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Verbal Working Memory Impairments in Individuals with Schizophrenia and Their First-Degree Relatives: Findings From the Consortium on the Genetics of Schizophrenia

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Abstract

Working memory (WM) impairment is a promising candidate endophenotype for schizophrenia that could facilitate the identification of susceptibility genes for this disorder. The validity of this putative endophenotype was assessed by determining whether 149 probands with schizophrenia and 337 of their first-degree relatives demonstrated WM impairment as compared to 190 unaffected community comparison subjects. Subjects were participants in the Consortium on the Genetics of Schizophrenia (COGS) project, a seven-site research network that was established to investigate the genetic

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architecture of endophenotypes for schizophrenia. Participants received comprehensive clinical assessments and completed two verbal WM tasks, one requiring transient on-line storage and another requiring maintenance plus complex manipulation of information by reordering the stimuli. Schizophrenia probands performed worse than the other groups on both tasks, with larger deficits found for the more challenging reordering WM task. The probands' relatives performed more poorly than community comparison subjects on both tasks, but the difference was significant only for the more challenging maintenance plus complex manipulation WM task. This WM impairment was not attributable to diagnoses of schizophrenia spectrum disorder, mood disorders, or substance use disorders in the relatives. In conjunction with evidence that WM abilities are substantially heritable, the current results support the validity and usefulness of verbal WM impairments in manipulation of information as endophenotypes for schizophrenia in large-scale genetic linkage and association studies.

Introduction

While there is strong evidence for genetic transmission of vulnerability to schizophrenia (Harrison and Weinberger 2005; Tsuang 2001), the heterogeneity and complexity of this "group" of clinical phenotypes pose great obstacles for research aimed at understanding the molecular genetic basis of liability to this disorder. A powerful alternative for identifying susceptibility genes is to study the transmission of endophenotypes that are presumed to be more closely linked to the underlying genes that mediate schizophrenia and are likely to have a simpler genetic architecture than the clinical phenotype (Braff and Freedman 2002; Braff et al 2007; Gottesman and Gould 2003). Furthermore, endophenotypes are presumed to vary quantitatively among individuals at risk for schizophrenia, making clinically unaffected relatives of schizophrenia patients informative for linkage and association studies.

The Consortium on the Genetics of Schizophrenia (COGS) was established across seven sites to investigate the genetic architecture of six carefully selected candidate neurocognitive and neurophysiological endophenotypes for schizophrenia (Calkins et al 2007). In this report, we evaluate whether one of the COGS candidate neurocognitive endophenotypes, verbal working memory (WM) impairment, characterizes schizophrenia probands and their relatives. WM is typically defined as a limited-capacity storage system used for the temporary online maintenance and manipulation of information (Baddeley 1986). The validity of WM deficits as endophenotypes for schizophrenia is supported by four main lines of evidence.

First, schizophrenia patients consistently demonstrate WM deficits across a diverse range of tasks (Lee and Park 2005). Two broad types of WM paradigms have typically been studied in schizophrenia (Perry et al 2001). The first type assesses transient, on-line maintenance functions that do not involve manipulation of the stored information, whereas the second type involves maintenance plus manipulation of information (sometimes called "executive functioning" WM). Schizophrenia patients often show more severe impairment on WM tasks that involve maintenance plus complex manipulation (Barch 2005). In the verbal domain, patients and healthy controls show average separations of about 0.71 - 0.82 standard deviations on both digit span forward and backward repetition tasks (Aleman et al 1999). However, group differences can exceed 1.4 standard deviations on more complex letter-number sequencing tasks (e.g., (Conklin et al 2005; Gold et al 1997; Perry et al 2001)).

Second, WM impairment appears to be a core feature of schizophrenia that is not attributable to other aspects of the illness. WM deficits show minimal cross-sectional correlations with severity of delusions and hallucinations, are detectable in clinically stabilized outpatients, and appear to be relatively stable across both time and fluctuations in clinical status, suggesting that they are not merely secondary manifestations of psychotic symptoms (Heaton et al

2001; Hill et al 2004; Park et al 1999). They are also not secondary to antipsychotic medication side effects or factors associated with chronicity, as deficits of comparable magnitude are present in neuroleptic-free patients (Barch et al 2001; Carter et al 1996) and during the immediate post-onset period (Hutton et al 1998; Lussier and Stip 2001).

Third, WM abilities appear to be genetically mediated. Heritability estimates for verbal and spatial WM tasks are moderately high in both healthy individuals (.43 - .49; (Ando et al 2001; Hansell et al 2005)) and schizophrenia patients (.36 - .42; (Tuulio-Henriksson et al 2002)). In addition, WM impairments in healthy siblings of schizophrenia probands scale in severity with genetic loading for this disorder in singleton versus multiplex families (Tuulio-Henriksson et al 2003) and in discordant dizygotic versus monozygotic twin pairs (Cannon et al 2000; Glahn et al 2003).

Fourth, similar, though attenuated, WM disturbances are also present in clinically unaffected biological relatives of schizophrenia patients. First-degree relatives of schizophrenia probands score about .25 to .50 standard deviations below healthy controls across verbal and spatial WM tasks (Snitz et al 2006; Trandafir et al 2006). Some, though not all, studies suggest that relatives may perform more poorly on tasks that require more demanding executive functions. For example, a recent study found that effect sizes between relatives of schizophrenia probands and controls increased as one moved from digit span forward ($d = .43$) to digit span backward ($d = .56$) and to letter-number sequencing ($d = .66$) (Conklin et al 2005).

These converging lines of evidence suggest that WM deficits, particularly those involving executive functions, reflect genetically-mediated susceptibility to schizophrenia. This study evaluated the performance of large number of well characterized schizophrenia patients, their first-degree relatives, and community comparison subjects on the Letter-Number Span (LNS) task (Gold et al 1997; Wechsler 1997), which included a forward span repetition condition and a reordering condition.

Although other studies have considered WM performance in first-degree relatives of schizophrenia patients, this study had several advantageous features. First, this is the largest study to date. Second, this study included comprehensive clinical assessments which, in combination with the good statistical power, enabled us to evaluate a variety of factors that may contribute to performance. Third, the COGS uses a highly rigorous data collection quality assurance program (described in (Calkins et al 2007)) that ensures the cross-site reliability of the dependent measures. Fourth, the recruitment scheme of COGS is somewhat unusual. As described below and elsewhere (Calkins et al 2007), the COGS employs a distinctive ascertainment strategy designed to optimize genetic analyses of quantitative endophenotypes. The minimal requirement for pedigree ascertainment includes a schizophrenia proband, both parents (at least one unaffected), and at least one unaffected sibling. Sampling both affected and unaffected individuals increases variation or “contrast” in the proposed endophenotypes (see (Braff et al 2007; Schork et al 2007) for reviews). This contrast-based approach differs from alternate recruitment strategies (e.g., affected sibling pairs and multiplex families) that are commonly used for studying qualitatively-defined disease phenotypes. Hence, the current study carefully evaluated whether working memory displays the expected patterns of performance in families using this contrast-based family recruitment method.

The primary variable for working memory for COGS was defined *a priori* as the Reordered condition of the LNS, which has a mental manipulation component. The secondary measure was the simple repetition condition was included to examine the genetics of WM maintenance. In line with expectations for an endophenotype (Gottesman and Gould 2003), the primary hypothesis was that schizophrenia patients would perform worse than their relatives, who would in turn perform worse than comparison subjects, on the LNS Reordered condition.

Methods

Overview

The COGS is a 7-site National Institute of Mental Health (NIMH) funded project designed to collect neurocognitive and neurophysiological endophenotypes and to perform genetic analyses on schizophrenia subjects, their first-degree relatives, and community comparison subjects (CCS). The COGS includes the University of California San Diego, University of California Los Angeles, University of Colorado, Harvard University, Mount Sinai School of Medicine, University of Pennsylvania, and University of Washington. The local institutional review boards of each site approved the study, and all subjects provided informed consent to participate.

Participants

In accordance with the COGS quantitative statistical genetics design, targeted families included those with a) at least one proband with schizophrenia, b) 2 living parents (including at least 1 non-psychotic parent) and c) at least 1 non-psychotic sibling who participated in the study. Other eligible family pedigrees included a) 1 living non-psychotic parent and at least 2 non-psychotic siblings who participated in the study, or b) no living parents but at least 3 non-psychotic siblings who participated in the study. Neurocognitive and neurophysiological assessments were sought for all probands, siblings, and parents between 18 – 65 years old. Additional details about the recruitment and ascertainment procedures of the COGS project are available in a major descriptive paper (Calkins et al 2007).

All probands, family members, and CCS underwent a standardized diagnostic and clinical assessment protocol that included a modified version of the Diagnostic Interview for Genetic Studies (Nurnberger et al 1994), the Family Interview for Genetic Studies (NIMH Genetics Initiative, 1992), the Scale for Assessment of Negative Symptoms (Andreasen 1983), the Scale for Assessment of Positive Symptoms (Andreasen 1984), and a medical record review. Family members and CCS also completed an assessment of schizotypal and other axis II cluster A personality features via a modified version of the Structured Interview for Schizotypy (SIS (Kendler 1989). For every proband at least one relative completed endophenotyping (range 1 – 6 relatives), for an average of 2.26 relatives per family. The Relatives group was comprised of 118 parents and 206 siblings.

Interviewers were trained to administer the diagnostic rating scales by experienced COGS faculty members using a standardized training protocol. Following the interview, each subject was assigned DSM-IV best-estimate final diagnoses through a consensus diagnostic process that included at least two faculty level clinicians. All probands fulfilled DSM-IV criteria for schizophrenia according to best estimate final diagnostic procedures. The majority of probands were taking antipsychotic medications at clinically determined dosages, with 7 taking typical antipsychotics, 124 taking atypical antipsychotics, 9 taking both typical and atypical antipsychotics, and 9 taking no antipsychotic medication.

CCS were included if they had no personal history of psychosis or Cluster A Personality Disorder and no family history of psychosis. To parallel comorbidity outside of the schizophrenia spectrum in relatives of probands, other non-psychotic Axis I psychopathology was acceptable, but clinical stability and/or remission at the time of assessment was required.

Across all three groups, individuals were excluded from these analyses for electroconvulsive therapy in the past six months; a positive drug or alcohol urine toxicology screen on day of assessment; a diagnosis of substance abuse disorder in the past 30 days or substance dependence in the past 6 months; an estimated premorbid IQ of less than 70 as determined by the Wide Range Achievement Test-3 (WRAT-3; (Wilkinson 1993); or a history of head injury

involving loss of consciousness greater than 10 minutes or subsequent cognitive changes, seizure disorder, or other neurological or major systemic medical problems that could influence performance on the neurocognitive tasks.

Procedures

The WM task was administered to each subject as part of the COGS research protocol, which consisted of 4 hours of clinical assessment and 6 hours of neurophysiological and neurocognitive testing. The assessment and testing procedures were identical across the proband, relative, and CCS groups. The neurophysiological and neurocognitive tasks were presented in one of two standardized orders. Participation typically took place over two separate visits conducted within a week of each other. All efforts were made to accommodate participants' time restrictions and participants were encouraged to take breaks whenever necessary to prevent fatigue during the lengthy assessments.

Prior to study initiation, neurocognitive testers and key faculty members from each site participated in a 2-day in-person training session and certification by a COGS neuropsychologist was required. The neurocognitive testers subsequently received annual, in-person refresher training on the WM and other tasks, as well as bi-weekly phone conferences and on-site visits from a QA coordinator to ensure the accuracy and standardization of assessments (see (Calkins et al 2007)). In addition, the QA site (UCLA) performed QA checks periodically by verifying any score that was an outlier; scores that were outside plus or minus 1.5 SD of the mean within each group were flagged. Every outlier score was then compared to the paper copy at the local site to confirm that it was accurate (not a data entry mistake), and that it was valid (the local tester confirmed that the subject understood the nature of the task).

Proband symptom assessment

From the SANS, a negative symptom score was based on the mean of the subscale global ratings (excluding the Attention subscale). From the SAPS, a psychotic symptom score was based on the mean of the Hallucinations and Delusions global ratings, and a disorganization symptom score was based on the mean of the Bizarre Behavior and Formal Thought Disorder global ratings.

Working Memory Assessment

Participants completed the Letter-Number Span (LNS) (Gold et al 1997; Wechsler 1997). Two task conditions are administered. In the "LNS Reordered" condition, the tester verbally presents increasingly longer sequences of intermixed numbers and letters (e.g., G4K2) at a rate of one per second. The "LNS Reordered" condition is the same as the Letter-Number Sequencing test used in the Wechsler Memory Scale-III (Wechsler 1997). After each sequence, the participant is asked to repeat the numbers in ascending order first and then the letters in alphabetical order. In the "LNS Forward" condition (Gold et al 1997), the tester verbally presents a different set of increasingly longer sequences of inter-mixed letters and numbers at a rate of one per second. After each sequence, the participant is asked to recall the numbers and letters in the same exact order, with no reordering of the stimuli.

In both conditions, the number of digits and letters increases by one on each trial, up to a maximum length of 8 stimuli. Three sequences of the same length are presented during each trial. Both conditions are discontinued when the subject fails three consecutive sequences of the same length. The score for each condition is the total number of correctly recalled sequences. LNS Reordered data were missing for 2 Relatives ($n = 322$) and 1 Proband ($n = 148$).

Statistical methods

Data analysis was conducted in four phases. First, group differences in demographics were evaluated with one-way ANOVA's for continuous variables and chi-square tests for categorical variables. Second, group differences on LNS Reordered and Forward were evaluated using separate mixed effects regression analyses (SAS Proc MIXED, specifying an unstructured covariance structure and Satterthwaite degrees of freedom). These models used LNS scores as the dependent variables, family as a random effect to account for the non-independence of observations among genetically-related family members in the proband and relative groups, and pre-selected fixed effects and covariates. The fixed factors were group, research site, and a group X research site interaction term. The covariates were age, sex, and parental education. Age was included as a covariate in all analyses because groups differed significantly in age. Relationships between age and working memory are typically linear in both general population samples (Bopp and Verhaeghen 2005; Myerson 2003) and in schizophrenia outpatients, at least up to age 60 (Kurtz 2005). The scatter plots in the current sample revealed no evidence of non-linearity between age and LNS scores in any of the groups, and higher-order non-linear effects did not account for significant additional variance beyond the linear effects. Tests for homogeneity of within-group regression were performed prior to analyses, because the usual covariance adjustment for linear effects is appropriate only when there is no meaningful evidence for group X covariate interactions. There were no significant interactions involving any of the covariates (age, sex, parental education). Personal education was not included as a covariate due to evidence that educational attainment is influenced by schizophrenia (Isohanni et al 2001). Significant group effects were evaluated with follow-up t-tests, as omnibus tests for comparisons of only three groups are protected against Type I errors.

To assess the psychometric properties of the LNS, we calculated the true score variance for the Reordered and Forward conditions. This was done by multiplying the observed variance by the reliability (Cronbach's alpha) in the control group for each task (Chapman 1973; Chapman 1978). For items not administered (i.e., were above the discontinuation cut off), zero's were inserted. These analyses were performed to address the possibility that differences in task sensitivity in the Reordered and Forward conditions could confound the interpretation of study results.

Third, to evaluate whether any differences between relatives and CCS on the LNS were attributable to the presence of schizophrenia spectrum disorders among relatives rather than genetic vulnerability per se, the previous analyses were re-run after excluding relatives with a history of schizophrenia spectrum disorder, including schizophrenia, schizoaffective disorder, or cluster A personality disorders. We also evaluated whether LNS scores were associated with subclinical features of cluster A personality disorders in the relative and CCS groups. Correlations between total scores from the Modified SIS and LNS scores were computed separately within each group.

Finally, the effects of non-spectrum psychiatric disorders, including substance use or mood disorders, on LNS performance were examined within the relative and CCS groups. These analyses excluded relatives with histories of schizophrenia spectrum disorders. For substance use disorders, a dichotomous variable coded the presence or absence of a lifetime history of abuse or dependence of alcohol or substances for each subject. Similarly, a dichotomous mood disorder variable coded the presence or absence of a lifetime diagnosis of Major Depressive Disorder, Dysthymic Disorder, Bipolar I, or Bipolar II. Three sets of mixed effects regression analyses examined performance in relatives of probands versus control groups using the same models described above, with additional fixed factors to assess the effects of substance disorder and mood. The first models included substance disorder as a fixed effect, the second included mood disorder as a fixed effect, and the third simultaneously included substance use and mood disorders as fixed effects. These models evaluated whether mood and substance use disorders

account for significant variance in LNS scores within groups and whether group differences remained significant after accounting for these disorders.

Results

Demographic and clinical characteristics

As shown in Table 1, probands had a higher proportion of males, fewer years of completed education (as expected), lower estimated pre-morbid intellectual functioning on the WRAT-3, and higher parental education than both other groups. The relatives were significantly older than the proband and CCS groups. Probands had a typical age of onset and demonstrated low to moderate levels of symptoms.

Within the proband group, there were several significant and trend-level correlations between symptoms and LNS scores, though the magnitudes of the correlations were small. Lower LNS Reordered scores significantly correlated with higher disorganized symptoms ($r = -.23$, $p < .01$), and showed trend-level associations with higher positive ($r = -.15$, $p = .07$) and negative ($r = -.15$, $p = .07$) symptoms. Lower LNS Forward scores significantly correlated with higher levels of positive ($r = -.19$, $p < .05$), negative ($r = -.21$, $p = .01$), and disorganized ($r = -.21$, $p = .01$) symptoms.

LNS differences among the proband, relative, and comparison groups

In the mixed effects regression models that examined LNS performance, it is important to note that there were no significant effects for research site or group $\times$ research site, or for sex (F 's < 1.00 , p 's $> .43$). Descriptive statistics and results of statistical tests for the other model parameters are presented in Table 2. Age was a significant covariate for both LNS Reordered and Forward. Parental education was a significant predictor of scores on LNS Forward, with lower parental education associated with lower LNS Forward scores. After accounting for age and parental education in the regression models, there were significant group effects for both conditions of the LNS.

For LNS Reordered, probands performed significantly worse than the relatives and CCS, reflecting medium and large effect sizes, respectively. In addition, the relatives performed significantly worse than the CCS. The magnitude of this difference was small to medium. For LNS Forward, probands performed significantly worse than both relatives and CCS, with the differences reflecting small and moderate effect sizes, respectively. Relatives and CCS did not significantly differ from each other.

Examination of the true score variance associated with LNS conditions enhances confidence that the relatives' impairment is not an artifact of the discriminating power of the tasks themselves. Within the control group, the true score variances were very similar across the two conditions: LNS-Reordered = 5.08, LNS-Forward = 5.75. Greater true score variance enhances the discriminating power of a task condition. Thus, the LNS-Forward had slightly higher discriminating power than the LNS-Reordered. These analyses suggest that the larger differences in LNS-Reordered scores in the Relatives as compared to the CCS are not simply attributable to a psychometric artifact.

To determine whether LNS differences between relatives and CCS merely reflected the presence of schizophrenia spectrum disorders among the relatives, the analyses were repeated after excluding the small number of "affected" relatives ($n = 15$; 6 schizophrenia, 2 schizoaffective disorder, 6 schizotypal personality disorder, 1 paranoid personality disorder). The results were nearly identical, with the relatives performing worse than CCS on LNS Reordered ($d = .33$) but not on LNS Forward ($d = .20$). Thus, the lower LNS Reordered scores

in the relatives group were not attributable to the presence of spectrum disorders among the relatives.

We next evaluated whether the schizotypy features assessed by the SIS were associated with LNS performance within the relative and CCS groups, excluding relatives with schizophrenia spectrum disorders. SIS scores were positively skewed in both groups and higher in the relative ($M = 13.9$, $SD = 9.3$) than the CCS group ($M = 10.9$, $SD = 8.4$), $t(525) = 3.61$, $p < .05$. A natural log transformation was applied to the Total SIS scores for correlational analyses. The correlations between SIS scores and LNS scores were very small and non-significant in the relative and CCS groups ($-.05$ to $.01$), suggesting minimal associations with schizotypal features.

LNS and non-spectrum psychiatric disorders in the relative and comparison groups

The final set of mixed effects regression analyses evaluated the impact of mood and alcohol / substance use on LNS performance within the relative and CCS groups, and on mean differences between the relative and CCS groups. Twenty-four percent of the relatives and 15% of the CCS had lifetime mood disorders, while 18% of the relatives and 13% of the CCS had lifetime substance use disorders.

The first models included all of the factors and covariates described in the previous analyses, as well as a mood disorder factor. For LNS Reordered, the effect of mood disorder was not significant, $F(1,144) = 1.32$, $p > .05$, and the relative versus CCS difference remained significant, $F(1,293) = 6.41$, $p < .05$. For LNS Forward, mood disorder was not significant, $F(1,145) = .27$, $p > .05$, and the relatives versus CCS effect remained non-significant, $F(1,294) = 1.05$, $p > .05$.

Analyses including substance use history as a factor indicated that, for LNS Reordered, substance use was statistically significant, $F(1,144) = 4.93$, $p < .05$, and the group effect remained significant with substance use in the model, $F(1,293) = 5.09$, $p < .05$. For LNS Forward, the substance use factor was not significant, $F(1,145) = 1.34$, $p > .05$, and the group difference remained non-significant when this factor was entered, $F(1,294) = 1.01$, $p > .05$. Finally, the group effect on LNS Reordered scores persisted after the mood and substance use disorder factors were simultaneously entered into the model, $F(1,293) = 5.84$, $p < .05$. Thus, the relative versus CCS difference on LNS Reordered were not merely attributable to the presence of non-schizophrenia spectrum disorders in both groups.

Discussion

This study evaluated verbal WM in what we believe to be the largest samples of schizophrenia probands, first-degree relatives, and CCS studied to date. Consistent with our hypotheses, probands performed worse than both other groups on both WM tasks, with larger effect sizes found for the WM task that required executive functions. Probands' relatives also performed worse than CCS on the more demanding maintenance plus manipulation task but not on the WM task that required only maintenance of information. The relatives' impairment on the executive functioning WM task does not appear to be attributable to psychometric properties (i.e., discriminating power) of the LNS conditions or the presence of schizophrenia spectrum disorders, and persisted after accounting for the effects of lifetime mood and substance use disorder histories. In conjunction with evidence that WM abilities are substantially heritable (Greenwood et al 2007), the current results support the validity and usefulness of executive functioning WM as an endophenotype for schizophrenia in large-scale genetic studies of quantitative endophenotypes.

In this report, there were no significant correlations between sub-clinical schizotypal features and WM within the relative or CCS groups. Prior studies of the relationship between WM and schizotypal features among unaffected relatives of schizophrenia probands have shown generally mixed results (e.g., (Conklin et al 2005; Delawalla 2006; Johnson et al 2003; Saperstein et al 2006), possibly reflecting differences in the schizotypy assessment instruments and WM tasks employed across studies. The current results suggest that the LNS Reordered impairments in the relatives reflect vulnerability to schizophrenia rather than the clinical or subclinical features of schizophrenia spectrum disorders assessed in this study.

Although verbal WM with an executive component clearly demonstrated some of the expected characteristics of an endophenotype for schizophrenia (Gottesman and Gould 2003), the magnitudes of the separations between probands and family members, compared to CCS, were somewhat smaller than those found in previous studies using the same or comparable WM tasks (e.g., an effect size between probands and CCS on LNS Reordered of $d = .94$ in this study compared with effect sizes of 1.4 or greater in other studies (Conklin et al 2005; Gold et al 1997; Perry et al 2001)). The medium patient versus CCS effect size for LNS forward ($d = .54$) was also somewhat smaller than previous studies of conventional digit span forward tasks (Aleman et al 1999). Similarly, the magnitude of the relatives' impairment on LNS Reordered ($d = .36$) was somewhat lower than that reported in a recent study ($d = .56$ (Conklin et al 2005), and the magnitude of the non-significant difference between relatives and CCS on LNS Forward fell at the low end of the range for effect sizes for digit span forward tasks (Snitz et al 2006; Trandafir et al 2006).

The somewhat smaller but still significant separations among groups in the current study could be associated with two key methodological features of the COGS. First, the central aim of the COGS is to investigate the genetic determinants of quantitative endophenotypic traits, which requires sufficient "contrast" among affected and unaffected relatives to obtain statistical power. Enrolled families included at least two unaffected first-degree relatives of schizophrenia probands, a minimum family structure that resulted in somewhat larger pedigrees than those of most previous family studies. These rigorous selection criteria might have led to the inclusion of relatively intact patients and family members who were willing to complete the lengthy clinical and endophenotype assessments. Thus, the less marked group separations might reflect the possibility that these families are unusually intact with less severe manifestations of the illness compared to other studies. The normal-range WRAT-3 scores in the probands and the low proportion of relatives with lifetime histories of schizophrenia spectrum disorders appear consistent with this possibility.

Second, to parallel lifetime psychiatric comorbidity in relatives of probands, the COGS project includes CCS with disorders outside of the schizophrenia spectrum, whereas previous studies often excluded such participants (see (Snitz et al 2006). This inclusion criterion enabled us to meaningfully examine the impact of comorbid diagnoses on LNS performance in the relatives and CCS, and minimizes potential confounds associated with the use of "super controls" (Kendler 1990). However, the inclusion of comparison subjects with non-spectrum psychiatric disorders might create additional "noise" in the control sample and could have reduced the between-group separations on the WM tasks in the current study.

Another issue that deserves consideration is the task demands of the LNS-Forward task, which did not significantly differentiate the relative and CCS groups. This task emphasizes one aspect of WM maintenance, namely the "load" or number of items to be recalled. Tasks that assess other maintenance-related functions, such as the delay interval over which information must be maintained before recall, may show larger separations between relatives of schizophrenia probands and controls (e.g., (Park et al 1995).

Genetic studies of complex quantitative endophenotypes require the use of large numbers of family cohorts, which are often only possible through multi-site collaborative efforts that use standardized recruitment and assessment procedures. The current findings demonstrate that the rigorous data collection and quality assurance procedures employed by the COGS can be successfully implemented for WM assessments across seven geographically diverse research sites, as we detected no significant site effects. Confidence in these methods is bolstered by initial heritability analyses of the primary endophenotypic measures from the COGS project, including the LNS Reordered (Greenwood et al 2007). Based on a sample that largely overlaps with the current one, the observed heritability for LNS-Reordered was .39, which was among the highest of the candidate endophenotypes. This figure closely approximates previous heritability estimates for verbal and visual working memory (36-42%) in families of schizophrenia probands from a Finnish isolate with a different pedigree structure (Tuulio-Henriksson et al 2002) and in non-clinical samples (Ando et al 2001; Hansell et al 2005).

These heritability figures, like those from other endophenotypes, are lower than the typical heritability estimates for schizophrenia (.80; (Owen et al 2002). However, the COGS project recognizes a key distinction between heritability and “mapability” in genetics. Though highly heritable, the clinical diagnosis of schizophrenia comprises a diverse range of signs and symptoms that likely reflect a complex genetic architecture that impacts multiple, largely unspecified, neurobiological systems. The LNS and other COGS endophenotypes were carefully selected in an effort to increase mapability. Although the genetic architecture of these endophenotypes may also be somewhat complex (Flint and Munafò 2007), the expectation is that they more directly reflect the activities of neuronal mechanisms than the illness itself. Several promising polymorphisms in candidate genes associated with WM performance have already been identified in both healthy subjects and in schizophrenia patients (e.g., (Cannon 2005; Goldberg and Weinberger 2004; Greenwood and Parasuraman 2003; Parasuraman et al 2005; Wedenoja et al in press). The endophenotype strategy adopted by COGS is complementary to 20 years of genetic linkage and association studies using the clinical diagnosis of schizophrenia that have had modest success in identifying disease-related genes.

It should be noted that this report describes only part of a larger research project that involved an extensive assessment battery. Therefore, the possibility of error associated with multiple comparisons must be kept in mind in this type of large-scale project. The COGS continues to collect WM and other neurocognitive and neurophysiological performance data, as well as DNA on all proband and family member participants. In future reports, associations between WM task performance, performance on other COGS candidate endophenotypes, and DNA will ultimately be evaluated in accordance with our mission to identify genes that contribute to vulnerability for schizophrenia.

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Table 1
Demographic and clinical characteristics in probands (n = 149), relatives (n = 337), and controls (n = 190)

	Probands	Relatives	Controls	Statistic
Age (years) (SD)	34.99 ^a (10.86)	45.41 ^b (13.92)	37.35 ^a (12.10)	$\bar{F} = 44.07^{**}$
Sex (% male)	72% ^a	42% ^b	45.9% ^b	$\chi^2 = 38.60^{**}$
Education (years) (SD)	13.52 ^a (2.08)	15.46 ^b (2.66)	15.38 ^b (2.81)	$\bar{F} = 31.48^{**}$
Parental education (years) (SD)	15.73 ^a (3.36)	14.60 ^b (3.68)	14.82 ^b (2.98)	$\bar{F} = 5.09^*$
WRAT-3 (standard score) (SD)	100.8 ^a (11.7)	105.6 ^b (11.2)	106.8 ^b (10.6)	$F = 12.43^{**}$
Age of onset (years) (SD)	21.4 (6.1)			
Duration of illness (SD)	13.5 (10.5)			
Positive symptoms (SD)	1.3 (1.4)			
Negative symptoms (SD)	2.4 (1.4)			
Disorganized symptoms (SD)	2.4 (1.5)			

Note: Groups with different subscripts are significantly different from each other. WRAT-3 = Wide Range Achievement Test, 3rd edition.

*
p < .05;

**
p < .001

Table 2

Letter-Number Sequencing performance across groups

	Probands (n = 149)	Relatives (n = 324)	Controls (n = 190)	Age F	Parental Education F	Group F	Post-hoc t-tests			Effect sizes		
							P v C	P v R	R v C	P v C	P v R	R v C
LNS Reorder (M) (SD)	9.18 (2.55)	10.67 (2.59)	11.59 (2.55)	25.90***	1.35	33.48***	8.10***	5.85***	2.52*	.94	.58	.36
LNS Forward (M) (SD)	12.84 (2.74)	13.74 (3.02)	14.38 (2.90)	43.17***	5.43*	12.90***	4.96***	3.76**	1.12	.53	.31	.22

Note: LNS = Letter-Number Sequencing. Means are based on raw scores. Statistical tests are based on mixed effects regression models including family as a random effect, age, sex, and parental education as covariates, and group, site, and group X site as fixed effects. Effect size estimates were calculated using the sample-size weighted pooled within-group standard deviations (LNS-Reordered = 2.56; LNS-Forward = 2.90). P v C: a comparison of schizophrenia probands and comparison groups. P v R: a comparison of relatives and schizophrenia probands. R v C: a comparison of relative and comparison groups.

*
p < .05;
**
p < .005;

p < .0001