ARCHIVAL REPORT

Discovering Schizophrenia Endophenotypes in Randomly Ascertained Pedigrees

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Background: Although case-control approaches are beginning to disentangle schizophrenia's complex polygenic burden, other methods will likely be necessary to fully identify and characterize risk genes. Endophenotypes, traits genetically correlated with an illness, can help characterize the impact of risk genes by providing genetically relevant traits that are more tractable than the behavioral symptoms that classify mental illness. Here, we present an analytic approach for discovering and empirically validating endophenotypes in extended pedigrees with very few affected individuals. Our approach indexes each family member's risk as a function of shared genetic kinship with an affected individual, often referred to as the coefficient of relatedness. To demonstrate the utility of this approach, we search for neurocognitive and neuroanatomic endophenotypes for schizophrenia in large unselected multigenerational pedigrees.

Methods: A fixed-effects test within the variance component framework was performed on neurocognitive and cortical surface area traits in 1606 Mexican-American individuals from large, randomly ascertained extended pedigrees who participated in the Genetics of Brain Structure and Function study. As affecteds were excluded from analyses, results were not influenced by disease state or medication usage.

Results: Despite having sampled just 6 individuals with schizophrenia, our sample provided 233 individuals at various levels of genetic risk for the disorder. We identified three neurocognitive measures (digit-symbol substitution, facial memory, and emotion recognition) and six medial temporal and prefrontal cortical surfaces associated with liability for schizophrenia.

Conclusions: With our novel analytic approach, one can discover and rank endophenotypes for schizophrenia, or any heritable disease, in randomly ascertained pedigrees.

Key Words: Coefficient of relatedness, cognition, cortical surface area, endophenotype, family study, schizophrenia

Susceptibility loci for schizophrenia were recently localized using population-based genome-wide association methods that focus on common variants (1–8). Although these loci represent an important advance toward unraveling the genetic architecture of the illness, the number of causal gene identifications is limited and identified loci explain only a small proportion of the heritable risk (9). A recent whole exome sequence study examined 2536 schizophrenia cases and 2543 control subjects, providing the strongest evidence to date for specific genetic variants that increase risk for psychosis (10). Purcell *et al.* (10) identified numerous rare (<1 in 10,000) mutations across many genes that when considered in aggregate are strongly associated with schizophrenia risk. However, no individual variant or gene-based test achieved statistical significance, suggesting a complex polygenic burden increases risk for schizophrenia through multiple targets within one or more metabolic pathways. Although it is possible that with additional samples individual rare variants identified through exome or whole genome sequencing may become significant, these findings clearly demonstrate the polygenic nature of schizophrenia risk (11). Going forward, it is critical to systematically examine the impact of risk variants on empirically derived gene sets or bioinformatically validated gene networks to elucidate how genetic processes predispose the complex behavioral symptoms that define schizophrenia. Yet, even for Mendelian disorders with known mutations, the biological mechanisms that span the space between genotype and clinical phenotype are often unclear. It is likely that polygenic diseases, like psychiatric illnesses, will have even more complex genotype-phenotype relationships. For this reason, quantitative traits, rather than bifurcated diagnoses, are better suited for modeling complex gene effects (12), as they provide a relative ranking of individuals along an assumed continuum. One dilemma for psychiatric genetics, then, is developing techniques for understanding the impact of sets of risk genes on the neurobiological antecedents of mental illness. Based primarily on work in other areas of medicine [e.g., (13)], it is clear that the use of well-designed and validated allied phenotypes, intermediate phenotypes, or endophenotypes should facilitate this process by characterizing the effects of disruptions in gene networks on traits closely aligned to the illness (14).

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Endophenotypes that are sensitive to the genetic liability for an illness can characterize the pathways through which genetic variation gives rise to clinical phenomenon (15). An endophenotype is a heritable trait that is genetically correlated with disease liability, providing greater power to localize and characterize the mechanisms of disease-related genes than diagnostic status alone (15–18). Typically, endophenotypes are identified through twin or family studies where probands are selected for a specific illness (19). Many studies have more complex recruitment strategies [e.g., (20–23)], requiring multiple affected individuals to maximize the potential that the proband has a genetic, rather than sporadic, form of the illness. However, such ascertainment strategies can complicate both genetic and endophenotypic inference (24). An alternate approach is to study families that were not selected for a specific phenotype. For common illnesses like major depression with lifetime prevalence rates approaching 15% (25), random epidemiologic sampling methods should provide adequate samples of affected individuals without obvious ascertainment bias. Utilizing a similar approach in large extended pedigrees, we recently discovered a number of behavioral, neuroanatomical, and transcriptional endophenotypes for major depression (18). Combining one of these endophenotypes, the *RNF123* lymphocyte-based transcript, in a bivariate quantitative trait locus localization analysis provided a novel locus for major depression (18), an illness whose genetic structure is still an enigma (26).

It is possible that even with rarer illnesses like schizophrenia (e.g., ~1% prevalence) endophenotypes can be identified in unselected samples, assuming pedigree sizes are large enough to model pleiotropy between endophenotype and illness. Using large unselected families could benefit our search for empirically validated schizophrenia endophenotypes and establish a foothold for disentangling the complex polygenic burden of the illness. To do so requires analytic approaches optimized for assessing endophenotypic variation of a relatively small number of affected individuals in the context of their larger family. One such analytic approach, developed here, indexes each person's illness risk as a function of genetic kinship with an affected individual. That is, a first-degree relative of an affected individual is expected to share approximately 50% of their genetic variation, while a second-degree relative is anticipated to have 25% of shared genetic variation with a similar halving of genetic sharing for each subsequent degree of relatedness. We show that such an index, often referred to as the coefficient of relationship, can be used to perform a fixed-effect single degree of freedom test within a variance component analysis, providing genetic correlation information between a trait of interest and the illness and thus showing that the measure is a candidate endophenotype for the disease.

In the present article, we search for neurocognitive and neuroanatomic endophenotypes for schizophrenia in large multigenerational pedigrees using a novel approach to the estimation of the endophenotypic ranking value (*ERV*), which is closely related to the genetic correlation between endophenotype and disease. Specifically, we test the hypothesis that individual brain-related traits are sensitive to genetic liability for schizophrenia, even in extended pedigrees with few affected individuals.

Methods and Materials

Participants

Mexican-American individuals ($n = 1606$) from large extended pedigrees (75 pedigrees, average family size 21.41 [2–126] people) who participated in the Genetics of Brain Structure and Function study were included in the analysis. Individuals in this cohort had actively participated in research for over 20 years and were selected from a single census tract in south San Antonio without regard to psychiatric diagnosis, with the constraints that they were of Mexican-American ancestry and part of a large family [see (27,28) for recruitment details]. No other inclusion or exclusion criteria were imposed in the initial study. However, individuals were excluded from the neurocognitive evaluation for history of neurological illnesses, stroke, or other major neurological event. Individuals were excluded from the neuroimaging evaluation for these criteria and for magnetic resonance imaging contraindications. Reported pedigree relationships were empirically verified, based on autosomal markers, and intrafamilial relationships were edited if necessary. All participants provided written informed consent on forms approved by the institutional review boards at the University of Texas Health Science Center San Antonio/Texas Biomedical Research Institute and at Yale University.

Diagnostic Assessment

All participants received face-to-face medical history and psychiatric interviews. The Mini-International Neuropsychiatric Interview Plus (MINI-Plus) (29), a semi-structured interview to facilitate diagnoses of DSM-IV and ICD-10 psychiatric illnesses, was augmented to include items on lifetime diagnostic history. Masters- and doctorate-level research staff, with established reliability ($\kappa \geq .85$) for psychotic and affective disorders, conducted all interviews. All subjects with possible psychopathology were discussed in case conferences that included licensed psychologists or psychiatrists, and lifetime consensus diagnoses were determined.

Neurocognitive Assessment

Each participant received a 90-min neuropsychological evaluation (21,30,31). Neuropsychological tests included standard clinical measures and well-validated computerized tasks (32–34). Twenty neurocognitive variables were derived from 16 neuropsychological tests, including measures of attention/concentration, executive processing, working memory, declarative memory, language processing, intelligence, and emotional processing. Eight percent of sample was tested in Spanish and test instructions were translated into Spanish and back-translated into English.

Neuroimaging Assessment

Images were acquired on a research-dedicated, Siemens (Erlangen, Germany) 3T Trio/TIM scanner with a 12-element high-resolution phase array head coil housed in the Research Imaging Institute, University of Texas Health Science Center San Antonio. Neuroanatomic images included seven high-resolution T1-weighted three-dimensional turbo fast low-angle shot (turbo-FLASH) sequences with an adiabatic inversion contrast pulse and the following parameters: echo time/repetition time/inversion time = 3.04/2100/785 msec, flip angle = 13°, 800 μm isotropic resolution, 200 mm field of view, 5-min duration (35 minutes total). A retrospective motion correction protocol was implemented to improve signal to noise (35). Image processing was based on cortical surface representations using FreeSurfer (http://surfer.nmr.mgh.harvard.edu/). The analysis followed previously described procedures (36,37) as implemented in our group (38). Images underwent inhomogeneity corrections, intensity normalization, linear alignment to a common atlas space, and skull removal. Next, white matter voxels were identified based on

location and relative intensity. The two hemispheres were separated and a tessellated mesh was built around the mass of white matter voxels. This mesh was smoothed with an algorithm that takes into account the local intensity in the original images and topological defects are corrected. The resulting smoothed mesh represented the white matter surface. The gray matter (pial) surface was generated by expanding the white surface to the gray matter/cerebrospinal fluid boundary while constraining the smoothness of the surface. Gray and white matter surfaces were visually inspected and manually edited if necessary. Next, the pial surface was inflated into a sphere, registered to an atlas utilizing cortical folding patterns, and segmented into regions of interest based on gyral and sulcal structure, surface curvature, and sulcal depth (39,40). More specifically, a Bayesian approach was applied to establish the probability that a given vertex belonged to a given label based on a probability atlas. Surfaces were parceled into 33 regions of interest per hemisphere defined by the Desikan-Killiany atlas (39). Eight subcortical regions were parceled using similar procedures and volumetric measures were calculated.

Quantitative Genetic Analysis

Quantitative genetic analysis was used to partition trait covariance among related individuals into genetic and environmental components. For a trait, the phenotypic covariance matrix ($\mathbf{\Omega}$) in a pedigree of n members was modeled as $\mathbf{\Omega} = \mathbf{R}\sigma^2_G + \mathbf{I}\sigma^2_E$, where $\mathbf{R}$ is the $n \times n$ kinship matrix for the pedigree, σ^2_G is the variance in the trait due to additive genetic effects, $\mathbf{I}$ is an $n \times n$ identity matrix, and σ^2_E is the variance due to random environmental effects. The additive genetic heritability (h^2) of a trait is defined as $h^2 = \sigma^2_G/(\sigma^2_G + \sigma^2_E)$. Before analysis, candidate endophenotypes were normalized using an inverse Gaussian transformation. Age, age^2, sex, and their interactions (age $\times$ sex, age^2 $\times$ sex) were included as covariates to model mean effects. In addition, intracranial volume was included as a covariate for FreeSurfer analyses. Regression terms were estimated for each covariate, and the likelihood of a model in which the covariate effect was estimated was compared with the likelihood of a model in which the covariate effects were constrained to zero. To control for multiple comparisons within each endophenotype class, the false discovery rate was set to q = .05.

Estimating the Endophenotypic Ranking Value in Randomly Ascertained Pedigrees

An endophenotype must be heritable and genetically correlated with disease liability (18). Glahn *et al.* (18) proposed the endophenotype ranking value to formally test for endophenotypic status and to rank potential endophenotypes. The *ERV* provides an unbiased and empirically derived method for identifying and choosing appropriate endophenotypes in a manner that balances the strength of the genetic signal for the endophenotype and the strength of its relation to the disorder of interest. It is defined as the product of the square root of the heritability of the disease $\left(h^2_D\right)$ on the continuous liability scale under the assumption of a normal threshold model, the square root of the heritability of the endophenotype $\left(h^2_E\right)$, and the genetic correlation (ρ_G) between liability and endophenotype. The *ERV* is expressed in the following formula:

$$ERV = \sqrt{h^2_D h^2_E}|\rho_G|$$

The *ERV* is a standardized genetic covariance with values varying between 0 and 1, where higher values indicate that the endophenotype and the illness are more strongly influenced by shared genetic factors.

The *ERV* was previously used in situations where all component parameters (h^2_D, h^2_E, ρ_G) were directly estimated from a given data set. Direct estimation of these parameters requires a pedigree-based study design with sufficient disease cases, either with relatively common illnesses (prevalence of ≥10%) or in heavily ascertained pedigrees. In this context, all three parameters (and the *ERV*) are simultaneously estimated using a standard bivariate quantitative genetic variance component model (18). However, when a disease is less common, such as schizophrenia, we show that it is possible to estimate the *ERV* from even randomly selected pedigree designs if there is a sufficient number of relatives of disease cases. Rewriting the underlying covariance model as a fixed effects model provides information on the *ERV* in terms of differences in the mean endophenotypic values of unaffected relatives of affected individuals versus those of unaffected individuals who have no known relatives with the disease.

Consider a disease that is determined by a normal threshold process on a continuous latent liability (l) such that the population prevalence can be written

$$K_P = \int_t^\infty f_N(l)dl$$

where $f_N(l)$ is a standard normal probability density function with mean 0 and unit variance and t is the threshold above which an individual's liability is scored as a disease. The heritability of the disease on the latent liability scale is closely related to that on the observed binary scale when affected individuals are scored as a 1 and unaffected individuals are scored as a 0:

$$h^2_B = \frac{f_N(t)^2}{K_P(1-K_P)} h^2_D$$

using the transformation first developed by Dempster and Lerner (41).

To rewrite the variance/covariance terms of the *ERV* in terms of observable mean effects, we consider the expectations for differences in means of a putative endophenotype between unaffected relatives of an individual with the disease versus unaffected individuals without an affected relative. Let μ_R be the mean of the endophenotype (y) in a set of individuals who are related to an affected individual with coefficient of relationship r_D and let μ_U be the mean in individuals who are unrelated to any affected individual. After some algebra relating mean effects to covariance components, the *ERV* can be rewritten on the binary scale in terms of the standardized difference in means between these two groups as:

$$ERV_B = \sqrt{K_P(1-K_P)}\frac{|\mu_R - \mu_U|}{r_D\sigma}$$

where σ is the standard deviation of the endophenotype. Transforming to the underlying normal liability scale yields

$$ERV_B = \frac{K_P(1-K_P)}{f_N(t)}\frac{|\mu_R - \mu_U|}{r_D\sigma} = \sqrt{h^2_D h^2_E}|\rho_G|.$$

This formula utilizing subgroup means to detect genetic correlation can be used for any pairwise relationship. However, we are interested in utilizing all joint information to make inferences about the identity and suitability of prospective endophenotypes. We can further generalize this model to any set of arbitrary

relatives by utilizing a linear model for the mean in the set of relatives of affected individuals in which $\mu_R = \mu_U + \beta \max(r_D)$, where the coefficient of relationship between every individual and his closest affected relative is employed as a covariate with regression coefficient β for the endophenotype. Additionally, covariates such as those including sex and age (as described above) can also be included as needed. Using this extended model, the *ERV* can be written as

$$ERV = \frac{K_P(1-K_P)}{f_N(t)}\frac{|\beta|}{\sigma}.$$

Thus, a test of the significance of β (using a standard likelihood ratio test statistic) represents a formal test of the *ERV* that requires that there be both a heritable basis for the disease and a genetic correlation of the endophenotype with the disease. Given that we perform this fixed effect estimation and testing in pedigree data, we can simultaneously (and explicitly) obtain an estimate of the heritability (h^2_E) of the endophenotype itself. We implemented the *ERV* estimation approach as a mixed linear model in the computer package, SOLAR (42).

Results

Family Profiles

Based upon our consensus diagnostic process, 6 of the 1606 individuals met criteria for lifetime schizophrenia. Individuals with schizophrenia were 46.87 years of age (13.45, SD 34–67), had 10.50 years of education (2.88, SD 7–14) and were male individuals. Each affected individual was from a unique pedigree and together they were related to 233 nonschizophrenic relatives, including 14 unaffected first-degree relatives, 17 unaffected second-degree relatives, and so on, as shown in Table 1.

Neurocognitive Endophenotypes

A total of 1560 individuals had valid neurocognitive data, including 220 nonschizophrenic relatives. As can be seen in Table 2, all of the neurocognitive tests were heritable and strongly influenced by age. Three neurocognitive tests were sensitive to genetic liability for schizophrenia: digit symbol substitution ($\hat{\beta} = -1.59$; *ERV* = .591), delayed facial memory ($\hat{\beta} = -1.48$; *ERV* = .550), and emotion recognition ($\hat{\beta} = -1.39$; *ERV* = .516). Each of these tests has previously been associated with schizophrenia risk (30,43).

Gray Matter Endophenotypes

Nine hundred ninety-seven individuals had T1-weighted images processed in FreeSurfer at the time of the analysis, including 137 nonschizophrenic relatives. Bilateral cortical surface area estimates were uniformly heritable (Figure 1, Table 3). After controlling for multiple comparisons, six regions were significantly and negatively associated with schizophrenia risk: the fusiform gyrus ($\hat{\beta} = -1.62$; *ERV* = .601), the entorhinal cortex ($\hat{\beta} = -1.73$; *ERV* = .643), the parahippocampal gyrus ($\hat{\beta} = -1.66$; *ERV* = .615), the precuneus ($\hat{\beta} = -1.56$; *ERV* = .581), inferior temporal gyrus ($\hat{\beta} = -1.49$; *ERV* = .553), and superior frontal gyrus ($\hat{\beta} = -1.49$; *ERV* = .554). These predominately frontal and temporal regions have been previously implicated in the pathophysiology of schizophrenia (44) and there is limited evidence that surface area in these regions is associated with risk for the illness (45).

In contrast to surface area measurements, no subcortical volume was statistically associated with risk for schizophrenia after correcting for multiple comparisons. However, amygdala volume trended toward statistical significance ($\hat{\beta} = -1.26$; *ERV* = .470).

Discussion

We used large extended pedigrees unselected for mental illness to identify and rank neurocognitive and neuroimaging endophenotypes for schizophrenia. Although our sample contained few cases with schizophrenia, our results strongly suggest that reliable genetic correlation information is embedded and available in these extended pedigrees. The identified neurocognitive measures are strikingly consistent with prior endophenotype searches in schizophrenia (22,23,46,47) and bipolar disorder (21), providing validity for our experimental approach and analytic procedure. The cortical surface area findings generally replicate and extend the prior literature (44,45,48). This method of *ERV* testing and estimation has several benefits over the standard variance/covariance approach. First, the method provides a test of means, which are consistently more powerful than tests of variances/covariances (49). Second, the endophenotypic data of affected individuals are not included in *ERV* estimation. Hence, the *ERV* is not influenced by disease state or medication usage. Finally, the test can be used even when there are few affected individuals in the sample, if there are sufficient numbers of unaffected relatives. The current analytic and experimental tactic provides a novel approach for discovering and ranking endophenotypes for schizophrenia or any heritable disease.

It is important to note that the use of large extended pedigrees is critical for this analytic strategy. Despite having sampled just 6 individuals with schizophrenia (who were not included in the focal analyses), our sample provided 233 individuals at various levels of genetic risk for the disorder. While our method could work with any family-based design (e.g., twin pairs or trios), an advantage of large extended pedigrees is that many unaffected relatives should be available for even a small number of cases, providing the statistical power needed to adequately test hypotheses about putative pleiotropy between endophenotype and illness. To further demonstrate the utility of

Table 1. Sample Demographics

Participant	Sample Size	Age	% Female	% Left-Handed	Education	% Employed
Schizophrenia	6	46.86 (13.45)	0	33	10.50 (2.88)	0
First-Degree Unaffected	14	49.35 (12.50)	21	7	9.29 (3.20)	43
Second-Degree Unaffected	17	45.11 (22.08)	64	12	8.26 (4.49)	64
Third-Degree Unaffected	36	52.10 (10.20)	69	8	11.00 (3.50)	50
Fourth-Degree Unaffected	69	34.58 (12.55)	68	16	12.48 (2.58)	61
Fifth-Degree Unaffected	42	35.77 (16.31)	48	29	10.86 (3.59)	71
Sixth-Degree Unaffected	34	34.12 (5.79)	59	0	12.09 (2.29)	71
Seventh-Degree Unaffected	15	20.58 (2.00)	60	13	11.67 (1.40)	53
Unrelated, Unaffected	1373	44.30 (15.48)	61	12	11.56 (3.17)	58

Table 2. Neurocognitive Endophenotypes

Traits	Heritability (h^2, p Value)	Age ($\hat{\beta}$, p Value)	Sex ($\hat{\beta}$, p Value)	Schizophrenia Relatedness ($\hat{\beta}$, p Value)	*ERV*
Semantic Fluency	.357, 8×10^{-16}[a]	−.018, 1×10^{-11}[a]	−.040, 6×10^{-1}	−.820, 2×10^{-1}	.305
Verbal Fluency	.462, 1×10^{-25}[a]	−.017, 1×10^{-10}[a]	.115, 9×10^{-2}	−1.340, 3×10^{-2}	.498
Digit-Symbol	.514, 4×10^{-27}[a]	−.043, 2×10^{-98}[a]	.142, 5×10^{-3}[a]	−1.590, 1×10^{-3}[a]	.591[a]
Trails A	.298, 1×10^{-11}[a]	.029, 2×10^{-34}[a]	−.325, 2×10^{-7}[a]	.998, 5×10^{-2}	.371
CPT-IP Hits	.246, 5×10^{-7}[a]	−.011, 1×10^{-4}[a]	−.089, 2×10^{-1}	.767, 2×10^{-1}	.285
Digit Span Forward	.499, 2×10^{-28}[a]	−.023, 1×10^{-21}[a]	−.072, 3×10^{-1}	−.366, 5×10^{-1}	.136
Digit Span Backward	.394, 8×10^{-19}[a]	−.020, 1×10^{-14}[a]	−.014, 8×10^{-1}	−1.296, 3×10^{-2}	.481
Letter-Number	.486, 2×10^{-28}[a]	−.030, 8×10^{-37}[a]	.115, 6×10^{-2}	−1.468, 1×10^{-2}	.545
PCET Categories	.092, 7×10^{-3}[a]	−.013, 5×10^{-7}[a]	.061, 4×10^{-1}	−.982, 3×10^{-2}	.126
SDRT Correct	.277, 6×10^{-10}[a]	−.010, 1×10^{-4}[a]	−.137, 5×10^{-2}	−.003, 1×10^{-0}	.001
Trails B	.453, 2×10^{-23}[a]	.024, 3×10^{-20}[a]	−.220, 7×10^{-4}[a]	1.448, 1×10^{-2}	.538
CVLT Learning	.394, 2×10^{-17}[a]	−.022, 1×10^{-19}[a]	.576, 3×10^{-19}[a]	.196, 7×10^{-1}	.073
CVLT Delay	.375, 5×10^{-18}[a]	−.018, 5×10^{-14}[a]	.500, 2×10^{-14}[a]	−.442, 4×10^{-1}	.164
CVLT Recognition	.302, 2×10^{-12}[a]	−.020, 9×10^{-19}[a]	.356, 9×10^{-9}[a]	−.865, 1×10^{-1}	.321
Facial Memory	.419, 5×10^{-21}[a]	−.013, 4×10^{-7}[a]	.236, 5×10^{-4}[a]	−.884, 1×10^{-1}	.201
Facial Memory Delay	.445, 6×10^{-19}[a]	−.014, 7×10^{-8}[a]	.277, 5×10^{-5}[a]	−1.481, 5×10^{-3}[a]	.550[a]
Penn Emotion	.264, 9×10^{-10}[a]	−.026, 6×10^{-26}[a]	.183, 4×10^{-3}[a]	−1.607, 2×10^{-3}[a]	.516[a]
Matrix Reasoning	.482, 2×10^{-28}[a]	−.034, 1×10^{-52}[a]	.011, 9×10^{-1}	−1.203, 3×10^{-2}	.447
Vocabulary	.762, 1×10^{-56}[a]	−.011, 6×10^{-5}[a]	−.012, 9×10^{-1}	−1.587, 3×10^{-2}	.589
WASI IQ	.714, 6×10^{-53}[a]	−.011, 2×10^{-5}[a]	−.010, 9×10^{-1}	−1.370, 6×10^{-2}	.509

$n = 1560$. *ERV* estimates assume an illness prevalence of 1%.

CPT-IP, Continuous Performance Test, Identical Pairs version; CVLT, California Verbal Learning Test; *ERV*, endophenotypic ranking value; FDR, false discovery rate; PCET, Penn Conditional Exclusion Test; SDRT, Spatial Delayed Response-Task; Trails A, Trail Making Test, Part A; Trails B, Trail Making Test, Part B; WASI, Wechsler Abbreviated Scale of Intelligence.

[a]Estimates significant after correction for multiple testing (FDR = .05).

extended pedigrees, we conducted additional analyses while excluding affected individuals as well as their unaffected first-degree relatives ($n = 14$), dramatically reducing common environmental influences between individuals with schizophrenia and their more distantly related family members. Results of these analyses were generally similar to those reported above (Supplement 1), speaking to the robustness of our approach and suggesting that common environmental influences did not drive our results. A benefit of phenotypically randomly selected large extended pedigrees is that many different endophenotypes can be analyzed in a single study, yielding substantial efficiency as phenotypes are added. As the genetic architectures of other mental illnesses are likely to be as complex as that of schizophrenia, involving multiple common and rare mutations within a common gene pathway (50,51), we anticipate that very well characterized unselected pedigrees will be critical for testing biological hypotheses about the impact of particular gene networks on illness risk.

Neurocognitive measures are quintessential endophenotypes for schizophrenia (52). Neurocognitive traits are highly heritable (53), patient deficits are generally severe or very severe (54), and their expression is often concordant in unaffected relatives (22,47,55–57). We identified three neurocognitive measures related to liability for schizophrenia: the number correct on the digit-symbol substitution task (23,32), a processing speed measure; the number of items recognized on the Penn Facial Memory Test (58), a test of declarative memory; and the number of correctly identified emotions portrayed on 40 actors (33). Each of these tests was previously associated with schizophrenia risk. Indeed, the identical digit-symbol substitution was also found to be the measure most strongly associated with schizophrenia in an independent sample of Latino pedigrees selected for a sibling pair

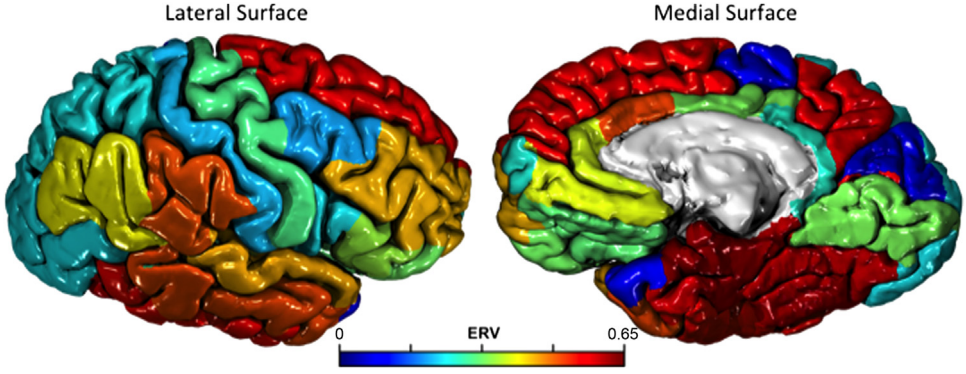

Figure 1. Endophenotypic ranking value (*ERV*) statistics associating variation in cortical surface area with schizophrenia risk. Applying a novel analytic approach for discovering and empirically validating endophenotypes in extended pedigrees with very few affected individuals, we demonstrate that common genetic factors influence liability for schizophrenia and the FreeSurfer derived cortical surface area in six medial temporal and prefrontal regions (see Table 3 for details).

Table 3. Cortical Surface Area and Subcortical Volumes

Trait	Heritability (h^2, p Value)	Age ($\hat{\beta}$, p Value)	Sex ($\hat{\beta}$, p Value)	Intracranial Volume ($\hat{\beta}$, p Value)	Schizophrenia Relatedness ($\hat{\beta}$, p Value)	*ERV*
Frontal Lobe						
Superior	$.679, 2 \times 10^{-22}$[a]	$-.011, 4 \times 10^{-6}$[a]	$-.384, 1 \times 10^{-7}$[a]	$.020, 1 \times 10^{-35}$[a]	$-1.491, 9 \times 10^{-3}$[a]	.554[a]
Middle (rostral)	$.595, 2 \times 10^{-18}$[a]	$-.011, 8 \times 10^{-6}$[a]	$-.509, 5 \times 10^{-12}$[a]	$.019, 1 \times 10^{-32}$[a]	$-1.217, 3 \times 10^{-2}$	.452
Middle (caudal)	$.597, 1 \times 10^{-19}$[a]	$-.008, 5 \times 10^{-3}$[a]	$-.247, 3 \times 10^{-3}$[a]	$.016, 4 \times 10^{-19}$[a]	$-.550, 4 \times 10^{-1}$	.204
Inferior (pars opercularis)	$.601, 8 \times 10^{-20}$[a]	$-.014, 6 \times 10^{-7}$[a]	$-.232, 5 \times 10^{-3}$[a]	$.016, 3 \times 10^{-18}$[a]	$-.651, 3 \times 10^{-1}$	.242
Inferior (pars triangularis)	$.617, 7 \times 10^{-21}$[a]	$-.012, 3 \times 10^{-5}$[a]	$-.343, 4 \times 10^{-5}$[a]	$.012, 3 \times 10^{-11}$[a]	$-.973, 1 \times 10^{-1}$	.361
Inferior (pars orbitalis)	$.406, 1 \times 10^{-9}$[a]	$-.013, 4 \times 10^{-6}$[a]	$-.476, 8 \times 10^{-9}$[a]	$.015, 4 \times 10^{-17}$[a]	$-.895, 1 \times 10^{-1}$	.333
Orbitofrontal (lateral)	$.567, 2 \times 10^{-16}$[a]	$-.017, 3 \times 10^{-11}$[a]	$-.308, 6 \times 10^{-5}$[a]	$.020, 2 \times 10^{-31}$[a]	$-.862, 1 \times 10^{-1}$	.320
Orbitofrontal (medial)	$.257, 1 \times 10^{-5}$[a]	$-.009, 4 \times 10^{-3}$[a]	$-.362, 5 \times 10^{-5}$[a]	$.013, 5 \times 10^{-12}$[a]	$-.710, 2 \times 10^{-1}$	.400
Frontal pole	$.497, 2 \times 10^{-12}$[a]	$-.003, 3 \times 10^{-1}$	$-.380, 1 \times 10^{-6}$[a]	$.020, 2 \times 10^{-32}$[a]	$-1.077, 6 \times 10^{-2}$	.264
Precentral	$.680, 3 \times 10^{-27}$[a]	$-.011, 1 \times 10^{-5}$[a]	$-.392, 2 \times 10^{-7}$[a]	$.019, 8 \times 10^{-31}$[a]	$-.829, 2 \times 10^{-1}$	.308
Paracentral lobule	$.535, 5 \times 10^{-14}$[a]	$-.007, 1 \times 10^{-2}$[a]	$-.134, 1 \times 10^{-1}$	$.020, 5 \times 10^{-29}$[a]	$-.244, 7 \times 10^{-1}$	.090
Medial Temporal						
Entorhinal cortex	$.479, 4 \times 10^{-15}$[a]	$.004, 2 \times 10^{-1}$	$-.322, 2 \times 10^{-4}$[a]	$.010, 1 \times 10^{-8}$[a]	$-1.732, 6 \times 10^{-3}$[a]	.643[a]
Parahippocampal	$.576, 2 \times 10^{-18}$[a]	$-.016, 4 \times 10^{-9}$[a]	$-.166, 4 \times 10^{-2}$[a]	$.018, 8 \times 10^{-24}$[a]	$-1.655, 6 \times 10^{-3}$[a]	.615[a]
Temporal pole	$.525, 1 \times 10^{-14}$[a]	$.004, 2 \times 10^{-1}$	$-.313, 4 \times 10^{-4}$[a]	$.011, 7 \times 10^{-9}$[a]	$.236, 7 \times 10^{-1}$	.088
Fusiform	$.493, 1 \times 10^{-12}$[a]	$-.015, 3 \times 10^{-9}$[a]	$-.364, 2 \times 10^{-6}$[a]	$.020, 1 \times 10^{-32}$[a]	$-1.617, 5 \times 10^{-3}$[a]	.601[a]
Lateral Temporal						
Superior	$.693, 1 \times 10^{-25}$[a]	$-.006, 2 \times 10^{-2}$[a]	$-.326, 1 \times 10^{-5}$[a]	$.021, 3 \times 10^{-38}$[a]	$-1.241, 3 \times 10^{-2}$	.461
Middle	$.586, 5 \times 10^{-18}$[a]	$-.012, 2 \times 10^{-6}$[a]	$-.485, 1 \times 10^{-10}$[a]	$.018, 2 \times 10^{-29}$[a]	$-1.392, 1 \times 10^{-2}$	.517
Inferior	$.446, 4 \times 10^{-13}$[a]	$-.016, 8 \times 10^{-10}$[a]	$-.363, 3 \times 10^{-6}$[a]	$.019, 3 \times 10^{-29}$[a]	$-1.489, 7 \times 10^{-3}$[a]	.553[a]
Transverse temporal	$.549, 3 \times 10^{-17}$[a]	$-.003, 3 \times 10^{-1}$	$-.128, 1 \times 10^{-1}$	$.016, 1 \times 10^{-17}$[a]	$-.998, 1 \times 10^{-1}$	.371
Bank (superior temporal)	$.453, 3 \times 10^{-12}$[a]	$-.011, 1 \times 10^{-4}$	$-.397, 2 \times 10^{-6}$[a]	$.015, 4 \times 10^{-17}$[a]	$-.804, 2 \times 10^{-1}$	.299
Parietal						
Postcentral	$.645, 5 \times 10^{-21}$[a]	$-.008, 8 \times 10^{-4}$[a]	$-.377, 3 \times 10^{-7}$[a]	$.023, 9 \times 10^{-46}$[a]	$-.589, 3 \times 10^{-1}$	.219
Supramarginal	$.503, 8 \times 10^{-15}$[a]	$-.009, 6 \times 10^{-4}$[a]	$-.370, 1 \times 10^{-6}$[a]	$.022, 1 \times 10^{-39}$[a]	$-1.364, 1 \times 10^{-2}$	.507
Superior	$.672, 8 \times 10^{-19}$[a]	$-.012, 1 \times 10^{-6}$[a]	$-.409, 1 \times 10^{-7}$[a]	$.020, 9 \times 10^{-32}$[a]	$-.682, 3 \times 10^{-1}$	.253
Inferior	$.471, 3 \times 10^{-15}$[a]	$-.015, 2 \times 10^{-9}$[a]	$-.370, 7 \times 10^{-7}$[a]	$.020, 7 \times 10^{-36}$[a]	$-1.118, 5 \times 10^{-2}$	.415
Precuneus	$.612, 5 \times 10^{-19}$[a]	$-.012, 5 \times 10^{-6}$[a]	$-.339, 7 \times 10^{-6}$[a]	$.021, 4 \times 10^{-37}$[a]	$-1.564, 7 \times 10^{-3}$[a]	.581[a]
Occipital						
Lingual	$.696, 3 \times 10^{-21}$[a]	$-.012, 1 \times 10^{-5}$[a]	$-.407, 3 \times 10^{-7}$[a]	$.016, 5 \times 10^{-21}$[a]	$-.919, 1 \times 10^{-1}$	.341
Pericalcarine	$.731, 7 \times 10^{-28}$[a]	$-.009, 1 \times 10^{-3}$[a]	$-.428, 1 \times 10^{-7}$[a]	$.012, 5 \times 10^{-12}$[a]	$-1.550, 2 \times 10^{-2}$	.576
Cuneus	$.612, 1 \times 10^{-18}$[a]	$-.010, 7 \times 10^{-5}$[a]	$-.524, 5 \times 10^{-11}$[a]	$.016, 4 \times 10^{-21}$[a]	$-.177, 8 \times 10^{-1}$	.066
Lateral	$.593, 1 \times 10^{-17}$[a]	$-.010, 3 \times 10^{-5}$[a]	$-.664, 1 \times 10^{-19}$[a]	$.019, 3 \times 10^{-32}$[a]	$-.660, 2 \times 10^{-1}$	.245
Cingulate						
Rostral anterior	$.386, 3 \times 10^{-8}$[a]	$-.005, 1 \times 10^{-1}$	$-.336, 5 \times 10^{-5}$[a]	$.021, 5 \times 10^{-31}$[a]	$-1.062, 4 \times 10^{-2}$	.395
Caudal anterior	$.410, 3 \times 10^{-9}$[a]	$-.008, 4 \times 10^{-3}$[a]	$-.191, 3 \times 10^{-2}$[a]	$.017, 4 \times 10^{-20}$[a]	$-1.390, 2 \times 10^{-2}$	.516
Posterior	$.601, 4 \times 10^{-17}$[a]	$-.012, 8 \times 10^{-6}$[a]	$-.313, 7 \times 10^{-5}$[a]	$.017, 3 \times 10^{-24}$[a]	$-.916, 1 \times 10^{-1}$	.340
Isthmus	$.562, 3 \times 10^{-19}$[a]	$-.003, 3 \times 10^{-1}$	$-.428, 7 \times 10^{-8}$[a]	$.018, 2 \times 10^{-24}$[a]	$-.723, 2 \times 10^{-1}$	.269
Insular	$.646, 3 \times 10^{-19}$[a]	$.005, 7 \times 10^{-2}$	$-.317, 4 \times 10^{-5}$[a]	$.020, 2 \times 10^{-33}$[a]	$-.869, 2 \times 10^{-1}$	.323
Subcortical Nuclei						
Accumbens	$.416, 3 \times 10^{-10}$[a]	$-.037, 2 \times 10^{-44}$[a]	$.048, 5 \times 10^{-1}$	$.016, 1 \times 10^{-22}$[a]	$-.425, 4 \times 10^{-1}$	.158
Amygdala	$.676, 2 \times 10^{-24}$[a]	$-.022, 6 \times 10^{-23}$[a]	$-.138, 4 \times 10^{-2}$[a]	$.025, 2 \times 10^{-61}$[a]	$-1.265, 2 \times 10^{-2}$	.470
Caudate	$.678, 2 \times 10^{-25}$[a]	$-.018, 2 \times 10^{-12}$[a]	$-.222, 3 \times 10^{-3}$[a]	$.019, 1 \times 10^{-29}$[a]	$-.747, 2 \times 10^{-1}$	.277
Hippocampus	$.654, 1 \times 10^{-22}$[a]	$-.021, 2 \times 10^{-21}$[a]	$-.153, 2 \times 10^{-2}$[a]	$.026, 2 \times 10^{-68}$[a]	$-.736, 2 \times 10^{-1}$	.273
Pallidum	$.470, 4 \times 10^{-12}$[a]	$-.027, 1 \times 10^{-25}$[a]	$-.355, 2 \times 10^{-6}$[a]	$.016, 2 \times 10^{-22}$[a]	$-.207, 7 \times 10^{-1}$	.077
Putamen	$.706, 5 \times 10^{-23}$[a]	$-.035, 2 \times 10^{-51}$[a]	$-.311, 3 \times 10^{-6}$[a]	$.014, 6 \times 10^{-22}$[a]	$-.359, 5 \times 10^{-1}$	.133
Thalamus	$.631, 2 \times 10^{-22}$[a]	$-.035, 8 \times 10^{-55}$[a]	$-.288, 4 \times 10^{-6}$[a]	$.019, 3 \times 10^{-42}$[a]	$-.666, 2 \times 10^{-1}$	.247
Ventral diencephalon	$.569, 5 \times 10^{-18}$[a]	$-.027, 2 \times 10^{-34}$[a]	$-.324, 6 \times 10^{-7}$[a]	$.022, 3 \times 10^{-52}$[a]	$-.286, 6 \times 10^{-1}$	.106

$n = 997$. *ERV* estimates assume an illness prevalence of 1.
ERV, endophenotypic ranking value; FDR, false discovery rate.
[a]Estimates significant after correction for multiple testing (FDR = .05).

concordant for the illness (30). Re-analyzing these data, the estimated *ERV* statistic for digit symbol performance was .493 in this sample, similar to the .591 observed here. Processing speed deficits, particularly those indexed by the digit-symbol substitution task, appear to be a central feature of the cognitive deficit in schizophrenia (59,60). Similarly, the identical facial memory was associated with schizophrenia risk in 35 multiplex multigenerational families of European ancestry (55). Facial memory impairment, and declarative memory more generally, is consistently linked to risk for the illness (22,47,55). While fewer investigators have examined the link between emotion recognition and schizophrenia risk, work by the Consortium on the Genetics of Schizophrenia recently demonstrated a link between the same task applied here and illness liability, potentially mediated through a locus on 1p36 (61). Other

neurocognitive measures were similarly sensitive to genetic liability for schizophrenia with high *ERV* values but did not exceed our correction for multiple comparisons: verbal fluency (*ERV* = .498); letter-number span (*ERV* = .545), a working memory measure; Trail Making Test, Part B (*ERV* = .538), an executive functioning task; and the Wechsler Abbreviated Scale of Intelligence IQ and vocabulary indices (*ERV* = .509 and .589, respectively).

Although meta-analyses report evidence for volumetric reductions in thalamus, hippocampus, anterior cingulate cortex, and corpus callosum area and increased ventricular size in schizophrenia (62,63), findings in unaffected relatives have been mixed (48,64,65). It is possible that the methods for measuring neuroanatomical variation are critical for this variability in the literature (38). Here, we focused on measures of cortical surface area, as there is increasing evidence for areal disruptions in schizophrenia (66) and in their unaffected relatives (45,67). We identified six regions with reduced surface area in those at risk for schizophrenia within the medial and lateral temporal lobes, the prefrontal cortex, and the precuneus cortex. Portions of the cingulate gyrus previously noted as schizophrenia endophenotypes were likewise associated with illness risk and as having high *ERV* values (e.g., caudal anterior cingulate gyrus *ERV* = .516). However, these measures did not survive correction for multiple comparisons. It is tempting to suggest that the medial temporal cortex regions associated with schizophrenia risk, which include the entorhinal and parahippocampal gyri, that are spatially proximal and involved in declarative memory, and the fusiform gyrus, which is involved in facial processing (68), could also be associated with the facial memory endophenotype identified in this sample, providing a parsimonious link between neurocognitive and neuroanatomic endophenotypes.

Using a newly derived variant of the endophenotype ranking value statistic based upon the coefficient of relationship, we demonstrate that large unselected pedigrees can provide evidence that a measure is a candidate endophenotype for schizophrenia and rank those endophenotypes according to their genetic covariance with the illness.

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