborn, No.	5	4	9	5	5	5	9	4
Smoker, No.	5 ^a	2	5	6 ^a	7	3	6	2
Cigarettes/d at follow-up	19.6 (23.3) ^a	1.0 (2.7)	4.3 (7.8)	4.1 (7.5) ^a	17.8 (14.6) ^a	5.0 (8.5)	3.3 (6.1)	2.1 (5.1)
Alcoholic drinks/wk at follow-up	4.7 (8.2) ^a	6.4 (10.9)	5.4 (5.3)	4.5 (3.8) ^a	4.5 (6.6)	10.8 (7.7)	8.0 (6.7)	4.4 (6.2)
Age at first psychotic symptoms, y	23.8 (5.0)				22.2 (6.2)			
Duration of illness, y	16.6 (11.5)				14.9 (8.8)			
GAF score at follow-up ^b	59.2 (5.9)				60.8 (22.9)			
CAN score at follow-up ^c	4.7 (3.1)				4.8 (3.6)			
Total PANSS score at follow-up	47.6 (11.8) ^a				50.0 (18.5)			
Medication, typical/atypical/both, No. ^d	4/3/2				3/3/3			
Medication intake, haloperidol equivalent/y								
Typical	2006.5				464.4			
Atypical	2264.7				2402.3			
Both	2257.5				2358.6			

Abbreviations: CAN, Camberwell Assessment of Need; GAF, Global Assessment of Functioning; MRI, magnetic resonance imaging; PANSS, Positive and Negative Syndrome Scale; R/L/A, right/left/ambidextrous.

^aData missing in 1 individual.

^bAvailable in 6 MZ patients and 6 DZ patients.

^cAvailable in 6 MZ and 5 DZ patients.

^dData missing in 1 DZ patient. Corrected for scan interval.

environmental (disease-related) factors to the progressive brain volume changes in schizophrenia. Therefore, we conducted a longitudinal magnetic resonance imaging (MRI) study in monozygotic (MZ) and dizygotic (DZ) same-sex twin pairs discordant for schizophrenia and compared them with healthy MZ and DZ twin pairs, with a scan interval of 5 years.

The twin model is a powerful approach for determining the relative contributions of genetic influences and common and unique environmental influences on variation in brain volumes and their common origin with disease liability.¹⁶ Moreover, morphologic findings in twins can be extended to the singleton population.¹⁷ For genetic influences, the determining factor is the extent to which MZ twin pairs resemble each other more than the DZ twin pairs. The presence of shared environmental factors is suggested when correlations in DZ twins are larger than half of the MZ correlation.¹⁸ The importance of unique environmental factors can first be obtained from the extent to which MZ twins do not resemble each other. In a similar manner, the extent to which genetic and environmental factors influence brain volume changes and schizophrenia liability can be determined by comparing their cross-trait/cross-twin correlations in MZ and DZ twins. A cross-trait/cross-twin correlation is the correlation of a trait in twin 1 (ie, liability for schizophrenia) with a second trait (ie, brain volume change) in twin 2 of the same pair. If the cross-trait/cross-

twin correlation is approximately twice as high in MZ as in DZ twins and is comparable with the within-twin/cross-trait correlation (ie, the association between the 2 traits within the individuals), then it can be inferred that genes common to both traits influence their association.

METHODS

PARTICIPANTS

Participants were recruited from the twin pair cohort¹⁹ at the University Medical Center Utrecht. A total of 9 MZ and 10 DZ twin pairs discordant for schizophrenia and 14 MZ and 13 DZ healthy twin pairs completed the longitudinal MRI study (N=92) after an interval of 5 years (mean, 4.86 years [SD, 0.57 years]). The control twin pairs were matched to the discordant twin pairs for zygosity, age, sex, birth order, handedness, their parents' socioeconomic status, and follow-up duration (**Table 1**). All twins participated after written informed consent was obtained. Zygosity was determined by DNA fingerprinting, using either the polymorphic markers D06S474, D07S1804, D07S1870, D12S811, D13S119, D13S126, D13S788, D20S119, D22S683, DXS1001, and ELN, or D13S317, VWA, D74520, D35158, TH01, TP0X, CSF1P0, and D55818. The study was approved by the medical ethics committee for research in humans of the University Medical Centre Utrecht and was carried out according to the directives of the Declaration of Helsinki (amendment of Edinburgh, 2000).

All participants underwent extensive psychiatric assessments at baseline and at follow-up with the Comprehensive Assessment of Symptoms and History.²⁰ Age at onset of illness was defined as the first time the patient experienced psychotic symptoms. Duration of illness was defined as the time between age at illness onset and age at first MRI scan. Outcome was assessed using the Global Assessment of Functioning.²¹ The Camberwell Assessment of Need²² was used to evaluate the need for care of the patient in daily life functioning. The Positive and Negative Syndrome Scale²³ was used to evaluate severity of symptoms. In the co-twins of the schizophrenic patients and the matched healthy twin pairs, the Schedule for Affective Disorders and Schizophrenia–Lifetime version²⁴ and the Structured Interview for DSM-IV Personality²⁵ were completed. Information about smoking status (number of cigarettes) and alcohol use was collected at follow-up.

Sixteen probands met criteria for schizophrenia and 3 met criteria for schizoaffective disorder. Furthermore, co-twins of the probands met diagnoses of schizoid personality disorder (1 MZ), schizotypal personality disorder (2 MZ), cannabis abuse (1 DZ), conduct disorder (1 MZ), and major depressive disorder (1 MZ and 1 DZ). In 2 healthy participants, diagnoses of adjustment disorder with depressed mood (1 DZ) and major depressive disorder (1 MZ) were made. At follow-up, 2 co-twins of probands had developed a major depressive disorder (2 DZ) and 1 developed a depressive disorder not otherwise specified (1 DZ). Four healthy participants (3 MZ and 1 DZ) developed major depressive disorders. Except for 1 control twin pair that was separated at 12 years of age when both of their parents died, all twins were reared together.

A table from the Dutch National Health Service²⁶ was used to calculate the cumulative dosage of typical antipsychotics during the scan interval and to derive the haloperidol equivalents. For atypical antipsychotic drugs, the respective pharmaceutical companies suggested the following conversions into haloperidol equivalents: clozapine, 40:1; olanzapine, 2.5:1; risperidone, 1:1; sulpiride, 170:1; quetiapine, 50:1; sertindole, 2:1, and aripiprazole, 2:1. During the scan interval, 6 patients had been taking atypical antipsychotic medication (including clozapine, olanzapine, and aripiprazole) exclusively and 7 patients were taking typical medication exclusively. Five patients switched between typical and atypical antipsychotic medications during the scan interval. From 1 patient, we did not have reliable information about medication intake. None of the co-twins (with the exception of 1 co-twin who had used antipsychotic medication for a short period) had ever used antipsychotic medication at the time of the second MRI scan. Five of the co-twins (3 MZ and 2 DZ) as well as 2 of the healthy participants (2 MZ) used antidepressants.

MRI ACQUISITION AND ANALYSIS

The MRI brain scans were acquired on a Philips NT scanner (Philips Medical Systems, Best, the Netherlands) operating at 1.5 T in all participants. T1-weighted 3-dimensional fast-field echo scans with 160 to 180 contiguous coronal slices (echo time/repetition time, 4.6 milliseconds/30 milliseconds; flip angle, 30°; voxel dimension, 1 × 1 × 1.2 mm³) and T2-weighted dual-echo turbo-spin-echo scans with 120 contiguous coronal slices (echo time 1/echo time 2/repetition time, 14 milliseconds/80 milliseconds/6350 milliseconds; flip angle, 90°; voxel dimension, 1 × 1 × 1.6 mm³) of the whole head were used for quantitative measurements. In addition, T2-weighted dual-echo turbo-spin-echo scans (echo time 1/echo time 2, 9 milliseconds/100 milliseconds; flip angle, 90°; voxel dimension, 0.98 × 0.98 mm²) with 19 axial 5-mm slices and a 1.2-mm gap of the whole head were used for clinical neurodiagnostic evaluation.

Processing was done on the neuroimaging computer network from our department of psychiatry. All images were coded

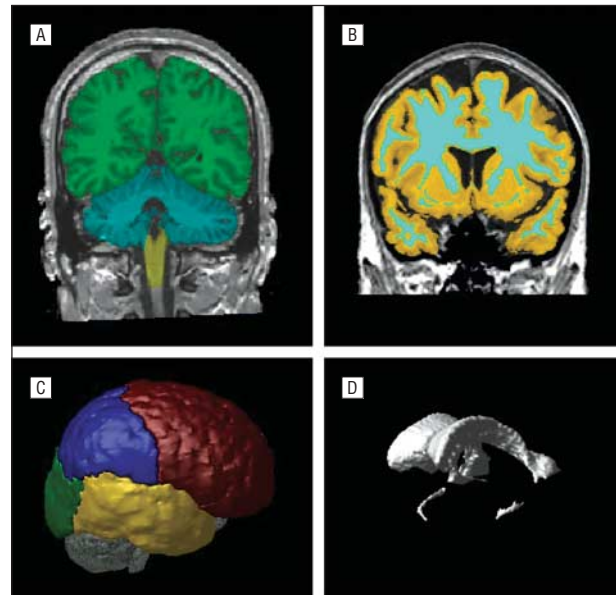


Figure 1. Segmentations of the whole brain and cerebellum (A), gray and white matter (B), the frontal, temporal, parietal, and occipital lobes (C), and the lateral and third ventricles (D). Quantitative assessments of the intracranial, total brain, gray and white matter of the cerebrum (total brain excluding cerebellum and stem) and lateral and third ventricle volumes were performed based on histogram analyses and series of mathematical morphology operators to connect all voxels of interest.

to ensure blindness of participant identification and diagnoses. Scans were manually put into Talairach frame (no scaling) for segmentation purposes and corrected for inhomogeneities in the magnetic field.²⁷ Quantitative assessment of intracranial, total brain, and gray and white matter of the cerebrum (total brain excluding cerebellum and stem), and lateral and third ventricle volumes was performed using histogram analyses and series of mathematical morphology operators to connect all voxels of interest validated previously.^{28,29} All images were checked after measurement and corrected manually if necessary. The interrater reliabilities of the volume measurements, determined by the intraclass correlation coefficient, were 0.95 and higher.

The frontal, parietal, temporal, and occipital lobes were segmented based on transformations to a model brain onto which the lobes had been manually demarcated.^{30,31} The model brain was selected earlier among 200 brain images of healthy individuals between 16 and 70 years of age. Brain images were registered to the model brain through the Automatic Nonlinear Image Matching and Anatomical Labeling algorithm³² to remove global differences in size and shape of individual brains. The inverse of the transformation process registered the manual segmentations of the model brain to all participants' brain images. The segments were visually checked (**Figure 1**).

STATISTICAL ANALYSIS

Brain volume changes are expressed in percentages: [(follow-up–baseline volume)/baseline volume] × 100. Data were examined for outliers, extreme values, and normality of distribution. No transformations were needed for this or any of the other measures.

For statistical analysis of the data, our approach was 2-fold. Multiple repeated-measures univariate analyses of covariance were applied to initial measurement of brain volume change in the 2 groups. This procedure did not allow for decomposition of the observed variance into genetic and environmental parts, but made the findings comparable with earlier twin studies in which a single MRI scan was made and was readily in-

terpretable. In repeated-measures univariate analysis of covariance, unstandardized residuals of percentage brain volume change were entered as dependent variables. Percentage brain volume change was corrected for age and sex. Twin status (twin 1 = patient with schizophrenia or healthy control, twin 2 = co-twin of patient or healthy control) was entered as a within-subjects factor. Between-subjects factors were group (discordant or healthy twin pair) and zygosity (MZ or DZ).

For estimating the significance of the relative contributions of genetic and environmental (family-related and unique) factors on variability in (progressive) brain volume changes in schizophrenia, structural equation modeling is the method of choice in twin studies. Implementing bivariate genetic models, structural equation modeling also yields valid tests on whether genetic or family-related factors explain the association between schizophrenia and brain volume changes. To what extent genes and/or environment are responsible for this association was expressed by bivariate heritability: the percentage of covariance that is accounted for by a common genetic factor. This method has been extensively described³³ and applied to schizophrenia before.^{16,34}

The analyses were done using the statistical package Mx.³⁵ Because the Mx software cannot handle ordinal and continuous data simultaneously, the residuals of the percentage brain volume changes (after regression on sex and age) were used to calculate a 5-category ordinal measure for brain volumes. This ordinal scale was created by dividing the residuals, so that each category covers 20% of the data following a normality distribution.

Both affected and unaffected individuals were assumed to have an underlying liability to develop schizophrenia (standard normal distribution).^{16,34} Thus, it was believed that if a person with a high value on the liability scale crossed a certain threshold, he or she would become ill (patient) or otherwise remain healthy (discordant co-twin of patient or healthy twin pairs).

The critical threshold for schizophrenia was not based on our sample, as it was approximately 25% schizophrenic patients, which would have resulted in an overestimation of the prevalence (ie, 1%). Also, our active attempts to find as many discordant twin pairs as possible and exclude concordant pairs would have affected heritability estimates. Instead, we constrained the heritability (h^2) at 81%, the influence of family-related environmental factors (c^2) at 11% ($r_{MZ}=0.92$, $r_{DZ}=0.52$) (based on a meta-analysis of twin studies¹²), and the prevalence at 1% (resulting in a critical threshold at 2.33) (based on epidemiological studies³⁶). This procedure was found to give valid and reliable estimates for bivariate heritability in such analyses.¹⁶

Using structural equation modeling, the phenotypic correlations (r_{ph}) between schizophrenia liability and percentage brain volume changes were calculated; phenotypic correlations can result from a common set of genes or environmental factors. These phenotypic correlations were then decomposed into genetic (r_g) and environmental components (r_e), thus providing information regarding the possible shared genetic and environmental influences of schizophrenia liability and brain volume changes. Decomposition of these sources was based on the comparison of cross-trait/cross-twin correlations for MZ and DZ twins, ie, the correlation between a trait (schizophrenia liability) of twin 1 with another trait of twin 2 (brain volume change), where twin 1 and twin 2 represent a twin pair. If the absolute value of the correlation between brain volume change of twin 1 and schizophrenia liability of twin 2 is larger in MZ twins than in DZ twins, this indicates that the genes that influence brain volume change (partly) overlap with genes that influence schizophrenia. The extent of the overlap is reflected by the magnitude of the genetic correlation (r_g). When the cross-trait/cross-twin correlations are similar and, for MZ and DZ twins, differ from 0, this suggests that environmental factors that are shared within families contribute to the phenotypic correlation between brain volume change

and schizophrenia. If both correlations are 0, only individual-specific environmental correlations exist.

The contribution of additive genetic (A), common environmental (C), and unique environmental (E) factors to the variance in brain volume changes (univariate heritability) and to the covariance between schizophrenia liability and brain volume changes (bivariate heritability) was expressed as a percentage of the total (co)variance: the percentage A was expressed as h^2 (heritability), C as c^2 (common or shared environment) and E as e^2 (unique environment). Random measurement error is part of e^2 . By combining the information from r_g , r_c , and r_e with h^2 , c^2 , and e^2 , the influence of genetic, common environmental, and unique environmental factors on the total phenotypic correlation between schizophrenia and brain volume change could be established. Specifically, the percentage of covariance between schizophrenia and percentage brain volume change that is accounted for by a common genetic factor is expressed as bivariate heritability: $h_{biv}^2 = \text{cov}_A / (\text{cov}_A + \text{cov}_C + \text{cov}_E)$.

By minimizing a goodness-of-fit statistic between observed and predicted covariance matrices, structural equation modeling programs estimate model parameters (a, c, and e). Effects of genetic and family-related factors were tested by comparing the likelihoods of nested models ($-2 \log$ likelihood, which is χ^2 distributed) in which it is tested whether, for example, a CE model fits as well as an ACE model, using the most simple model that best explains the data. A χ^2 value greater than 3.84 (1 df) indicates a significant difference at $\alpha=0.05$, which means that the reduced model provides a significantly worse fit to the data and indicates that the discarded effect (eg, disease effect) cannot be left out of the model without seriously deteriorating the goodness of fit.

Using the full model, estimates (including 95% confidence intervals [CIs]) were obtained that reflect the increase or decrease in brain volume over time in patients or in individuals with a familial background of schizophrenia.

RESULTS

REPEATED-MEASURES ANALYSIS OF COVARIANCE

Over time, a significant group effect was found for percentage whole brain volume change (**Table 2** and **Figure 2**). Combined, patients and their co-twins showed a progressive decrease in whole brain volume over time compared with the control twin pairs ($F_{1,42}=4.60$; $P=.04$). Furthermore, a significant twin \times zygosity interaction was found ($F_{1,42}=8.18$; $P=.01$), which indicates that the MZ co-twins show a more prominent decrease in whole brain volume than the DZ co-twins compared with the patients with schizophrenia. The group \times zygosity interaction was significant for lateral ventricle volume ($F_{1,42}=13.20$; $P=.01$) owing to a more prominent progressive increase in lateral ventricle volume in discordant DZ compared with MZ twin pairs, whereas the controls did not show a difference between MZ and DZ pairs. Moreover, a significant group effect was found in frontal, temporal, and (gray matter) occipital lobes. No significant group effects were found for cerebral white matter ($F_{1,41}=0.24$; $P=.63$), cerebellum ($F_{1,42}=2.05$; $P=.16$), or third ventricle ($F_{1,42}=0.39$; $P=.54$) volumes.

STRUCTURAL EQUATION MODELING

Over time and irrespective of disease, a decrease in whole brain volume was found, which was moderately correlated within twin pairs. A significant familial effect was found

Table 2. Brain Volume Change After a 5-Year Follow-up in Twins With and Without Schizophrenia

Brain Structure	Mean (SD) % Brain Volume Change ^a				Discordant Compared With Healthy Twin Pairs ^b			
	MZ Twins		DZ Twins		Discordant vs Healthy Twin Pairs		Schizophrenic Patients and Co-twins Difference vs HCs 1 and HCs 2 Difference	
	Discordant Twin Pairs (n=9 Pairs)	Healthy Twin Pairs (n=14 Pairs)	Discordant Twin Pairs (n=10 Pairs)	Healthy Twin Pairs (n=13 Pairs)	F _{1,42}	P Value	F _{1,42}	P Value
Whole brain	-1.28 (3.09)	-0.37 (2.18)	-2.39 (2.87)	-0.30 (2.08)	4.60	.04	0.10	.76
Frontal lobe	-2.05 (3.62)	-1.12 (2.16)	-3.29 (3.02)	-1.02 (2.25)	4.79	.03	1.40	.24
Temporal lobe	-0.77 (3.28)	+0.14 (2.43)	-2.01 (2.70)	+0.15 (1.91)	4.64	.04	0.25	.62
Parietal lobe	-1.46 (4.00)	-0.90 (2.74)	-3.07 (3.84)	-0.73 (3.12)	2.36	.13	0.00	.99
Occipital lobe	-1.84 (3.24)	+0.30 (2.45)	-1.53 (2.98)	+0.07 (2.07)	7.13	.01	0.12	.73
Cerebral GM	-3.33 (6.54)	-2.42 (2.77)	-4.16 (5.27)	-1.81 (3.39)	2.34	.13	0.16	.69
Frontal lobe	-3.87 (6.77)	-2.94 (3.08)	-4.82 (4.85)	-2.41 (3.09)	2.36	.13	0.75	.39
Temporal lobe	-1.37 (5.80)	-0.64 (2.98)	-3.34 (4.24)	-0.13 (2.51)	3.50	.07	0.96	.33
Parietal lobe	-3.86 (5.76)	-3.21 (3.48)	-4.68 (6.39)	-2.49 (3.82)	1.27	.27	0.05	.82
Occipital lobe	-5.07 (7.34)	-1.97 (4.66)	-4.52 (6.92)	-0.53 (5.09)	4.55	.04	0.00	.99
Cerebral WM	+1.38 (3.71)	+1.87 (4.03)	+0.26 (4.03)	+1.01 (4.45)	0.24	.63	0.10	.76
Frontal lobe	+0.77 (3.26)	+1.54 (3.07)	-0.29 (3.25)	+1.04 (4.30)	1.22	.28	0.00	.95
Temporal lobe	+0.74 (4.50)	+1.65 (4.35)	+1.49 (4.24)	+0.71 (5.23)	0.00	.99	1.68	.20
Parietal lobe	+2.18 (5.47)	+2.37 (4.04)	-0.04 (4.17)	+1.71 (5.45)	0.50	.48	0.01	.92
Occipital lobe	+3.41 (6.96)	+2.97 (5.05)	+2.85 (5.96)	+0.87 (5.60)	0.76	.39	0.02	.97
Cerebellum	+0.55 (3.02)	+1.01 (3.05)	-0.73 (3.38)	+1.34 (2.35)	2.05	.16	0.01	.91
Lateral ventricle	+2.71 (15.65)	+7.26 (9.06)	+13.26 (13.05)	+0.99 (8.36)	1.53	.22	1.43	.24
Third ventricle	-6.07 (19.44)	+1.03 (14.98)	+5.17 (29.16)	+1.43 (20.55)	0.39	.54	0.00	.96

Abbreviations: DZ, dizygotic; GM, gray matter; HC, healthy control; MZ, monozygotic; WM, white matter.

^aPercentage volume change from baseline to follow-up: [(follow-up - baseline volume)/baseline volume] × 100. Gray matter and WM volumes of 1 DZ co-twin are missing.

^bMultiple repeated measures analysis of covariance of percentage brain volume change with twin as a within-group variable; group (discordant twin pair or healthy twin pair) and zygosity (MZ and DZ) as between-group variables; and age and sex as covariates. Significant main effects for group (discordant vs healthy twin pairs) suggest familiar or genetic influences on percentage brain volume change; significant interaction effects of group × twin (schizophrenic patients and co-twins difference vs HCs 1 and HCs 2 difference) suggest that possible (nongenetic) disease-related factors influence percentage brain volume change after the 5-year follow-up.

(h^2 and c^2 together, 55%), but it was not possible to disentangle the influence of h^2 and c^2 in this sample. Around half of the variance was accounted for by e^2 (45%) (**Table 3**).

When adding schizophrenia as a trait in the bivariate analysis (**Table 4**), the decrease in whole-brain volume became more pronounced, with higher schizophrenia liability due to genetic factors (within-twin/cross-trait correlation, $r_{ph} = -0.22$). The influence of genes involved in schizophrenia liability and whole-brain volume change was found to be significant and was estimated to account for 66% (95% CI, 51%-100%). This means that at least 51% of the covariance of whole-brain volume change and schizophrenia can be explained by genetic factors. In addition, shared environmental factors, though not of significant influence, explained approximately 23% (95% CI, 0%-40%) of the shared variance between schizophrenia liability and brain volume change. The remaining (non-significant) approximately 11% (95% CI, 0%-38%) shared variance between schizophrenia liability and brain volume change was due to unique environmental factors. In addition, a significant influence of additive genetic factors on volume change and schizophrenia was found for frontal (76%; 95% CI, 54-100%) and temporal (79%; 95% CI, 56-100%) lobe volume change.

ASSOCIATION WITH CLINICAL VARIABLES

No associations were found between clinical measurements or level of parental education and brain volume

changes. Participants who met diagnosis did not differ from those who were healthy (for whole-brain volume, MZ individuals: $F_{1,35} = 0.14$; $P = .71$; and DZ individuals: $F_{1,34} = 0.01$; $P = .94$). The correlation between whole-brain volume change and number of cigarettes smoked ($r = -0.13$; $P = .22$) and amount of alcohol consumed ($r = 0.12$; $P = .27$) at follow-up was nonsignificant. When repeating repeated-measures univariate analyses of covariance with participants who had used drugs, the co-twin who had used antipsychotic drugs briefly, with participants who consumed more than 15 drinks per week at follow-up excluded, the group effect remained significant ($F_{1,27} = 4.36$; $P = .046$).

COMMENT

This study examined the relative contributions of genetic and environmental factors on percentage brain volume changes over time in schizophrenia. In a longitudinal study with a 5-year interval, MZ and DZ twin pairs discordant for schizophrenia were compared with healthy twin pairs. To our knowledge, this is the first longitudinal MRI study in twin pairs discordant for schizophrenia.

Our main finding is that progressive decreases in whole brain and frontal and temporal lobe volumes were found both in patients with schizophrenia and in their unaffected co-twins compared with the healthy twin pairs. This was largely due to decreases in gray matter volume over

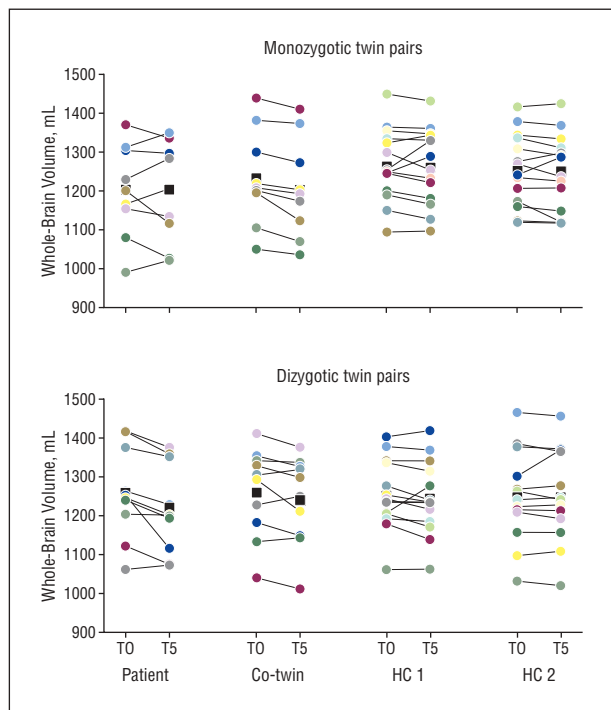


Figure 2. Whole-brain volume at baseline (T0) and follow-up (T5) in twin pairs discordant for schizophrenia and healthy twin pairs. Each line represents an individual. Twins correspond to each other by color; patients are associated with their co-twins, and healthy controls (HCs) 1 are associated with their co-twins (HCs 2). The black squares and lines represent mean whole brain volume. For monozygotic twin pairs, percentage volume change was -0.37% for patients and -2.18% for their co-twins; for monozygotic HCs 1, it was -0.23% , and for their co-twins (HCs 2), it was -0.51% . For dizygotic twin pairs, percentage volume change was -3.26% for patients and -1.52% for their co-twins; for dizygotic HCs 1, it was -0.65% , and for their co-twins it was 0.05% .

time. Furthermore, by applying structural equation modeling, we demonstrated that at least 51% of the correlation of -0.22 between whole-brain, frontal lobe, and temporal lobe volume loss and schizophrenia liability could be explained by genetic factors that are also directly implicated in the disease. Thus, genes that are directly involved in the etiology of schizophrenia may also contribute to the (frontal and temporal) brain volume loss observed in the patients and their co-twins. The results also imply that the genes that play a role in (frontal and temporal) brain volume loss in (healthy) aging may be suitable candidate genes for schizophrenia. Finally, the finding of progressive brain volume loss in the unaffected co-twins of the patients indicates that the progressive brain volume loss in schizophrenia can no longer be explained solely as the result of disease-associated factors, such as antipsychotic medication intake, smoking, or outcome.

The brain volume change over time in the chronically ill patients was approximately 5 times that found in individuals with normal aging (as expressed in the brain volume changes in the control participants during the 5-year interval). This finding is in keeping with longitudinal MRI studies that reported progressive brain changes in chronically ill patients.^{6,8,37} By including co-twins who were discordant for schizophrenia, we were able to measure the relative contributions of common environmental and genetic factors on these progressive brain volume changes.

The extent of the tissue loss in the co-twins of the patients approximated that found in the patients themselves and was considerably more prominent than in controls. This finding strongly suggests that progressive brain volume change in schizophrenia has a familial background and is consistent with the results of a study in siblings discordant for childhood-onset schizophrenia.¹⁵ Additionally, based on the cross-trait/cross-twin comparison of disease liability with brain volume change in the MZ and DZ twin pairs in our study, the relative influence of genetic, common environmental, and unique environmental factors could be calculated. These results demonstrated that approximately 66%, at least 51%, of the variation in whole-brain volume loss that is associated with schizophrenia could be explained by genetic factors.

That frontal lobe volume loss in particular can be explained by genetic factors (76%; 95% CI, 54%-100%) that also directly contribute to schizophrenia may not be entirely surprising. The frontal lobe has been implicated in the genetic risk for the disease in a cross-sectional study in the current sample¹⁹ and in others.³⁸ The genetic influences on temporal lobe volume change in schizophrenia (79%; 95% CI, 56%-100%) found in this study are consistent with findings of decreased temporal cortical thickness in family members of patients.³⁹ To what extent genetic risk is associated with cortical thickness change in local areas in the cortex is currently being assessed.

Shared environmental factors implicated in the disease explained another 23% (nonsignificant) of the variation in whole-brain volume loss in schizophrenia (8% of the variation in frontal lobe and 3% in temporal lobe volume). Unique environmental factors did not significantly contribute to the progressive whole-brain volume change found in patients, as the remaining shared variance between schizophrenia liability and brain volume change due to unique environmental factors or measurement error was small, approximately 11% (comparable with the frontal lobe [16%] and the temporal lobe [18%]). Antipsychotic medication intake can be considered such a unique environmental factor. While all the patients were taking antipsychotic medication (except for 1 co-twin who took them briefly), none of the co-twins used antipsychotic medication. Consequently, antipsychotic medication is unlikely to cause the progressive brain volume changes in the patients with schizophrenia.

Our sample size did not allow a statistical analysis to assess possible gene \times environment interactions, such as in the case of epigenetic mechanisms, which exert lasting control over gene expression without altering the genetic code.⁴⁰ However, even if gene \times environment interactions were involved in preventing the co-twins from developing schizophrenia, this does not explain why it would leave the co-twins with a symptom-free progressive brain volume loss later in life. Indeed, there is suggestive evidence that the progressive brain volume loss in co-twins may not be without consequences: studies examining cognitive function in unaffected co-twins of patients with schizophrenia find impairments on cognitive tests and educational attainment.⁴¹⁻⁴³ Cognitive impairments may thus be associated with the progressive brain volume loss observed in the unaffected co-twins of patients with schizophrenia. However, in this study, we did not find an asso-

Table 3. Within-Trait/Cross-Twin Correlations and Cross-Trait/Cross-Twin Correlations on Percentage Brain Volume Change

Brain Structure	Correlation (95% Confidence Interval) ^a			
	Within-Trait/Cross-Twin Correlation ^b		Cross-Trait/Cross-Twin Correlation ^c	
	23 MZ Twin Pairs	23 DZ Twin Pairs	23 MZ Twin Pairs	23 DZ Twin Pairs
Whole brain	0.46 (0.09 to 0.71)	0.35 (-0.18 to 0.69)	-0.32 (-0.51 to -0.10)	-0.05 (-0.31 to 0.21)
Frontal lobe	0.47 (0.07 to 0.73)	0.56 (0.16 to 0.77)	-0.35 (-0.54 to -0.12)	-0.11 (-0.36 to 0.13)
Temporal lobe	0.49 (0.14 to 0.72)	0.48 (-0.06 to 0.77)	-0.38 (-0.56 to -0.16)	-0.16 (-0.41 to 0.09)
Parietal lobe	0.29 (-0.11 to 0.60)	0.21 (-0.31 to 0.62)	-0.22 (-0.42 to 0.01)	-0.14 (-0.40 to 0.14)
Occipital lobe	0.10 (-0.27 to 0.44)	-0.16 (-0.60 to 0.40)	-0.34 (-0.52 to -0.13)	-0.11 (-0.36 to 0.15)
Cerebral GM	-0.03 (-0.42 to 0.37)	0.17 (-0.32 to 0.58)	-0.26 (-0.45 to -0.06)	-0.07 (-0.33 to 0.19)
Frontal lobe	0.29 (-0.14 to 0.62)	0.40 (-0.07 to 0.71)	-0.29 (-0.48 to -0.07)	-0.03 (-0.29 to 0.23)
Temporal lobe	0.30 (-0.12 to 0.62)	0.59 (0.15 to 0.82)	-0.36 (-0.55 to -0.14)	-0.26 (-0.50 to -0.00)
Parietal lobe	-0.03 (-0.47 to 0.42)	0.13 (-0.30 to 0.51)	-0.23 (-0.42 to -0.02)	-0.06 (-0.22 to 0.31)
Occipital lobe	0.35 (-0.08 to 0.66)	0.49 (0.06 to 0.76)	-0.31 (-0.51 to -0.08)	-0.11 (-0.35 to 0.15)
Cerebral WM	0.34 (-0.11 to 0.66)	0.25 (-0.25 to 0.63)	0.01 (-0.22 to 0.24)	0.03 (-0.25 to 0.29)
Frontal lobe	0.01 (-0.45 to 0.46)	0.16 (-0.26 to 0.53)	-0.06 (-0.28 to 0.15)	0.02 (-0.25 to 0.28)
Temporal lobe	0.27 (-0.16 to 0.60)	0.06 (-0.43 to 0.52)	0.01 (-0.22 to 0.23)	0.12 (-0.16 to 0.38)
Parietal lobe	0.53 (0.13 to 0.77)	0.24 (-0.26 to .63)	0.08 (-0.15 to 0.30)	-0.03 (-0.30 to 0.24)
Occipital lobe	0.39 (-0.03 to 0.69)	0.02 (-0.48 to 0.40)	0.11 (-0.11 to 0.32)	0.11 (-0.16 to 0.36)
Cerebellum	0.63 (0.33 to 0.82)	0.45 (-0.13 to 0.76)	-0.19 (-0.40 to 0.05)	-0.04 (-0.32 to 0.22)
Lateral ventricle	0.13 (-0.27 to 0.48)	0.29 (-0.33 to 0.68)	0.18 (-0.05 to 0.39)	0.26 (-0.03 to 0.55)
Third ventricle	-0.06 (-0.43 to 0.33)	0.27 (-0.26 to 0.65)	0.05 (-0.17 to 0.27)	-0.08 (-0.33 to 0.18)

Abbreviations: DZ, dizygotic; GM, gray matter; MZ, monozygotic; WM, white matter.

^aThe 95% confidence intervals including 0 indicate statistical nonsignificance.

^bIrrespective of disease. Within-trait/cross-twin correlation, or intraclass correlation, is the correlation of twin 1 with his/her co-twin (twin 2) on percentage brain volume change: $\{[(\text{follow-up} - \text{baseline volume})/\text{baseline volume}] \times 100_{\text{twin 1}}\} - \{[(\text{follow-up} - \text{baseline volume})/\text{baseline volume}] \times 100_{\text{twin 2}}\}$.

^cAssociated with schizophrenia. Correlation of percentage brain volume change of twin 1 with genetic liability to schizophrenia (SZ) of his/her co-twin (twin 2): $\{[(\text{follow-up} - \text{baseline volume})/\text{baseline volume}] \times 100_{\text{twin 1}}\} - \text{SZ}_{\text{twin 2}}$. The schizophrenia within-trait/cross-twin correlation ($\text{SZ}_{\text{twin 1}} - \text{SZ}_{\text{twin 2}}$) is constrained to 0.92 in MZ twins and 0.52 in DZ twins based on the genetic point estimates of a meta-analysis and a 1% prevalence.

ciation between outcome, level of parental education, and brain volume changes.

As to the (patho)physiological processes that are responsible for the progressive brain changes, we can only speculate. The brain volume loss in the discordant twin pairs may represent altered plasticity in adulthood. It is now clear that the brain continues to show plasticity during adulthood, at least in some areas. Neurogenesis is known to occur in the adult human hippocampus⁴⁴ and olfactory bulb.⁴⁵ However, adult neurogenesis in several other areas, including the neocortex, striatum, amygdala, and substantia nigra, has also been suggested.⁴⁶ Electron microscopy studies in rodent brains have demonstrated that neural circuits are sculpted by spontaneous activity and sensory experience.⁴⁷ Also, evidence is accumulating that functional rewiring takes place in the adolescent and adult rodent brain, which may involve structural plasticity with synapse formation and elimination.^{48,49} Moreover, action potential firing was found to influence myelination.⁵⁰ Thus, it is tempting to hypothesize that the progressive volume loss associated with the liability to develop schizophrenia represents aberrant plasticity of adult functional neural networks. Indeed, several lines of evidence suggest there is abnormal neurogenesis and aberrant expression of developmental genes in schizophrenia and a role of candidate schizophrenia susceptibility genes in adult neurogenesis.⁵¹ Some of the candidate schizophrenia susceptibility genes have been associated with brain volumes in healthy subjects.⁵² In schizophrenia, the disrupted in schizophrenia 1 (DISC1), translin-associated factor X (TRAX),⁵³ and GAD1⁵⁴ genes were found to contribute to decreased gray matter volumes.

Common environmental factors implicated in the disease itself explained 23% of the brain volume loss in patients, though this effect was not significant. Possible common environmental factors shared among patients with schizophrenia and their close relatives are stress factors.^{55,56} The emotional burden of the disease can also be considerable in siblings of patients with schizophrenia.⁵⁷ For twins, who often have a close emotional relationship with each other, the experience of a co-twin having a severe psychiatric disease like schizophrenia may represent a more pronounced burden. Other possible common environmental factors that patients share with their co-twins that have been associated with schizophrenia are viral infections,^{58,59} psychosocial factors,⁶⁰ prenatal environment,⁶¹ and delivery complications.⁶² Delivery complications have been associated with decreased brain volumes in twin pairs discordant for the disease.^{63,64} Early (prenatal or perinatal) neurodevelopment lesions that render the brain vulnerable and anomalous late neurodevelopmental processes may interact with other causative factors associated with the onset of psychosis.^{56,65-68}

This study has several limitations that have to be considered. We were able to retrieve 91% of the original twin sample, and no sample bias was present at the time of the second MRI scan. However, the relatively small sample size (N=92) did give room for possible chance variations between the subgroups. The smaller brain tissue loss and ventricular volume increase in MZ compared with DZ patients is counterintuitive. When selecting discordant twin pairs and assuming the genetic liability to have an underlying continuum (as hypothesized here), the MZ patients with schizophrenia may have had a relatively

Table 4. Phenotypic Correlations and Estimated Influences of Additive Genetic and Common and Unique Environmental Factors on Brain Volume Change^a

Brain Structure	% (95% Confidence Interval) ^b						
	Sources of Variance on Brain Volume Change ^c			Phenotypic Correlation ^d	Sources of Covariance Between Schizophrenia and Brain Volume Change ^e		
	<i>h</i> ²	<i>c</i> ²	<i>e</i> ²		<i>h</i> ² _{biv}	<i>c</i> ² _{biv}	<i>e</i> ² _{biv}
Whole brain	32 (0 to 76)	23 (0 to 59)	45 (23 to 76)	-0.22 (-0.41 to -0.01) ^f	66 (51 to 100) ^f	23 (0 to 40)	11 (0 to 38)
Frontal lobe	17 (0 to 70)	37 (0 to 65)	46 (26 to 74)	-0.26 (-0.44 to -0.05) ^f	76 (54 to 100) ^f	8 (0 to 36)	16 (0 to 38)
Temporal lobe	19 (0 to 71)	32 (0 to 62)	49 (28 to 78)	-0.28 (-0.47 to -0.08) ^f	79 (56 to 100) ^f	3 (0 to 32)	18 (0 to 36)
Parietal lobe	16 (0 to 60)	14 (0 to 51)	71 (40 to 99)	-0.17 (-0.36 to 0.04)	62	20	18
Occipital lobe	13 (0 to 40)	0 (0 to 25)	87 (60 to 99)	-0.21 (-0.37 to 0.01)	74 (52 to 100) ^f	1 (0 to 27)	25 (0 to 47)
Cerebral GM	8 (0 to 45)	7 (0 to 40)	85 (55 to 100)	-0.19 (-0.37 to 0.01)	73	5 (5 to 100)	22 (0 to 51)
Frontal lobe	22 (0 to 61)	16 (0 to 52)	62 (36 to 95)	-0.20 (-0.39 to 0.01)	65 (49 to 89) ^f	21 (0 to 40)	14 (0 to 35)
Temporal lobe	3 (0 to 55)	39 (0 to 64)	58 (34 to 86)	-0.25 (-0.43 to -0.04) ^f	32 (0 to 90)	44 (0 to 92)	24 (4 to 42)
Parietal lobe	9 (0 to 41)	3 (0 to 26)	88 (57 to 100)	-0.12 (-0.31 to 0.08)	64	14 (0 to 45)	22 (0 to 77)
Occipital lobe	14 (0 to 65)	31 (0 to 57)	55 (32 to 85)	-0.20 (-0.40 to 0.01)	72 (49 to 100) ^f	8 (0 to 38)	20 (0 to 50)
Cerebral WM	1 (0 to 60)	26 (0 to 54)	73 (40 to 100)	-0.06 (-0.27 to .15)	35	38	28
Frontal lobe	3 (0 to 46)	8 (0 to 39)	89 (54 to 100)	-0.12 (-0.32 to .08)	50	29	21
Temporal lobe	12 (0 to 56)	12 (0 to 47)	77 (43 to 100)	-0.08 (-0.28 to .13)	35	37	28
Parietal lobe	45 (0 to 76)	7 (0 to 62)	48 (24 to 85)	0.01 (-0.20 to 0.22)	51	28	21
Occipital lobe	33 (0 to 66)	2 (0 to 74)	65 (34 to 100)	0.05 (-0.15 to 0.25)	38	27	35
Cerebellum	45 (0 to 83)	21 (0 to 71)	34 (17 to 63)	-0.11 (-0.33 to .11)	63	20	17
Lateral ventricle	0 (0 to 47)	41 (1 to 63)	59 (36 to 86)	0.06 (-0.14 to 0.25)	0 (0 to 40)	58 (0 to 80)	42 (20 to 80)
Third ventricle	0 (0 to 44)	6 (0 to 34)	94 (56 to 100)	-0.10 (-0.30 to 0.11)	27	36	37

Abbreviations: biv, bivariate; *c*², shared environmental effects; *e*², nonshared environmental effects; GM, gray matter; *h*², heritability; WM, white matter.

^aAll analyses are for percentage brain volume change and corrected for age and sex. Parameters for schizophrenia are fixed based on a prevalence of 1% and the following genetic model: *h*²=0.81, *c*²=0.11, *e*²=0.08.

^bThe 95% confidence intervals from 0 to 100 are not shown.

^cIrrespective of disease. Reflects the influence of additive genetic and common and unique environmental factors on brain volume change, ie, additive genetic effects account for 32% of the variance on whole-brain volume change.

^dReflects the correlation between schizophrenia liability and percentage brain volume change within individuals, ie, the correlation between schizophrenia and whole-brain volume change = -0.22, which indicates that patients with schizophrenia have a larger reduction in whole-brain volume during the 5-year interval than healthy comparison participants.

^eSources of covariance between schizophrenia and percentage brain volume change are represented by *h*²_{biv}, *c*²_{biv}, and *e*²_{biv} on the correlation between schizophrenia liability and brain volume change within individuals, ie, genetic influences that are shared by percentage brain volume change and schizophrenia account for 66% of the phenotypic correlation between brain volume change and schizophrenia.

^fSignificant at $\alpha = .05$.

lower genetic liability for schizophrenia. However, this could not have explained our findings since it would have resulted in an underestimation of the genetic liability for progressive brain changes in schizophrenia.

The within-trait/cross-twin correlations of the MZ twin pairs were not significantly higher than the within-trait/cross-twin correlations of the DZ twin pairs. Based on the current sample, we cannot conclude that progressive brain volume change (irrespective of disease) is due to genetic factors. However, in a larger sample (approximately 100 healthy twin pairs), we found a comparable and significant heritability for whole-brain volume change (R.G.H.B., unpublished data, 2008). Thus, there indeed appears to be a heritable component to brain volume change.

Also, the sample size did not allow for measurement of the effect of interactions between genes and environmental factors on brain volume changes. Confidence intervals were large and therefore the environmental influences should be interpreted cautiously: the extent of common and unique environmental factors common to schizophrenia and progressive brain volume change in the patients remains inconclusive. For the other brain volume changes over time, we were not able to disentangle the influence of genetic, common environmental, and unique environmental factors.

In conclusion, we found progressive brain volume loss during a 5-year interval both in patients with schizophrenia and their unaffected co-twins. A significant proportion of this effect could be attributed to genes that are implicated in schizophrenia. Localizing and characterizing the genes involved in dynamic brain changes may prove to be a valuable approach in studying the pathophysiology of progressive brain changes in schizophrenia.

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