Archival Report

Biological Psychiatry

Discovering Schizophrenia Endophenotypes in Randomly Ascertained Pedigrees

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ABSTRACT

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.

Keywords: Coefficient of Relatedness, Cognition, Cortical Surface Area, Endophenotype, Family Study, Schizophrenia

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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

SEE COMMENTARY ON PAGE 3

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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).

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