

Cognitive Endophenotypes Inform Genome-Wide Expression Profiling in Schizophrenia

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Objective: We performed a whole-genome expression study to clarify the nature of the biological processes mediating between inherited genetic variations and cognitive dysfunction in schizophrenia. **Method:** Gene expression was assayed from peripheral blood mononuclear cells using Illumina Human WG6 v3.0 chips in twins discordant for schizophrenia or bipolar disorder and control twins. After quality control, expression levels of 18,559 genes were screened for association with the California Verbal Learning Test (CVLT) performance, and any memory-related probes were then evaluated for variation by diagnostic status in the discovery sample ($N = 190$), and in an independent replication sample ($N = 73$). Heritability of gene expression using the twin design was also assessed. **Results:** After Bonferroni correction ($p < 2.69 \times 10^{-6}$), CVLT performance was significantly related to expression levels for 76 genes, 43 of which were differentially expressed in schizophrenia patients, with comparable effect sizes in the same direction in the replication sample. For 41 of these 43 transcripts, expression levels were heritable. Nearly all identified genes contain common or de novo mutations associated with schizophrenia in prior studies. **Conclusion:** Genes increasing risk for schizophrenia appear to do so in part via effects on signaling cascades influencing memory. The genes implicated in these processes are enriched for those related to RNA processing and DNA replication and include genes influencing G-protein coupled signal transduction, cytokine signaling, and oligodendrocyte function.

Keywords: schizophrenia, gene expression, cognition, endophenotype, genome-wide association study

Supplemental materials: <http://dx.doi.org/10.1037/neu0000244.supp>

Schizophrenia is a highly heritable, genetically complex, and heterogeneous psychiatric syndrome. Twin and family studies estimate 80%–85% of variance in disease liability can be accounted for by genetic factors (Cannon, Kaprio, Lonnqvist, Huttenen, & Koskenvuo, 1998). Thus, substantial efforts have been dedicated toward uncovering genetic loci increasing risk for schizophrenia. Though many methodologies have been used in-

cluding genome-wide surveys of common genetic polymorphisms (Purcell et al., 2009; Ripke et al., 2013; Ripke et al., 2014), exome sequencing investigating rare variants (Fromer et al., 2014; Purcell et al., 2014), gene expression analyses (Gardiner et al., 2013; Guillozet-Bongaarts et al., 2014; Gulsuner et al., 2013), and integration of multiple methods (Hertzberg, Katsel, Roussos, Haroutunian, & Domany, 2015; Luo et al., 2015; van Eijk et al., 2014),

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Amanda B. Zheutlin and Rachael W. Viehman contributed equally to this article. The work was supported by NIH Grant Number MH052857 to TDC.

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nearly all gene-finding efforts in schizophrenia to date have used diagnostic-level classification as the phenotype of interest. However, there is substantial evidence that risk-increasing genes confer vulnerability via impacting intermediate levels of impairment, such as cognitive dysfunction, suggesting that these may also be useful phenotypic targets in gene discovery studies (Cannon & Keller, 2006; Greenwood, Light, Swerdlow, Radant, & Braff, 2012; Lencz et al., 2014; Tan, Callicott, & Weinberger, 2008; Touloupoulou et al., 2007).

Compared to the diagnosis of schizophrenia, which could imply dysfunction across a host of brain systems and signaling pathways, a given endophenotype provides a more constrained framework within which to interpret the functional-biological relevance of statistically identified genes (Heck et al., 2014; Ibrahim-Verbaas et al., 2015; Lencz et al., 2014). One of the most reliable endophenotypes in schizophrenia—and most profound areas of cognitive impairment—is verbal memory (Cannon et al., 2000; Greenwood et al., 2013; Schaefer, Giangrande, Weinberger, & Dickinson, 2013; van Erp et al., 2008). The California Verbal Learning Test (CVLT), a list-learning exercise, is a robust measure of memory impairment in schizophrenia (Haut et al., 2015; Stone et al., 2011; van Erp et al., 2008), is heritable (Carmelli, Swan, DeCarli, & Reed, 2002; Greenwood et al., 2007; Kremen et al., 2014; Panizon et al., 2011), and shows intermediate levels of affection among relatives of patients with schizophrenia in twin and family studies (Greenwood et al., 2013; van Erp et al., 2008), supporting its role as a candidate endophenotype in genetic studies.

Initial molecular genetic investigations of endophenotypes for schizophrenia have provided evidence that genes associated with putative cognitive endophenotypes at least partially overlap with those associated with schizophrenia (Heck et al., 2014; Lencz et al., 2014). However, so far this approach has been used largely to demonstrate the broad, shared genetic etiology between these phenotypes, rather than to identify specific genetic loci impinging on both, which is critical for elucidating the functional-physiologic significance of the underlying genetic associations. Furthermore, all of these studies have assayed common polymorphisms. Over 90% of common polymorphisms previously related to psychiatric illness are located in regulatory regions of DNA, rather than in coding regions where mutations more directly affect protein structure, suggesting that many of these loci may exert their effects via regulation of gene expression (Kim et al., 2014; Maurano et al., 2012). Thus, examining gene expression in relation to a cognitive endophenotype for schizophrenia may yield insights into genotypic effects on disease status and help to elucidate mechanisms by which cognitive dysfunction manifests in this disease.

In the current study, we used a discordant twin design to identify genes differentially expressed in relation to verbal memory performance, a cognitive endophenotype for schizophrenia. Gene expression was assayed from peripheral blood mononuclear cells (PBMCs), the most feasible way to assess expression levels in living patients. Gene expression in PBMCs is broadly heritable and correlates with central nervous system expression patterns (Cheung et al., 2003). While it is likely that some genes influencing memory performance will not be schizophrenia related, given the endophenotypic pattern of the CVLT, which suggests shared genetic etiology between memory and schizophrenia, we expected some proportion of these memory-related genes to show differential expression by diagnostic status (Glahn et al., 2007; Greenwood

et al., 2007; van Erp et al., 2008). This subset would represent a selection of genes potentially involved in those systems underlying memory impairment in schizophrenia. A twin design further allowed us to test the heritability of expression for each of these genes, dissociating potentially etiologically relevant genetic alterations from disease-related secondary effects.

Method

Participants

Twins born between 1940 and 1975 were identified on a population basis through the Swedish Twin Registry and screened for psychiatric status by linkage with the Swedish Hospital Discharge Registry (Lichtenstein et al., 2002). Pairs in which one or both cotwins had a diagnosis of schizophrenia or bipolar disorder were considered potential index pairs, and pairs in which neither twin had a history of hospitalization for either disorder were considered potential control pairs. Once recruited, all subjects were assessed by a psychiatrist using the Structured Clinical Interview for DSM-IV (SCID-IV; Spitzer, Williams, Gibbon, & First, 1992). Diagnoses were assigned according to *Diagnostic and Statistical Manual for Mental Disorders* (4th ed.; DSM-IV; American Psychiatric Association, 1994) criteria, using a consensus procedure taking into account the interview results as well as hospitalization and treatment records. Exclusion criteria included neurological disorder, history of head injury with loss of consciousness, mental retardation, substance dependence within the past 6 months, and inability to read or comprehend Swedish. Control twins were additionally excluded if they had a personal history of schizophrenia, schizoaffective disorder, or bipolar disorder. Patient twin pairs were included if the proband was given a consensus diagnosis of either schizophrenia/schizoaffective disorder or bipolar I disorder. Individuals with schizoaffective disorder were included in the schizophrenia group. All included probands were clinically stable at the time of testing and the blood draw, which occurred contemporaneously. Control twin pairs matched to the index pairs for age, sex, and zygosity were recruited from the Swedish Twin Registry. Zygosity was determined by DNA using a 46 single nucleotide polymorphism panel. In total, 190 individuals who were interviewed, underwent cognitive testing, and provided blood samples that were assayed for gene expression were included in the discovery sample for the present report. Of these individuals, 36 had a diagnosis of schizophrenia, 34 were unaffected cotwins of patients with schizophrenia, 23 individuals had a diagnosis of bipolar disorder, 16 were unaffected cotwins of patients with bipolar disorder, and 81 individuals were control twins. Included in the control twin group were 20 pairs ($N = 39$) where one or both individuals had a lifetime history of major depressive disorder. Across the sample, 46% of participants were male, and subjects were on average 49.9 years old (95% confidence intervals [CIs] [48.5, 51.4]). No significant differences between diagnostic groups in age, sex, or zygosity were observed ($p > .05$). Group-specific demographic characteristics for schizophrenia patients, cotwins, and controls are listed in Table 1.

An independent twin sample from Finland was used to test for replication. Information about recruitment, clinical evaluation, and cognitive testing used for this study was described in detail elsewhere (Oresic et al., 2012). From this study, 18 schizophrenia

patients, 18 cotwins, and 37 control twins provided blood samples for gene expression ($N = 73$). Although cognitive test data were available on many of these subjects, the testing had been performed 2–10 years prior to the blood draw, and thus the test data were not used in the present analyses. Demographic information for these subjects is summarized in Table 1.

Cognitive Assessment

Swedish participants underwent a standard neuropsychological battery including the Vocabulary and Block Design subtests of the Wechsler Abbreviated Scale of Intelligence Scale (WASI) and the CVLT. These measures had previously been translated into Swedish. The CVLT is a measure of verbal learning and memory. Individuals were read a list of 16 words and then asked to recall as many of the words as they could remember. This was repeated across four subsequent trials. The sum of words recalled across all five learning trials was used as the performance metric in all analyses. Though previous studies have demonstrated that CVLT performance is both heritable and related to schizophrenia (Glahn et al., 2007; Greenwood et al., 2007; Stone et al., 2015; van Erp et al., 2008), we conducted analyses to confirm these patterns in our sample as well. Diagnostic effects on CVLT performance were ascertained using a mixed-effect analysis of variance (ANOVA) model, where family ID was included as a random variable, as programmed in R using the nlme package (Pinheiro, Bates, DebRoy, Sarkar, & Team, 2015). Structural equation modeling was performed to assess genetic and environmental contributions to CVLT performance in Mx (Neale, Boker, Xie, & Maes, 2003), including all subjects within the sample (69 monozygotic twin pairs, 89 dizygotic twin pairs), rather than the subset with RNA expression data (for a full description of the sample, see Higier et al., 2014). Details of this procedure were identical to those for evaluating heritability of gene expression described below, substituting CVLT as the variable of interest.

We additionally calculated a measure of general cognitive ability to determine whether any effects were specific to memory. Z scores based on the means and standard deviations of control subjects were generated for the Vocabulary and Block Design subtests of the WASI. The average of these scores was used as our

measure of global cognitive ability. Diagnostic effects on general ability, as well as heritability, were assessed as described for CVLT performance.

RNA Microarray Analysis

For both Swedish and Finnish samples, RNA was extracted from PBMCs drawn from a 10-mL blood sample (ABI Tempus system). RNA aliquots at 100 ng/ μ L were sent to the University of California Los Angeles (UCLA) Biological Samples Processing Core for analysis. Technical replicates were run on all samples using the Illumina Human WG6 v3.0 chip, and correlations between these samples for each person were evaluated. All samples had a usable quantity of RNA, and all subjects had good correlations between their technical replicate samples (intra-class correlation coefficients ranged from 0.902 to 0.998). Samples were preprocessed using the Illumina Bead Studio package. Technical replicates were averaged to produce one vector per person, and samples were log-transformed to ensure normalization of the sample distributions. The Illumina chip indexed a total of 24,526 markers. After rank normalization and background subtraction, 5,967 probes were excluded on the basis of low signal strength (not significantly greater than 0 at a Bonferroni-corrected $\alpha \leq .05$), leaving 18,559 genes to be carried forward for analysis of association with CVLT performance, general ability, and diagnosis.

Genome-Wide Association Between Expression Levels and Memory Performance

All patients, cotwins, and controls from the Swedish sample ($N = 190$) were examined to test associations between peripheral gene expression and CVLT performance. Mixed-effect linear regression models of gene expression on CVLT performance were run for each probe. Family identity was included as a random variable, and age and sex were included as covariates. Gene markers that met the Bonferroni-corrected significance level of $\alpha = 2.69 \times 10^{-6}$ (i.e., .05/18,559) were considered hits. We conducted a genome-wide assay of global cognitive ability using the same design to ascertain if any effects were specific to memory. The same Bonferroni-corrected alpha level was considered

Table 1
Demographic Information for Swedish and Finnish Twins

Patient group	<i>N</i>	Age (years)	Sex (% male)	Zygoty (% MZ)	SANS	SAPS
<i>Swedish sample</i>						
SZ	36	50.2 [47.1, 53.4]	56%	42%	40.7 [32.7, 48.7]	21.6 [14.5, 28.6]
SZ cotwins	34	51.1 [47.8, 54.4]	53%	26%	9.6 [4.4, 14.7]	.6 [0, 1.3]
Controls	81	49.6 [47.4, 51.9]	49%	46%	5.2 [3.1, 7.4]	.4 [.1, .8]
Statistic	—	1.091	.409	3.701	73.25	58.299
Significance	—	.343	.815	.157	<.0001	<.0001
<i>Finnish sample</i>						
SZ	18	47.3 [44.1, 50.6]	28%	33%	31.8 [13.2, 50.4]	27.1 [17.9, 36.3]
SZ cotwins	18	47.3 [44.1, 50.6]	28%	33%	—	—
Controls	37	49.4 [47.3, 51.4]	32%	41%	—	—
Statistic	—	.498	.188	7.619	—	—
Significance	—	.490	.910	.107	—	—

Note. Group means, 95% confidence intervals, and *F* statistics from linear mixed-effect models of diagnosis on age, SANS, and SAPS, with family ID as a random variable, were reported when possible. Chi-square values were reported for sex and zygoty. MZ = monozygotic; SANS = Scale for the Assessment of Negative Symptoms; SAPS = Scale for the Assessment of Positive Symptoms; SZ = schizophrenia patients.

here. Analyses were conducted in R using the lme4 package (Bates, Maechler, Bolker, & Walker, 2014) and pbrtest package (Halekoh & Hojsgaard, 2014).

Associations Between Expression Levels and Diagnostic Status

Genes found to be related to memory performance were further examined to determine if their expression patterns varied by diagnostic group. Expression levels for schizophrenia patients, schizophrenia cotwins, and controls ($N = 151$) were analyzed using mixed-effect ANOVAs with diagnosis as the predictor, and family included as a random variable. Bipolar patients and cotwins were not included, because these groups were not represented in the Finnish twin sample available for use in testing replication of the associations. For genes whose expression varied significantly by diagnosis, fixed-effect comparisons between schizophrenia patients and each of the nonpatient groups (i.e., unaffected cotwins and controls) were examined. The significance threshold for the analyses by diagnosis was kept at $\alpha = .05$, with no correction, as we had previously applied a conservative Bonferroni threshold to limit the scope to those transcripts relating to memory performance, and we had a second, independent sample in which to test replication of any diagnostic differences.

Genes differentially expressed with respect to diagnosis were examined in an independent sample of schizophrenia patients, cotwins, and controls from Finland ($N = 73$). Mixed-effect ANOVAs, including family as a random variable, were conducted to examine whether expression of these genes varied by diagnostic status. Because we predicted that the diagnostic effects would mirror the discovery sample with respect to direction, we used a one-tailed $\alpha = .05$ significance threshold (with no correction for multiple comparisons at this replication stage). Statistical analyses were conducted in R using the nlme package (Pinheiro et al., 2015). Power analyses, when relevant, were conducted using G*Power 3.1 (Faul, Erdfelder, Lang, & Buchner, 2007) to estimate requisite sample sizes for detecting observed effect sizes given 80% power ($1 - \beta$) at $\alpha = .05$.

Heritability Analyses of Gene Expression

For those RNA probes found to be related to both memory and schizophrenia, we used structural equation modeling to compare gene expression covariance in monozygotic (MZ) twin pairs ($N = 26$ pairs) to that in dizygotic (DZ) twin pairs ($N = 37$ pairs) in order to assess the relative contribution of additive genetics (A), dominant genetics (D), common environment (C), and unique environment (E) (Plomin, DeFries, Knopik, & Neiderhiser, 2013). Models were run in Mx, which uses the maximum likelihood estimate to fit models to covariance matrices (Neale et al., 2003). Best-fitting models were selected on the basis of the root-mean-square error of approximation (RMSEA) and the Akaike Information Criterion (AIC), for both of which lower values reflect better fit (Akaike, 1974). AIC also penalizes the addition of parameters, so best-fit models by this metric are also relatively more parsimonious (Kline, 2011). We also tested the significance of the genetic and familial environmental contributions to each model by means of chi-square difference tests (i.e., directly comparing models in which each of these parameters was present vs. absent).

Gene Classification

Illumina probe IDs were converted to standard gene symbols using the Bioconductor R package illuminaHumanv4.db (Dunning, Lynch, & Eldridge, n.d.). Gene symbols for all probes significantly associated with both CVLT performance and diagnostic status were input into the Database for Annotation, Visualization, and Integrated Discovery (DAVID) for functional annotation clustering (Huang, Sherman, & Lempicki, 2009). This function can group genes together on the basis of classification terms from multiple gene ontology databases; for the current study, we included the default sources and clustering criteria. Clusters identified by this tool were assigned enrichment values and corresponding significance statistics based on the number of genes in the input list matching the included annotation terms relative to the number expected by chance.

Results

Cognitive Assessment

Consistent with previous studies (Glahn et al., 2007; Greenwood et al., 2007; Stone et al., 2015; van Erp et al., 2008), we found that CVLT performance was impaired in schizophrenia, correlated with general cognitive ability, and partially heritable. CVLT performance (total words recalled across the five learning trials; maximum score = 80) was significantly associated with diagnostic status, $F(5, 77) = 10.66$, $p < .0001$, marginal $R^2 = .220$. Schizophrenia patients on average scored significantly lower ($M = 38.8$, 95% CIs [34.5, 43.2]) than all other groups (schizophrenia cotwins: $M = 50.8$, 95% CIs [46.8, 54.8]; bipolar patients: $M = 51.6$, 95% CIs [46.9, 56.2]; bipolar cotwins: $M = 57.8$, 95% CIs [53.9, 61.6]; controls (history of depression): $M = 50.4$, 95% CIs [47.0, 53.8]; controls (no history of depression): $M = 55.7$, 95% CIs [52.7, 58.7]). CVLT performance also accounted for a significant portion of the variance in general cognitive ability, $F(1, 71) = 26.65$, $p < .0001$, marginal $R^2 = .108$. The best-fitting model of genetic and environmental contributions to variance in CVLT performance included a significant genetic component, accounting for 53% of the variance, $\chi^2(4, N = 316) = 1.885$, $p = .757$, RMSEA = 0, AIC = -6.115 , which is consistent with the correlations within zygosity groups, MZ: $r(67) = .566$, $p = 3.95e^{-7}$; DZ: $r(87) = .237$, $p = .025$.

General cognitive ability was also significantly associated with diagnostic status, though schizophrenia patients showed a relatively less profound deficit than was observed for verbal memory, $F(5, 67) = 3.11$, $p = .0138$, marginal $R^2 = .061$. Schizophrenia patients on average scored significantly lower ($M = -.49$, 95% CIs [-.89, -.10]) than did schizophrenia cotwins ($M = -.27$, 95% CIs [-.67, -.12]) and controls (history of depression: $M = -.01$, 95% CIs [-.34, .33]; no history of depression: $M = .01$, 95% CIs [-.23, .25]). No other groups scored significantly differently than controls (bipolar patients: $M = -.47$, 95% CIs [-.98, .03]; bipolar cotwins: $M = .14$, 95% CIs [-.50, .78]). The best-fitting model of genetic and environmental contributions to variance in general ability included a significant genetic component, accounting for 73% of the variance, $\chi^2(4, N = 292) = 0.626$, $p = .960$, RMSEA = 0, AIC = -7.374 , consistent with within-zygosity correlations, MZ: $r(60) = .719$, $p = 4.77e^{-11}$; DZ: $r(82) = .434$, $p = 3.53e^{-5}$.

Table 2
Heritability Estimates and Statistical Associations Between Gene Expression, CVLT, and Diagnostic Status

Illumina probe	Gene symbol	Swedish discovery sample									Finnish replication sample			
		Association with CVLT				Association with diagnosis				Heritability	Association with diagnosis			
		<i>t</i>	<i>df</i>	<i>p</i> _{Bon}	Partial <i>R</i> ²	<i>F</i>	<i>df</i>	<i>p</i>	Partial <i>R</i> ²		<i>h</i> ²	<i>F</i>	<i>df</i>	<i>p</i>
ILMN_1752111	SMARCAL1	5.15	171.82	.013	.134	6.26	2, 61	.003	.078	<i>.47</i>	1.95	2, 42	.155	.046
ILMN_2224143	MCM3	5.35	167.21	.005	.146	6.25	2, 61	.003	.080	<i>.47</i>	2.61	2, 42	.085	.062
ILMN_1728298	SBK1	5.03	178.74	.022	.124	6.07	2, 61	.004	.075	<i>.33</i>	2.78	2, 42	.073	.063
ILMN_1777740	THEM6	5.02	183.32	.023	.121	5.99	2, 61	.004	.075	<i>.47</i>	2.25	2, 42	.118	.055
ILMN_1750800	ACO1	5.33	174.07	.006	.140	5.81	2, 61	.005	.075	<i>.54</i>	1.98	2, 42	.151	.047
ILMN_2073012	TMEM203	5.06	173.37	.020	.128	5.75	2, 61	.005	.075	<i>.35</i>	2.19	2, 42	.124	.049
ILMN_1676745	ZNF142	5.32	187.98	.005	.131	5.74	2, 61	.005	.074	<i>.48</i>	2.06	2, 42	.141	.048
ILMN_1787461	RUNX3	5.46	186.37	.003	.138	5.49	2, 61	.006	.068	<i>.00</i>	2.59	2, 42	.087	.059
ILMN_1775522	MAGED1	6.14	178.43	<.0001	.174	5.32	2, 61	.007	.073	<i>.52</i>	3.02	2, 42	.060	.072
ILMN_1665943	GAR1	5.31	173.46	.006	.140	5.26	2, 61	.008	.070	<i>.45</i>	2.74	2, 42	.076	.064
ILMN_1689953	CD81	5.49	183.50	.002	.141	5.22	2, 61	.008	.066	<i>.41</i>	1.45	2, 42	.245	.034
ILMN_1701731	AKR1B1	5.04	182.99	.021	.122	4.86	2, 61	.011	.065	<i>.41</i>	1.88	2, 42	.165	.042
ILMN_1686871	PARP1	5.70	168.60	.001	.161	4.68	2, 61	.013	.060	<i>.40</i>	2.49	2, 42	.095	.060
ILMN_1786024	POLR3H	5.01	182.79	.024	.121	4.56	2, 61	.014	.049	<i>.58</i>	2.41	2, 42	.102	.058
ILMN_1733696	IMP3	5.32	164.72	.006	.147	4.56	2, 61	.014	.058	<i>.24</i>	1.82	2, 42	.174	.042
ILMN_1721978	CARD11	5.27	183.58	.007	.131	4.44	2, 61	.016	.050	<i>.46</i>	2.86	2, 42	.069	.068
ILMN_1700604	RBM14	4.88	172.74	.045	.121	4.41	2, 61	.016	.052	<i>.31</i>	1.38	2, 42	.262	.034
ILMN_2097793	ZBTB4	5.32	177.36	.006	.138	4.38	2, 61	.017	.056	<i>.25</i>	2.52	2, 42	.092	.059
ILMN_1673991	ATIC	5.92	187.94	<.0001	.157	4.28	2, 61	.018	.056	<i>.34</i>	3.09	2, 42	.056	.071
ILMN_1766010	YARS	5.31	172.45	.006	.140	4.21	2, 61	.019	.053	<i>.44</i>	2.67	2, 42	.081	.063
ILMN_2162989	TMEM189	4.88	187.02	.042	.113	4.12	2, 61	.021	.049	<i>.53</i>	1.69	2, 42	.197	.041
ILMN_1665943	MAP4K1	5.29	187.88	.006	.130	4.10	2, 61	.021	.054	<i>.38</i>	2.00	2, 42	.149	.046
ILMN_2334296	IL18BP	5.18	187.67	.011	.125	4.08	2, 61	.022	.046	<i>.50</i>	2.15	2, 42	.129	.047
ILMN_2044453	LPAR5	4.96	187.39	.029	.116	4.05	2, 61	.022	.053	<i>.51</i>	1.22	2, 42	.305	.030
ILMN_2153787	ARRDC5	4.87	185.73	.043	.113	4.03	2, 61	.023	.055	<i>.54</i>	1.85	2, 42	.169	.046
ILMN_1788538	NCALD	4.99	187.95	.025	.117	3.89	2, 61	.026	.050	<i>.47</i>	2.23	2, 42	.120	.055
ILMN_1670948	TSR2	4.98	185.88	.026	.118	3.77	2, 61	.029	.048	<i>.42</i>	1.67	2, 42	.201	.039
ILMN_1671257	DKC1	5.33	169.07	.006	.144	3.69	2, 61	.031	.050	<i>.45</i>	1.87	2, 42	.167	.045
ILMN_1663954	NELFCD	5.18	185.13	.011	.127	3.68	2, 61	.031	.053	<i>.49</i>	2.32	2, 42	.111	.054
ILMN_1761479	ZC3HC1	5.49	156.95	.003	.161	3.60	2, 61	.033	.046	<i>.52</i>	2.23	2, 42	.120	.053
ILMN_2183687	LIME1	4.98	183.23	.027	.119	3.60	2, 61	.033	.045	<i>.00</i>	1.97	2, 42	.152	.050
ILMN_1801348	GOT2	5.15	178.21	.013	.129	3.54	2, 61	.035	.044	<i>.51</i>	2.78	2, 42	.073	.062
ILMN_1779185	SPCC1L	4.99	162.99	.028	.133	3.46	2, 61	.038	.042	<i>.42</i>	2.28	2, 42	.115	.052
ILMN_1753885	YTHDF1	5.05	185.90	.019	.121	3.38	2, 61	.040	.046	<i>.29</i>	1.91	2, 42	.161	.046
ILMN_1740265	ACOT7	4.96	185.07	.029	.117	3.38	2, 61	.041	.044	<i>.49</i>	2.32	2, 42	.111	.054
ILMN_2306189	MAGED1	4.99	187.34	.025	.117	3.36	2, 61	.041	.050	<i>.50</i>	2.66	2, 42	.082	.065
ILMN_1748894	GTPBP3	4.87	173.16	.047	.120	3.35	2, 61	.042	.044	<i>.49</i>	2.48	2, 42	.096	.059
ILMN_1685580	CBLB	5.47	184.39	.003	.140	3.33	2, 61	.042	.043	<i>.57</i>	2.34	2, 42	.109	.058
ILMN_2343097	NCALD	5.12	188.00	.014	.122	3.33	2, 61	.043	.044	<i>.43</i>	2.47	2, 42	.097	.062
ILMN_1708328	RAB11FIP3	5.01	185.32	.023	.119	3.32	2, 61	.043	.044	<i>.52</i>	2.11	2, 42	.134	.050
ILMN_1751338	NUP133	5.20	167.84	.011	.139	3.31	2, 61	.043	.047	<i>.49</i>	2.01	2, 42	.146	.047
ILMN_1744068	ELP3	5.25	173.50	.008	.137	3.20	2, 61	.048	.043	<i>.51</i>	1.90	2, 42	.162	.043
ILMN_1763460	NHP2L1	5.02	183.07	.022	.121	3.19	2, 61	.048	.041	<i>.27</i>	1.80	2, 42	.179	.039

Note. Seventy-six genes were associated with the California Verbal Learning Test (CVLT) after correcting for multiple comparisons ($p < 2.69 \times 10^{-6}$), and of those, 43 were also associated with diagnostic status ($p < .05$) in the discovery sample. Of those 43 genes, 14 replicated significantly in the replication sample ($p < .05$, one-tailed). For CVLT- and diagnosis-associated genes, the effect of each probe on CVLT adjusted for age and sex in the discovery sample, as well as the overall effect of diagnosis on each probe in both samples, is reported. Boldface type indicates significance. Italics denote heritability models in which the chi-square was not significant, indicating good model fit. For all but four of the heritable genes (IMP3, ZBTB4, NHP2L1, YTHDF1), removal of the genetic term resulted in a significant decrement in model fit, $p < .05$. Boldface type indicates significance.

Genome-Wide Association Between Expression Levels and Memory Performance

The expression levels for 76 of the 18,559 probes retained following quality control were significantly associated with CVLT performance after Bonferroni correction ($p < 2.69 \times 10^{-6}$). For all of these probes, higher expression was associated with higher CVLT performance. For the subset of these genes also related to diagnostic status, the *t* and *p* values for the fixed effect of each

probe on CVLT are reported in Table 2. Effects for all 76 are reported in the online Supplemental Material (Table S1). The average effect size for the 76 probes significantly associated with CVLT (partial $R^2 = .131$, 95% CIs [.128, .134]) was reduced only moderately when excluding schizophrenia and bipolar patients (partial $R^2 = .095$, 95% CIs [.090, .099]), indicating that for the most part, the effects are independent of factors secondary to disease expression, such as chronicity (online supplemental Table

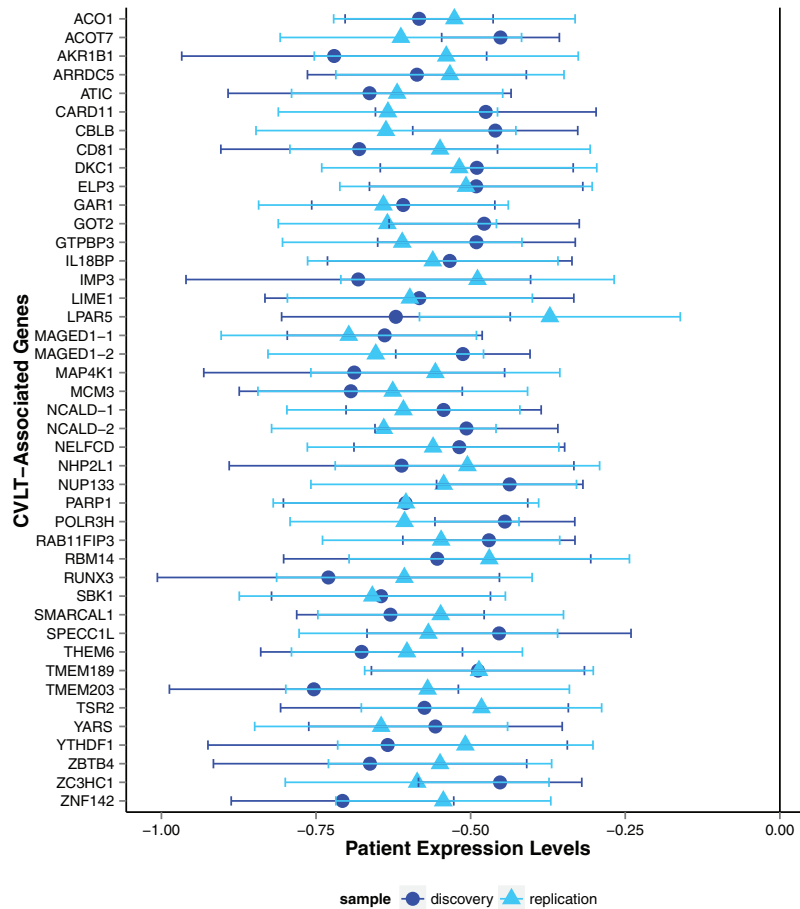


Figure 1. Forty-three memory-related genes were underexpressed in patients with schizophrenia as compared to controls. Gene expression values for schizophrenia patients in the discovery (Swedish) and replication (Finnish) samples are displayed as z scores normed within sample to the control mean and standard deviation for each gene. For some genes, two corresponding probes were identified; in these cases, probes were arbitrarily denoted gene-1 or gene-2. Error bars represent plus-or-minus the standard error. CVLT = California Verbal Learning Test. See the online article for the color version of this figure.

S1). Additionally, among the schizophrenia probands, gene expression for all markers associated with CVLT was not significantly correlated with positive symptoms, as measured by the Scale for the Assessment of Positive Symptoms (p 's > 0.25), nor did it differ between probands on ($N = 19$) or off ($N = 17$) antipsychotic medication (p 's > 0.25), suggesting that these effects are not secondary to clinical state or medication exposure either. No genes were significantly related to global cognitive ability after correcting for multiple comparisons.

Associations Between Expression Levels and Diagnostic Status

Of the 76 probes whose expression correlated with CVLT performance, 43 showed expression patterns that varied significantly in relation to diagnosis (Figure 1; Table 2). In fixed-effect comparisons between schizophrenia patients and each of the non-patient groups (i.e., unaffected cotwins and controls), patients showed significantly lower expression than controls at all 43 probes, and significantly lower expression than cotwins at 19 of

the 43 probes ($p < .05$; online supplemental Table S2). As all 76 probes were positively correlated with CVLT performance, reduced expression in patients is consistent with behavioral performance deficits observed in this group. Unaffected cotwins were intermediate between probands and controls on mean expression values for most of these genes, but did not differ significantly from controls for any. See the Supplemental Material (Figure S1 and Table S2) for further descriptive information on the distributions of expression levels by diagnostic group.

Fourteen of the 43 probes related to diagnosis in the discovery sample (Swedish twins) were confirmed as showing significantly lower expression in patients with schizophrenia compared to controls in the replication sample (Finnish twins; $p < .05$, one-tailed; online supplemental Table S3). However, the effect sizes for all 43 genes in the replication sample ($N = 73$) were similar in magnitude and in the same direction (i.e., reduced expression in patients) as those observed in the larger Swedish sample ($N = 151$). A power analysis revealed that, given the observed effect sizes for these 43 probes in the Finnish sample, if the replication sample had

been comparable in size to the discovery sample (i.e., $N = 151$ instead of 73), the diagnostic effects would have been statistically significant for all 43 probes in the replication sample (see Figure 1) (Faul et al., 2007). Patients with schizophrenia also had significantly lower expression than did their unaffected cotwins for 13 probes ($p < .05$, one-tailed), but again effect sizes were similar in magnitude and in the same direction as in the discovery sample across all of the genes. Unaffected cotwins and controls did not differ significantly in expression levels. Mean expression levels for each group in the replication sample are reported in the Supplemental Material (Table S3).

Heritability Analyses of Gene Expression

Heritability was assessed for the 43 probes related to both memory performance and schizophrenia. For 41 of the 43 probes, the best-fitting model included a genetic component, ranging from 24% to 58% ($M = 44%$, 95% CIs [42%, 47%]), indicating moderate heritability of expression levels of the genes relating to both memory and schizophrenia. All of these models included a genetic term (A, D) and a unique environment term (E), but no shared environment term (C). As such, the genetic term was reported in Table 2, and the E term was simply the remaining variance. Heritability models are considered to fit the data well if there is no significant difference, as tested using chi-square, between the model and the measured data (Plomin et al., 2013). Forty of the 43 best-fitting models had nonsignificant chi-square values ($p > .05$; see Table 2). For each model, we also explicitly tested whether the genetic and common environmental terms accounted for significant proportions of variance by performing a chi-square difference test with the genetic (A or D) or common environmental (C) terms in and out of the model. The C term was not significant for any of the 43 probes or CVLT performance ($p > .05$). Conversely, exclusion of the genetic term resulted in a significant decrement in model fit for CVLT and 37 of the 41 heritable markers ($p < .05$; see Table 2), confirming that the observed data are best explained by a model including a genetic factor.

Gene Classification

The genes associated with both CVLT and diagnostic status were input into DAVID for functional annotation clustering. Twelve clusters were identified, two of which exceeded the significance threshold for enrichment ($p < .05$); these two clusters contained genes related to RNA processing and DNA replication (online supplemental Table S4). Cluster assignments for each of the genes are reported in Table 3. All clusters—including annotation terms, number of genes assigned to each term, cluster enrichment values, and cluster significance values—are listed in the online Supplemental Material (Table S4).

Discussion

In this study we have found evidence of heritable gene expression contributing to both memory and schizophrenia, suggesting that some gene expression changes may increase risk for schizophrenia via effects on cognitive endophenotypes, such as memory impairment. Specifically, after correction for multiple testing at the whole genome level, we identified 76 RNA probes whose expres-

sion varied significantly in relation to memory function, 43 of which—reflecting 41 independent genes—also showed differential expression in schizophrenia patients compared with their unaffected cotwins and control twins. For all of these genes, higher expression correlated with better memory performance in the overall sample, and expression was reduced in patients with schizophrenia as compared to cotwins and controls. While many genes negatively correlated with both CVLT and diagnostic status (online supplemental Figure S1), none of these survived correction for multiple comparisons. As the samples were run in duplicate and averaged, it is unlikely that the lack of significant negative correlations were due to technical artifact. A smaller, independent sample of schizophrenia patients, cotwins, and control twins confirmed diagnostic differences for 14 of the probes, which represented nearly all functional clusters present in the full set of 43 genes. However, for all 43 probes, effect sizes in the smaller replication sample were consistent with those of the larger discovery sample both in direction and magnitude (see Figure 1); power analyses confirmed that all 43 effects would be significant with a replication sample of comparable size to the discovery sample. Heritability analyses utilizing the twin design revealed a genetic contribution for 41 genes within this subset, indicating that these effects are at least partially related to inherited variations. We did not find any genes significantly expressed in relation to general ability after correction. This is likely because our sample included a high proportion of patients with schizophrenia, who, consistent with previous work (Cannon et al., 2000; Schaefer et al., 2013), had a much more severe deficit in verbal memory than in general cognitive ability (i.e., 1.8 SD vs. .49 SD decrement compared with controls), conferring relatively higher power to detect schizophrenia-related gene expression impacting memory as opposed to general ability. In a general population sample, in which schizophrenia would occur at the population base rate of ~1%, there would likely be no increased sensitivity to detect memory-related genes than those impacting general ability.

The specific genes identified as differentially expressed in relation to both memory and diagnostic status in this study are largely overlapping with genes for which there is prior evidence of association with schizophrenia and related disorders, with neurocognition from linkage, exome sequencing, and genome-wide association studies, or with both (see Table 2). Specifically, 37 of the 41 genes have been previously related to schizophrenia (Fromer et al., 2014; Gulsuner et al., 2013; Purcell et al., 2014), one was recently identified in a genome-wide association study of cognitive phenotypes (Ibrahim-Verbaas et al., 2015), and two have been identified in studies of bipolar disorder (Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011; Sprooten et al., 2011), which is estimated to share 40% of its genetic etiology with schizophrenia (Lichtenstein et al., 2009).

Seven genes from the current study (SMARCAL1, MCM3, SBK1, ZNF142, ATIC, CBLB, ELP3) are in regions significantly associated with schizophrenia in a meta-analysis of 32 European ancestry linkage studies (Jia, Sun, Guo, & Zhao, 2010; Ng et al., 2009). An exome sequencing study comparing schizophrenia patients to controls identified de novo mutations in patients in 37 of the 41 genes identified here (Purcell et al., 2014). De novo mutations in four of these genes (ACO1, GTPBP3, YARS, and RUNX3) were also found in exome sequencing studies comparing

Table 3
Biological Relevance of CVLT-Associated Genes Expressed Differentially by Diagnostic Status

Illumina probe	Chr	Gene symbol	Gene description	Functional annotation clusters	Previous associations
ILMN_1740265	1	ACOT7	acyl-CoA thioesterase 7		SZ-exome
ILMN_1751338	1	NUP133	Nucleoporin 133kDa	RNA processing; protein localization and transport; membrane	SZ-exome
ILMN_1686871	1	PARP1	Poly (ADP-ribose) polymerase 1	RNA processing; DNA replication; nucleotide and ATP binding; regulation of transcription; ion binding	SZ-exome
ILMN_1787461	1	RUNX3	Runt-related transcription factor 3	RNA processing; regulation of cell proliferation and death; nucleotide and ATP binding; regulation of transcription; ion binding	SZ-exome
ILMN_1766010	1	YARS	tyrosyl-tRNA synthetase	RNA processing; nucleotide and ATP binding	SZ-exome
ILMN_1673991	2	ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase / IMP cyclohydrolase		SZ-exome; SZ-linkage
ILMN_1752111	2	SMARCAL1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a-like 1	RNA processing; DNA replication; nucleotide and ATP binding; regulation of transcription; ion binding	SZ-exome; SZ-linkage
ILMN_1676745	2	ZNF142	Zinc finger protein 142	RNA processing; regulation of transcription; ion binding	SZ-exome; SZ-linkage
ILMN_1685580	3	CBLB	Cas-Br-M (murine) ecotropic retroviral transforming sequence B	Regulation of cell proliferation and death; calcium ion binding; ion binding; catabolic processes; protein localization and transport	SZ-exome; SZ-linkage
ILMN_2412549	4	GAR1	GAR1 ribonucleoprotein homolog	RNA processing; DNA replication	SZ-exome
ILMN_2224143	6	MCM3	Minichromosome maintenance complex component 3	RNA processing; DNA replication; nucleotide and ATP binding; regulation of transcription; ion binding	SZ-exome; SZ-linkage
ILMN_1701731	7	AKR1B1	Aldo-keto reductase family 1, member B1 (aldose reductase)		SZ-exome
ILMN_1721978	7	CARD11	Caspase recruitment domain family, member 11	Regulation of cell proliferation and death; regulation of transcription; ion binding; cell membrane; membrane	BP; SZ-exome
ILMN_1761479	7	ZC3HC1	Zinc finger, C3HC-type containing 1	RNA processing; regulation of cell proliferation and death; ion binding; catabolic processes	SZ-exome
ILMN_1744068	8	ELP3	Elongation protein 3 homolog	RNA processing; DNA replication; regulation of transcription; ion binding	SZ-exome; SZ-linkage
ILMN_1788538	8	NCALD	Neurocalcin delta	Calcium ion binding; ion binding	COG; SZ-exome
ILMN_2343097	8	NCALD	Neurocalcin delta	Calcium ion binding; ion binding	COG; SZ-exome
ILMN_1777740	8	THEM6	Thioesterase superfamily member 6		
ILMN_1750800	9	ACO1	Aconitase 1	RNA processing; ion binding	SZ-exome
ILMN_2073012	9	TMEM203	Transmembrane protein 203	Cell membrane; membrane	SZ-exome
ILMN_1689953	11	CD81	CD81 molecule	Regulation of cell proliferation and death; protein localization and transport; phosphorylation; cell membrane; membrane	SZ-exome
ILMN_2334296	11	IL18BP	Interleukin 18 binding protein		SZ-exome
ILMN_1700604	11	RBM14	RNA binding motif protein 14	RNA processing; DNA replication; nucleotide and ATP binding; regulation of transcription; ion binding	BP; SZ-exome

(table continues)

Table 3 (continued)

ILLUMINA PROBE	CHR	GENE SYMBOL	GENE DESCRIPTION	FUNCTIONAL ANNOTATION CLUSTERS	PREVIOUS ASSOCIATIONS
ILMN_2044453	12	LPAR5	Lysophosphatidic acid receptor 5	Cell membrane; membrane	SZ-exome
ILMN_1733696	15	IMP3	IMP3, U3 small nuclear ribonucleoprotein homolog	RNA processing	SZ-exome
ILMN_1801348	16	GOT2	Glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate aminotransferase 2)	RNA processing; cell membrane; membrane	SZ-exome
ILMN_1708328	16	RAB11FIP3	RAB11 family interacting protein 3	Calcium ion binding; ion binding; protein localization and transport; cell membrane; membrane	SZ-exome
ILMN_1728298	16	SBK1	SH3-binding domain kinase 1	Nucleotide and ATP binding; phosphorylation	SZ-exome; SZ-linkage
ILMN_2097793	17	ZBTB4	Zinc finger and BTB domain containing 4	RNA processing; regulation of transcription; ion binding	SZ-exome
ILMN_2153787	19	ARRDC5	Arrestin domain containing 5		SZ-exome
ILMN_1748894	19	GTPBP3	GTP binding protein 3 (mitochondrial)	RNA processing; nucleotide and ATP binding	SZ-exome
ILMN_1665943	19	MAP4K1	Mitogen-activated protein kinase kinase kinase 1	Nucleotide and ATP binding; phosphorylation	SZ-exome
ILMN_2183687	20	LIME1	Lck interacting transmembrane adapter 1	Cell membrane; membrane	SZ-exome
ILMN_1663954	20	NELFCD	Negative elongation factor complex member C/D		
ILMN_2162989	20	TMEM189	Transmembrane protein 189	RNA processing; regulation of transcription; ion binding; catabolic processes; cell membrane; membrane	SZ-exome
ILMN_1753885	20	YTHDF1	YTH domain family, member 1		SZ-exome
ILMN_1763460	22	NHP2L1	NHP2 non-histone chromosome protein 2-like 1	RNA processing	
ILMN_1786024	22	POLR3H	Polymerase (RNA) III (DNA directed) polypeptide H (22.9kD)	RNA processing; DNA replication; ion binding	SZ-exome
ILMN_1779185	22	SPECC1L	Sperm antigen with calponin homology and coiled-coil domains 1-like		SZ-exome
ILMN_1671257	X	DKC1	Dyskeratosis congenita 1, dyskerin	RNA processing; DNA replication	SZ-exome
ILMN_1775522	X	MAGED1	Melanoma antigen family D1	Regulation of cell proliferation and death; regulation of transcription; ion binding; cell membrane; membrane	SZ-exome
ILMN_2306189	X	MAGED1	Melanoma antigen family D1	Regulation of cell proliferation and death; regulation of transcription; ion binding; cell membrane; membrane	SZ-exome
ILMN_1670948	X	TSR2	TSR2, 20S rRNA accumulation, homolog	RNA processing	

Note. CVLT = California Verbal Learning Test; SZ-exome = previously associated with schizophrenia in an exome sequencing study; SZ-linkage = previously associated with schizophrenia in a linkage study; BP = previously associated with bipolar disorder; COG = previously associated with a cognitive phenotype.

patients to parents (Fromer et al., 2014) and unaffected siblings (Gulsuner et al., 2013).

A SNP in NCALD, a gene producing a calcium-binding protein associated with regulation of G-protein coupled signal transduction, which has also been associated with schizophrenia (Purcell et al., 2014), has recently been associated with processing speed in a large genome-wide association study of control individuals (Ibrahim-Verbaas et al., 2015). Here, we report a strong association between gene expression of NCALD and memory performance, a related cognitive process, as well as reduced expression in patients with schizophrenia. Schizophrenia has consistently been associated with substantial deficits in memory and processing speed (Schaefer et al., 2013), as well as mutations in calcium-associated genes (Hertzberg et al., 2015; Ripke et al., 2013). This

is promising evidence that at least some schizophrenia-related genes confer risk via impacting cognition.

Schizophrenia and bipolar disorder share substantial genetic overlap, many endophenotypes, and several symptom-level similarities, including cognitive impairment and psychosis (Lichtenstein et al., 2009). Two memory-related, schizophrenia-associated genes identified in the current study, which have both previously been associated with schizophrenia (Purcell et al., 2014), have also previously been associated with bipolar disorder (Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011; Sproun et al., 2011). A SNP in BMP14, a gene that regulates gene transcription splicing, was identified in a genome-wide association study of bipolar disorder (Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011). Changes in gene transcription

may underlie some of the shared etiology between bipolar disorder and schizophrenia, potentially mediated by effects on cognition. Additionally, a SNP in *CARD11* has previously been associated with decreased white matter in siblings of bipolar patients as compared to controls (Sprooten et al., 2011). Decreased white matter integrity is a shared endophenotype between bipolar disorder and schizophrenia, which suggests that *CARD11* could plausibly confer risk for these disorders via impacts on white matter integrity (Camchong, Lim, Sponheim, & Macdonald, 2009; Sprooten et al., 2011).

Four genes implicated in our study are novel genes not previously associated with schizophrenia, bipolar disorder, or cognition: *NELFCD*, *NHP2L1*, *THEM6*, and *TSR2*. *NELFCD*, an essential component of the NELF complex, which suppresses the elongation of transcription by RNA polymerase II, *NHP2L1*, a highly conserved nuclear protein associated with RNA binding, and *TSR2*, a pre-rRNA processing protein with a known ASD-related de novo mutation (O’Roak et al., 2012), are all related to transcription and translation (Safran et al., 2002). Furthermore, a cluster analysis using DAVID, a bioinformatics platform that accesses many gene ontology databases to group lists of genes, identified a significant enrichment of DNA- and RNA-regulating genes within the memory- and schizophrenia-related genes (online supplemental Table S4) (Huang da et al., 2009). Twenty-one of the 41 identified genes fell into this category, suggesting a significant role of transcriptional and translational regulation in cognitive impairment in schizophrenia. This lends further evidence to the hypothesis that many of the genetic effects relevant in schizophrenia reflect regulatory processes rather than coding changes per se (Kim et al., 2014; Maurano et al., 2012).

We also identified six genes related to T and B cell signaling pathways, consistent with previous work implicating immune-related genes (Ripke et al., 2014; Wang, Yang, Gelernter, & Zhao, 2015), altered proinflammatory cytokine expression and protein levels (Dimitrov et al., 2013; Na, Jung, & Kim, 2014; Pandey, Ren, Rizavi, & Zhang, 2015; Song et al., 2014), and inflammation during pregnancy (Meyer, Feldon, & Dammann, 2011; Miller, Culpepper, Rapaport, & Buckley, 2013) in risk for schizophrenia (for review see: Fineberg & Ellman, 2013).

The primary competing explanation of the findings in this study are that the gene expression changes associated with memory and schizophrenia may be secondary to illness expression (e.g., symptoms) or treatment. While we cannot fully rule out that such factors contribute, they are unlikely to completely account for the present results, for several reasons. First, in considering the sources of the relationships between gene expression and CVLT scores, if the effects were driven purely by such secondary factors, they would disappear when probands with schizophrenia or bipolar disorder are excluded. However, the average effect size for the 76 probes significantly associated with CVLT (partial $R^2 = .131$, 95% CIs [.128, .134]) was reduced only moderately when excluding schizophrenia and bipolar patients (partial $R^2 = .095$, 95% CIs [.090, .099]) (online supplemental Table S1). A power analysis to determine the sample size required for detecting the average effect size found among the nonpatient participants (partial $R^2 = .095$) at genome-wide significance revealed that only 100 additional subjects would be necessary (Faul et al., 2007). Furthermore, expression levels of the genes associated with memory were found to be moderately heritable. Taken together, these results are consistent

with an inherited basis for the relationships between expression levels of these genes and memory in the general population. Future work may discern the specificity of these genotype–phenotype relationships, because genes can both impinge on a variety of cognitive processes (e.g., BDNF; Ahmed, Mantini, Fridberg, & Buckley, 2015), or only certain components of a single process (Kremen et al., 2014; Panizzon et al., 2011; Papassotriopoulos & de Quervain, 2011). Furthermore, as memory impairment is a feature of many psychiatric illnesses, schizophrenia only one among them, it is possible that some of these genes confer risk for psychopathology more broadly.

Given that probands with schizophrenia and bipolar disorder are enriched (relative to nonaffected individuals) for genetic variations conferring cognitive impairment, larger effect sizes for the associations between gene expression and memory performance would be expected when probands are included compared to those when probands are excluded, as was observed. Alternatively, the higher effect sizes when probands are included could reflect the impacts of clinical state, medications, or both on gene expression and memory performance. However, the assessments of memory performance and gene expression were done when probands were clinically stable, and expression levels of the genes associated with both memory and schizophrenia were not related to positive symptom severity or current antipsychotic medication status. These findings, combined with the fact that previous work has shown associations of genotypic variations in nearly all of the identified genes with schizophrenia, indicate that a shared inherited substrate is likely to represent a major source of the overlap between gene expression patterns associated with reduced memory performance and schizophrenia. That there were no significant expression differences between the nonaffected cotwins of probands and controls somewhat complicates this interpretation; however, as would be expected from a heritable source, cotwins’ expression levels were intermediate between probands and controls on most of the markers, and power was limited for this contrast. In any case, because these are correlational data, they do not provide direct evidence for causal relationships between expression of these genes and either memory performance or schizophrenia. Animal model studies using genetic manipulations could more directly test whether decreased expression of these genes cause decrements in memory performance or changes on other behavioral assays related to schizophrenia.

Other methodological limitations of this study include the assay of gene expression from peripheral tissue and the relatively small sample size. We examined gene expression in PBMCs, which has been shown to correlate with central nervous system expression and is heritable in our sample, but is nonetheless not as direct a read-out of expression levels in the brain as would be true of samples in neurons or glia (Cheung et al., 2003). Peripheral gene expression is a pragmatic approach—It is relatively low cost and less invasive to assay than other tissues, and biomarkers of disease ascertainable at this level are advantageous for future research and clinical application (Cattane et al., 2015). Furthermore, to protect against identifying gene expression changes irrelevant to brain function, we first screened the genome for genes associating with a cognitive phenotype. We also used a microarray assay to measure gene expression, which only captures expression of primary transcripts and cannot quantify absolute levels of expres-

sion. Future work using RNA sequencing could be useful in this regard.

Additionally, our sample size is relatively modest. Because we used Bonferroni correction for multiple testing on the whole genome level at the discovery stage and found evidence of replication of effect sizes and direction of schizophrenia-related variations in an independent sample, the small sample size is of concern primarily with regard to Type II (false negative) rather than Type I (false positive) error. That is, there are likely many hundreds of additional genes relating to both memory and schizophrenia that were not detected in this study. Even in the case that some or all of the genes not meeting statistical significance in the replication sample are false positives, the interpretation of the findings would remain the same as the 14 genes meeting statistical significance represent nearly all the functional clusters identified in the full set of 43 probes. Nonetheless, individual RNA transcripts within these functional groups that did not show statistical replication may not be genes of high effect.

In summary, consistent with substantial previous work linking schizophrenia-related genotypic variation to memory (Greenwood et al., 2012; Greenwood et al., 2013; Heck et al., 2014; Karlsgodt et al., 2011; Kauppi et al., 2015; Nicodemus et al., 2014; Touloupoulou et al., 2007; van Erp et al., 2008; Walton et al., 2013), we have provided evidence that a subset of genes expressed in relation to memory performance are underexpressed in schizophrenia patients relative to unaffected cotwins of patients and controls and that this expression is moderately heritable. Thus, genes increasing risk for schizophrenia may do so via effects on memory-related processes. Furthermore, enrichment of translation and transcription-related genes identified in the current study suggests that this may be a functional mechanism by which genes promoting memory impairment in schizophrenia may specifically confer risk. This study represents only a first step in uncovering gene expression changes that promote memory impairment in schizophrenia. Investigations of other endophenotypes for schizophrenia may uncover relevant genetic overlap or dissociation between cognitive processes, revealing important functional connections between risk-conferring genes and specific neurobiological systems.

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Received June 11, 2015

Revision received September 3, 2015

Accepted September 9, 2015 ■