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Adjudicating Neurocognitive Endophenotypes for Schizophrenia

David C. Glahn,[1]* Laura Almasy,[2] John Blangero,[2] Gary M. Burk,[1] Jose Estrada,[1] Juan Manuel Peralta,[2] Naxhielli Meyenberg,[3] Mariana Pereira Castro,[4] Jennifer Barrett,[1] Humberto Nicolini,[3] Henriette Raventós,[4] and Michael A. Escamilla[1,5]

[1]*Department of Psychiatry, University of Texas Health Science Center San Antonio, San Antonio, Texas*
[2]*Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, Texas*
[3]*Grupo de Estudios Médicos y Familiares Carracci, Mexico City, DF, Mexico*
[4]*Centro de Investigación en Biología Molecular y Celular, Universidad de Costa Rica, San José, Costa Rica*
[5]*Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, Texas*

Although genetic influences on schizophrenia are well established, localization of the genes responsible for this illness has proven extremely difficult. Given evidence that genes predisposing to schizophrenia may be transmitted without expression of the clinical phenotype, efforts have focused on developing endophenotypes. While several neuropsychological measures have been proposed to be endophenotypes, few studies have systematically assessed batteries of neurocognitive tests to determine which tests are most sensitive to liability for the illness. Two hundred sixty-nine Latino individuals were administered a standard neuropsychological battery. Two hundred fourteen of these were members of families with at least two siblings diagnosed with schizophrenia or schizoaffective disorder. The remaining were community controls without history of major psychiatric illness. Neurocognitive measures found to be heritable were entered into analyses designed to determine which tests covary with the degree of genetic relationship to affected individuals. Although five measures were found to uniquely model genetic liability for schizophrenia, digit symbol coding was the most sensitive. To assess the specificity of these endophenotypes, performance on these measures were compared to family members with bipolar and unipolar affective disorders. These markers clearly distinguished between individuals with psychotic illnesses and those with major depression. As measures contributed uniquely to discriminate individuals at varying risk for schizophrenia, our findings imply multiple independently inherited elements to the liability for the illness. We present a practical model for adjudicating endophenotypes and determining which measures are best suited for use in linkage analyses. © 2006 Wiley-Liss, Inc.

KEY WORDS: schizophrenia; endophenotype; genetics; family studies; neurocognitive; neuropsychological; bipolar disorder

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INTRODUCTION

Despite considerable evidence that risk for schizophrenia is inherited [Gottesman and Shields, 1967, 1982; Cannon et al., 1998], the molecular genetic basis for this illness remains elusive. Given that genes predisposing to schizophrenia may be transmitted without expression of the clinical phenotype, interest has arisen in developing quantitative endophenotypes [Gottesman and Gould, 2003], indicators of processes mediating between genotype and phenotype. Endophenotypes can be measured in all individuals (both affected and unaffected) and provide much greater power to localize and identify disease-related quantitative trait loci (QTLs) than affection status alone [Almasy and Blangero, 2001; Blangero et al., 2003]. Such QTLs should lead to the discovery of gene(s) involved in specific biological pathways disrupted in at least a portion of patients with schizophrenia, which in turn could lead to refinements in the diagnostic nosology [Tsuang et al., 2000]. Furthermore, established endophenotypic markers could help identify the relevant behavioral or functional correlates of susceptibility genes identified through traditional linkage or association approaches or help to define subpopulations in which to pursue more focused genetic hypotheses [Gottesman and Gould, 2003].

The great promise of endophenotypes for providing insights into the pathophysiology of schizophrenia has led to significant scientific activity and the proposal of numerous potential markers, including neuropsychological impairments [Faraone et al., 1999; Cannon et al., 2000; Egan et al., 2001a; Myles-Worsley and Park, 2002; Glahn et al., 2003; Hoff et al., 2005], neuroanatomic abnormalities [Cannon et al., 2002; McDonald et al., 2002; Seidman et al., 2002], and electrophysiological deviations [Myles-Worsley, 2002; Bramon et al., 2005]. However, relatively little research has focused on determining

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*Correspondence to: David C. Glahn, Ph.D., Department of Psychiatry, The University of Texas Health Science Center at San Antonio, Mail Code 7792, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900. E-mail: glahn@uthscsa.edu

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which of these putative endophenotypes are likely to be effective in linkage or association studies, raising the possibility that measures significantly influenced by environmental or epigenetic influences could be employed, leading to ambiguous or spurious findings. Gottesman and Gould [2003] warn "the use of endophenotypes in genetic research must be tempered by the realization that without controls and limits, their usefulness may be obscured." One goal of this article is to outline a strategy for prioritizing endophenotypes based on the extent to which they are influenced by genetic factors and the ability of the proposed endophenotypes to discriminate between individuals in different liability classes related to serious medical illnesses. While in this article we focus on appraising neurocognitive endophenotypes for schizophrenia, the procedures described herein could in principal be applied to any set of candidate phenotypes for any complex genetic illness.

In addition to assessing the utility of neuropsychological endophenotypes, we examine the relevance of these markers for other psychiatric phenotypes found in the families of persons with schizophrenia. As it is possible that genes predisposing schizophrenia also relay risk for other psychiatric disorders [Berrettini, 2000], particularly bipolar affective disorder, we evaluate the specificity of candidate endophenotypes to schizophrenia by comparing performance between individuals with schizophrenia to their family members with affective illnesses. To these ends, we studied extended pedigrees with at least two siblings with schizophrenia or schizoaffective disorder, and community comparison subjects with similar socioeconomic status and educational achievement.

MATERIALS AND METHODS

Subjects

Two hundred sixty-nine Latino individuals from the central valley of Costa Rica (35% of the sample), Mexico City, Mexico (30%), or San Antonio, Texas (35%) participated in the study. Two hundred fourteen of these persons were members of 46 families with at least two siblings diagnosed with schizophrenia or schizoaffective disorder. Subjects were recruited through advertisements and screening of patients at outpatient and inpatient facilities in these cities. Inclusion and exclusion criteria were the same across sites and required a previous diagnosis of schizophrenia in at least two siblings. Recruiters at each site were asked to recruit all available 1st and 2nd degree relatives in the families being studied. With few exceptions, relatives approached participated in the study. Age of onset, course of illness, and gender distribution of the patients with schizophrenia are comparable with studies of schizophrenia drawn from these and other populations. The remaining 55 subjects were community controls drawn in equal numbers for each site and without personal or family history of major psychiatric illness, as assessed by a structured clinical interview [Spitzer et al., 1994]. Community controls were recruited through advertisements in local news papers and via fliers placed in medical clinics.

Procedures

After providing written consent on forms approved by the review boards of all participating study sites, each subject received the South Texas Assessment of Neurocognition (STAN), a 90-min neuropsychological evaluation consisting of standard and computerized measures. Tests included in this battery were selected based on evidence of heritability, sensitivity to schizophrenia, and minimizing the effects of language (culture) or the availability of parallel English and Spanish forms. The STAN has parallel Spanish and English versions. Instructions for the computerized neuropsychological tests were translated to Spanish by bilingual psychologists and translated back into English by professional translators. Other tests (e.g., the California Verbal Learning Test) have published English [Delis et al., 2000] and Spanish [Fortuny et al., 2001] editions. All subjects received the same battery of tests in a fixed order and were allowed breaks as needed.

One member of each family completed the Family Interview for Genetic Studies (FIGS; [Maxwell, 1992]). Individuals indicated by the FIGS to have possibly had any form of mental illness subsequently received the Diagnostic Interview for Genetic Studies (DIGS; [Nurnberger et al., 1994]). Final diagnoses for each subject were assigned by a best estimate consensus process [Leckman et al., 1982] based on the DIGS and FIGS interviews and available medical records. Family members who did not receive a FIGS diagnosis of mental illness (including substance abuse) did not undergo a direct diagnostic interview.

Statistical Analyses

Statistical analyses were performed to determine which neuropsychological measures were (1) highly heritable, (2) sensitive to genetic liability for schizophrenia, and (3) specific to schizophrenia.

1. Heritability analyses were performed with Sequential Oligogenic Linkage Analysis Routines (SOLAR; [Almasy and Blangero, 1998]) and based on modeling the covariance among family members as a function of genetic proximity (kinship). Indices with stronger covariance between genetically more similar individuals than between genetically less similar individuals have higher heritability. Neuropsychological variables with significant heritabilites ($P < 0.01$) were included in subsequent analyses.
2. Neuropsychological variables were analyzed for association with genetic liability status for schizophrenia or schizoaffective disorder (i.e., unaffected 1st degree relative without an Axis I diagnosis, unaffected 2nd degree relative without an Axis I diagnosis, and healthy unrelated control without an Axis I diagnosis). An individual's genetic liability for schizophrenia/schizoaffective disorder was defined as the shortest genetic distance to an affected individual. Initially we performed a canonical discriminant analysis, a multivariate approach which derives the combination of predictor variables that best discriminates among the different levels of a classification variable (genetic distance from an affected individual) while controlling for redundancy (intercorrelation) by evaluating their unique contributions to the discrimination. In addition, we used a stepwise recursive elimination algorithm, excluding, in successive steps, predictors with insignificant ($P > 0.5$) contributions to the discrimination to determine those markers that independently discriminate between liability classes. Discriminant analyses were performed in SAS 9.1 (SAS Institute, Cary, NC). Since both genetic and non-shared environmental or disease-specific effects (e.g., medication usage, hospitalization, other illness sequela) potentially impact neuropsychological performance in patients with schizophrenia or schizoaffective disorder, including these individuals in the discriminant analysis could overestimate the influence of genetic factors. Thus, these subjects were excluded from these analyses. Similarly, family members diagnosed with major psychiatric illnesses other than schizophrenia or schizoaffective disorder were excluded.

To complement the canonical discriminant analysis, a Bayesian selection analysis was performed to determine which neurocognitive variables best predict liability class [Blangero et al., in press). In a Bayesian framework, comparison of two competing hypotheses can be performed by the evaluation of the Bayes factor, which is the ratio of the integrated likelihoods of the competing models [Kass and Raftery, 1995]. Bayes factors provide a direct evaluation of the superiority of one model (neurocognitive variable), over another [Kass and Raftery, 1995]. Furthermore, Bayes factor can be used to assess whether a specific model explains sufficient variation in the phenotype to justify the number of parameters used. We systematically assessed 2^n models, where n is the number of heritable neurocognitive measures assessed independently, in groups of two and in all possible sets of three, to determine the neuropsychological indices that best predict liability for schizophrenia/schizoaffective disorder.

3. To determine the specificity of impairment on neuropsychological measures found to be sensitive to liability for schizophrenia, comparisons between individuals with schizophrenia or schizoaffective disorder, and their relatives with either bipolar or unipolar depression were conducted. A mixed-effects ANCOVA model was performed. Single degree of freedom geometric contrasts were applied to test-specific hypotheses about group differences (i.e., schizophrenia/schizoaffective vs. bipolar disorder). Degrees of freedom were estimated with the Satterthwaite procedure and all variables found not to conform to the assumptions of normalcy (Shapiro–Wilk test, $P > 0.01$) were log- or power-transformed to approximate a normal distribution. The significance criterion for all hypothesis testing was set at alpha = 0.05, two-tailed.

RESULTS

Sample Characteristics

Of the 214 subjects from extended pedigrees, 55 had a best estimate consensus DSM-IV diagnosis of either schizophrenia or schizoaffective disorder (either depressive (n = 5) or bipolar subtypes (n = 12)), and are considered "affected," 21 were diagnosed with bipolar disorder (67% of whom had significant psychotic features), 16 with other affective illnesses, and 12 with other Axis I diagnoses, primarily alcohol dependence (see Table I). None of the remaining subjects was diagnosed with an Axis I illness (these subjects are designated as "unaffected" in the subsequent discussion). Of these unaffected relatives, 40 were 1st degree and 29 were 2nd degree relatives of individuals with schizophrenia or schizoaffective disorder. Ninety-six subjects were not biologically related to affected individuals and were used to form a genetic "unrelated" control sample; 41 were subjects who had married into selected families and 55 were unaffected subjects with no biologic or familial ties to the other subjects. Since liability groups (schizophrenia/schizoaffective "affected" group, 1st degree relatives, 2nd degree relatives, unrelated controls) differed in average age ($F[6,262] = 10.33$, $P < 0.001$) and in gender distributions ($\chi^2 = 15.39$, $P < 0.02$), these demographic variables were controlled in subsequent analyses.

Impact of Location, Language, and Education on Cognitive Performance

To determine if neuropsychological performance varied between countries, a MANOVA was performed with location as a between subjects variable (Costa Rica, Mexico, or United States) and with all of the cognitive indices modeled as a single repeated measure (genetic liability for schizophrenia/schizoaffective disorder was covaried). A significant main effect for location ($F[2,261] = 3.79$, $P = 0.02$) indicates that neurocognitive performance differs between study sites. These effects were decomposed with single degree of freedom contrasts which revealed non-significant differences between those subjects assessed in Mexico and the United States ($F = 0.01$, $P = 0.91$), but significant differences between the persons tested in the United States and Costa Rica ($F = 6.03$, $P = 0.01$) and between individuals assessed in Mexico and Costa Rica ($F = 4.60$, $P = 0.02$). Between site differences cannot easily be explained by assessment language, as both the Mexican and Costa Rican assessments were conducted entirely in Spanish and the United States assessments were done primarily in English.

While neuropsychological test performance is influenced by a vast array of moderating variables, learning opportunities vary significantly across countries and educational attainment has a more profound impact on neurocognitive performance than other factors [Ponton et al., 1996; Anger et al., 2000; Chung et al., 2003; Harvey et al., 2003]. In the current sample, educational attainment varied significantly between individuals studied in different countries ($F[2,226] = 39.16$, $P < 0.0001$), with higher levels of education in the United States (average ± standard deviation: 11.5 ± 2.8 years) than in Mexico (8.9 ± 5.0) and Costa Rica (7.1 ± 3.1). When educational attainment was entered as a covariate, between site differences in neuropsychological performance were attenuated ($F[2,261] = 0.33$, $P = 0.71$). Given these findings, location of assessment and level of education were entered as covariates in subsequent analyses.

Heritability Analysis

Estimated heritabilities for 18 variables are presented in Table II. These variables were grouped into theoretically

TABLE I. Sample Characteristics

Groups	n	Age	Education	Female (%)	Costa Rica (%)	Mexico (%)	Texas (%)
Schizophrenia/ Schizoaffective	55	37.5+15 [15–72]	7.8+4 [0–20]	38%	29	40	31
Bipolar disorder	21	42.7+12 [18–59]	7.5+4 [0–15]	67%	52	19	29
Unipolar disorder	16	35.1+10 [19–52]	9.4+3 [5–14]	63%	14	43	43
Other axis I	12	48.4+14 [24–70]	8.9+5 [5–20]	42%	25	23	52
Unaffected 1st degree	40	48.0+15 [19–79]	9.8+5 [0–22]	68%	35	45	20
Unaffected 2nd degree	29	30.0+9 [20–51]	8.2+3 [3–16]	66%	56	21	23
Unrelated individuals	96	33.0+12 [15–63]	11.7+3 [5–16]	66%	34	30	36

Age and education presented in years (Mean ± Standard Deviation [range]). The final three columns reflect the percentage of the sample recruited from each location.

TABLE II. Neuropsychological Measures and Their Heritabilities

Neurocognitive task	Dependant measure	Full sample (n = 269)		Excluding affected (n = 244)	
		h^2	P	h^2	P
Speed of processing					
Letter fluency[a]	Words beginning with a specific letter generated in 60 sec	0.378	9×10^{-4}	0.584	1×10^{-4}
Semantic fluency[a]	Animal names generated in 60 sec	0.446	0.002	0.290	0.050
Digit-symbol coding[b]	Correctly identified digit-symbol pairs in 90 sec	0.782	4×10^{-8}	0.746	2×10^{-9}
Trail Making Test, Part A[a]	Time needed to connect letters in ascending order	0.463	3×10^{-5}	0.344	8×10^{-4}
Attention/vigilance					
CPT a′[c]	Discriminability index for a 6 min identical pairs continues performance test	0.366	0.003	0.211	0.095
CPT Beta[c]	Bias index for a 6 min identical pairs continues performance test	0.472	4×10^{-4}	0.366	0.010
Digit span forward[b]	Correctly recalled digits strings in original order of presentation	0.770	2×10^{-7}	0.788	3×10^{-6}
Working memory					
Digit span backward[b]	Number of correctly recalled digits strings in reverse order of presentation	0.192	0.150	0.089	0.308
Spatial working memory[d]	Number of correct trials on a spatial delayed response test	0.466	6×10^{-5}	0.388	2×10^{-4}
Verbal episodic memory					
CVLT learning recall[e]	Number of items recalled over 5 repeated exposures of a 16 word list from the California Verbal Learning Test (CVLT)	0.376	2×10^{-4}	0.496	6×10^{-5}
CVLT semantic cluster[e]	Proximal recall of semantically related list items	0.492	9×10^{-5}	0.584	1×10^{-4}
CVLT delayed recall[e]	Number of list item recalled after a 20 min delay	0.495	6×10^{-5}	0.386	0.003
CVLT recognition[e]	Number of items recognized after a 20 min delay	0.454	4×10^{-4}	0.186	0.136
Digit-symbol recall[b]	Number of digits recalled when presented with corresponding symbols	0.530	6×10^{-5}	0.772	4×10^{-6}
Reasoning & problem solving					
Test of non-verbal intelligence[f]	Number of correctly completed progressive matrices	0.145	0.199	0.177	0.195
Abstraction[g]	Number of correct abstraction trials from the AIM paradigm	0.191	7×10^{-4}	0.219	0.043
Abstraction plus memory[g]	Number of correct abstraction plus memory trials from the AIM paradigm	0.014	0.462	0.000	0.500
Trail Making Test, Part B[a]	Time needed to connect alternating letters and numbers	0.414	4×10^{-4}	0.325	0.005

[a]Delis et al. [2004] or Fortuny et al. [2001].
[b]The Psychological Corporation [1997]) or Fortuny et al. [2001].
[c]Based on a measure similar to Cornblatt and Malhotra [2001].
[d]Glahn et al. [2003].
[e]Delis et al. [2000] or Fortuny et al. [2001].
[f]Brown et al. [1997].
[g]Glahn et al. [2000].
h^2=Estimated sample-specific heritability calculated in SOLAR.

distinct cognitive domains consistently shown to be impaired in schizophrenia [Nuechterlein et al., 2004]. Age, gender, education level, and location of assessment were included as covariates in both analyses. Three measures failed to reach significant levels of heritability ($P < 0.01$): digit span backwards, abstraction plus memory from the AIM, and the test for non-verbal intelligence (TONI). Performance on the digit span backwards subtest and the TONI were significantly correlated with educational attainment and when this covariate was omitted both markers were significantly heritable ($h^2 = 0.903$, $P < 1 \times 10^{-2}$; $h^2 = 0.548$, $P < 2 \times 10^{-6}$, respectively). In contrast, minimal heritability on the abstraction plus memory subtest may be due to reduced variance associated with floor effects.

Heritability analyses were repeated while excluding affected individuals. Seven variables failed to reach significance in this analysis: semantic fluency, the CPT a′ index, digit span backwards, long delay recognition from the CVLT, the TONI, and both the abstraction and the abstraction plus memory conditions of the AIM task. Disparities between these two analyses might reflect exaggerated heritability estimates when including affected individuals who tend to have poor

performance, or may be due to reduced sample size. However, given this disparity, subsequent analyses were performed initially with the 15 traits found to be heritable in the full sample followed by an analysis restricted to the 11 variables that survived the second analysis.

Liability for Schizophrenia

Results of the canonical discriminant analyses predicting genetic liability class are given in Table III. These analyses were performed on neuropsychological measures after statistically controlling (via multiple regression) for age, gender, educational attainment, and location of assessment. Although the canonical correlation of the 15 cognitive measures found to be significant in the complete sample with liability status was highly significant ($r = 0.643 \pm 0.05$, $F[15,149] = 7.18$, $P < 0.0001$), only five measures contributed a significant ($P < 0.05$) proportion of *unique* variance to the discrimination: Digit-Symbol Coding, Spatial Working Memory, Trail Making Test A, Letter Fluency, and California Verbal Learning Test (CVLT) Learning Recall. The same five measures were uniquely discriminate when these analyses were restricted to the 11 cognitive variables found to be heritable excluding affected individuals and the empirically derived canonical variable fit the data equally well ($r = 0.61 \pm 0.05$, $F[11,153] = 9.42$, $P < 0.0001$). Figure 1A indicates the magnitude of impairment on each measure separately for each liability class.

In addition to the canonical discriminate analyses, we performed a Bayesian model selection analysis to determine which set of these neuropsychological variables best predicts genetic liability for schizophrenia. We evaluated all 2^{15} (32,768) possible liability models and obtained strong evidence that digit-symbol coding is the best predictor of affection status with posterior probability of effect of 0.9899 (schizophrenia or schizoaffective patient vs. healthy unrelated subjects). This index also best discriminated healthy first (posterior probability of effect >0.95) and second (posterior probability of effect >0.95) degree relatives from unrelated subjects.

Specificity for Schizophrenia

As can be seen in Figure 1B, with the exception of letter fluency, performance by family members with bipolar disorder was indistinguishable from their relatives with schizophrenia or schizoaffective disorder ($F[1,936] = 0.90$, $P < 0.34$, NS). In contrast, individuals with unipolar depression were clearly dissociable from their "affected" relatives ($F[1,936] = 17.36$, $P < 0.0001$).

DISCUSSION

Using an extended pedigree design, 18 neurocognitive measures were evaluated as putative endophenotypes for schizophrenia. A canonical discriminant analysis indicated that five separate measures were independently sensitive to genetic liability for the illness: digit-symbol coding, spatial working memory, CVLT recall, trail making test part A, and letter fluency. In a separate Bayesian analysis, which accounted for pedigree size and structure, digit-symbol coding was shown to be the best predictor of who has schizophrenia/schizoaffective disorder, who is a first degree relative and who is a second degree relative of someone with these disorders, versus who is a control. These findings are generally consistent with previous reports that measures of processing speed and working and declarative memory are sensitive to the liability for schizophrenia [Cannon et al., 2000; Egan et al., 2001a; Hoff et al., 2005]. However, they extend the current literature by demonstrating that multivariate analytic methods can be successfully applied to quantitative measurements collected within extended pedigrees.

Digit-symbol coding was by far the most sensitive index of genetic liability for schizophrenia. While speed of processing can be influenced by a host of environmental or illness-related factors and by age-related declines [Joy et al., 2004], these measures are thought to index neuronal efficiency and to be sensitive to subtle brain dysfunction. Current cognitive neuroscience models of processing speed tasks, like digit-symbol coding, trail making and to some extent letter fluency,

TABLE III. Results of Canonical Discriminate Analysis

	15 Variable Model				11 Variable Model			
Neurocognitive task	Rank	Partial r^2	F	*P*	Rank	Partial r^2	F	*P*
Speed of processing								
Letter fluency	4	0.086	7.52	0.001	4	0.086	7.52	0.001
Semantic fluency	8	0.017	1.34	0.264				
Digit-symbol coding	1	0.226	23.66	0.001	1	0.23	23.66	0.001
Trail Making Test, Part A	3	0.063	5.39	0.005	3	0.063	5.39	0.005
Attention/vigilance								
CPT a′	7	0.024	1.89	0.155				
CPT Beta	12	0.007	0.55	0.576	8	0.016	1.22	0.298
Digit span forward	9	0.017	1.31	0.272	7	0.020	1.63	0.200
Working memory								
Digit span backward								
Spatial working memory	2	0.083	7.24	0.001	2	0.083	7.24	0.001
Verbal episodic memory								
CVLT learning recall	5	0.036	3.08	0.049	5	0.038	3.08	0.049
CVLT semantic cluster	6	0.026	2.05	0.132	6	0.026	2.05	0.136
CVLT delayed recall	10	0.015	1.13	0.327	9	0.016	1.29	0.279
CVLT recognition	15	0.002	0.14	0.867				
Digit-symbol recall	11	0.008	0.59	0.557	10	0.009	0.73	0.485
Reasoning & problem solving								
Test of non-verbal intelligence								
Abstraction	14	0.002	0.13	0.878				
Abstraction plus memory								
Trail Making Test, Part B	13	0.003	0.20	0.818	11	0.004	0.30	0.739

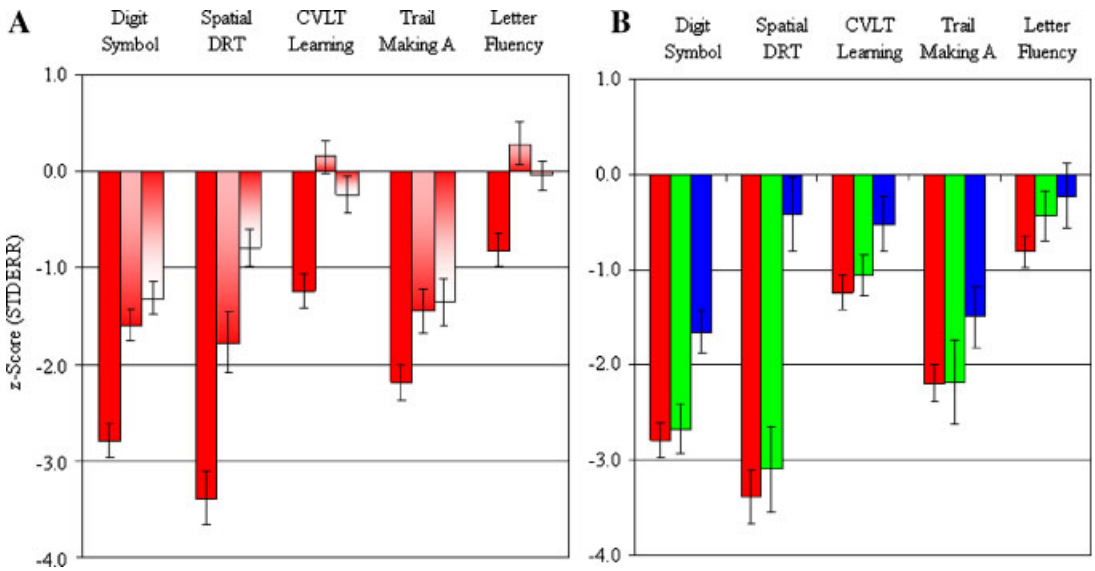

Fig. 1. **Panel A**: Performance scores on each of the five putative endophenotypic markers for individuals affected with either schizophrenia or schizoaffective disorder (black bars), unaffected 1st degree relatives (gray bars), and unaffected 2nd degree relatives (unfilled bars). Affected individuals perform worse on all measures incomparison to their unaffected family members, who are typically more impaired based on genetic proximity to an affected individual. **Panel B**: Performance by individuals with either schizophrenia or schizoaffective disorder (black bars), bipolar disorder (gray bars), and unipolar depression (unfilled bars). While family members with bipolar disorder are generally as impaired as patients with schizophrenia or schizoaffective disorder, those with unipolar depression show considerably less impairment. Data are presented as z-transforms based on the unrelated subjects performance with a mean of 0 and standard deviation units, while covering for the effects of age, gender, education level, and location of assessment. Error bars represent the standard error of the mean.

highlight the timely interplay of a prefrontal decision-making node, and posterior heteromodal regions including areas of parietal and temporal cortex [Fry and Hale, 2000; Hansell et al., 2005]. These models emphasize the integration of information across spatially distinct brain regions, rather than the activity of a specific region, suggesting that cognitive slowing as indexed by processing rate is directly related to neuronal efficiency [Rypma et al., 2005].

In addition to the digit symbol coding, trail making and verbal fluency measures, spatial working memory and verbal declarative memory indices were also associated with liability for schizophrenia, implicating large-scale neural networks including prefrontal and temporal cortices, respectively. While it is possible that a single brain region, common to each of these networks, causes these relatively distinct neuropsychological deficits, the functional neuroimaging literature has yet to identify this locus of dysfunction [Glahn et al., 2005]. Rather the field is beginning to focus on how various brain regions interact when engaged in task-specific networks. Indeed, Andreasen and colleagues proposed that the "cognitive dysmetria" present in schizophrenia is closely associated with poor cortical-cortical and cortical-subcortical connectivity [Andreasen, 1999]. Poor functional connectivity may result from a number of different sources, including aberrant neurotransmitter regulation (e.g., reduced dopaminergic tone [Egan et al., 2001b]), inefficient neural synchronization [Kwon et al., 1999], or subtle changes in neuronal architecture (e.g., reduced neuropil [Selemon and Goldman-Rakic, 1999]).

We present a systemic analytic path designed to determine the best putative endophenotypic markers from a group of inner-correlated neurocognitive measures. Such analysis strategies are becoming critical given the increasing number of endophenotypes proposed for psychiatric illnesses [Gottesman and Gould, 2003; Glahn et al., 2004; Hasler et al., 2004] and the necessity to minimize the total number of markers applied in linkage studies to protect against type I error. Our proposed analysis path requires that before a measure is considered to be a marker of genetic liability for an illness, it must demonstrate significant levels of heritability. While requiring that an index is influenced by inherited factors is seemingly obvious for a genetics study, few previous reports document the heritability of measures [Tuulio-Henriksson et al., 2002]. It should be noted that the heritability estimates derived in the current sample did not explicitly control for the impact of shared environment. However, since the focus of the current study was on extended pedigrees the potential for the inflation of heritability estimates by shared familial environment is reduced.

Once heritability requirements are met, analysis methods should model potential relationships between measures (correlation/redundancies) and preferably model relationships between family members (e.g., Faraone et al., 1999; Cannon et al., 2000; Hoff et al., 2005). We applied two multivariate methods: a canonical discriminate analysis and Bayesian analysis. While the canonical analysis was more sensitive than the Bayesian method, the Bayesian selection accounted for the pedigree structures. Regardless of the analysis procedure employed, assessment of endophenotypes should include statistical methods that determine which markers independently predict liability for the illness in question. This two step analytic approach, initially requiring that a measure is heritable followed by a multivariate procedure designed to limit the number of indices analyzed should focus linkage studies of quantitative endophenotypes and minimize spurious findings.

Although the five putative neurocognitive endophenotypes for schizophrenia were insensitive to group differences between individuals with psychotic illnesses (schizophrenia/schizoaffective disorder and bipolar affective illness), these same markers clearly distinguished between individuals with psychotic illnesses and those with major depression. Such findings could suggest overlapping etiology in schizophrenia and psychotic bipolar disorder [Potash et al., 2003]. However the bipolar patients studied here were selected through family

members with schizophrenia and thus may be representative of only a subset of individuals with bipolar disorder. A more rigorous evaluation of the specificity of these markers for schizophrenia/schizoaffective disorder would include assessing unaffected relatives ascertained by family members with other mental illnesses and is currently underway.

The neuropsychological data examined in the current manuscript was collected as part of a larger study designed to find genes predisposing schizophrenia. In this context, we did not exclude individuals with an Axis I disorder and comorbid current or historical substance abuse/dependence. However, when analyses were repeated while excluding subjects who met criteria for current DSM-IV alcoholism or substance abuse, findings remained consistent with those described above. A limitation of the current study is that family members found to be unaffected by FIGS interview did not receive a direct psychiatric interview. Furthermore, we did not utilize Axis II diagnoses (personality disorders) in the current analyses. Thus it is possible that some of the unaffected family members or healthy comparison subjects met criteria for schizotypal personality disorder, an illness associated with neuropsychological impairments [Siever and Davis, 2004]. Yet, as part of the spectrum of schizophrenia-related illnesses, schizotypal personality disorder is strongly related to genetic liability for schizophrenia and excluding these individuals from analyses designed to adjudicate potential endophenotypes would be unadvisable [Kendler et al., 1993].

Our findings should facilitate the search for susceptibility genes for schizophrenia given that the proposed cognitive endophenotypes should significantly increase statistical power to localize genetic loci. The neuropsychological endophenotypes identified here could also be used to index the behavioral or functional impact of previously proposed susceptibility genes or help to define subpopulations in which to pursue more focused genetic hypotheses. In addition, the method outlined above for the selection of an endophenotype most closely associated with a specific phenotype should assist comparable studies proposing correlated measures as endophenotypes for any genetically mediated disorder.

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