Cognitive Analysis of Schizophrenia Risk Genes That Function as Epigenetic Regulators of Gene Expression

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Epigenetic mechanisms are an important heritable and dynamic means of regulating various genomic functions, including gene expression, to orchestrate brain development, adult neurogenesis, and synaptic plasticity. These processes when perturbed are thought to contribute to schizophrenia pathophysiology. A core feature of schizophrenia is cognitive dysfunction. For genetic disorders where cognitive impairment is more severe such as intellectual disability, there are a disproportionally high number of genes involved in the epigenetic regulation of gene transcription. Evidence now supports some shared genetic aetiology between schizophrenia and intellectual disability. GWAS have identified 108 chromosomal regions associated with schizophrenia risk that span 350 genes. This study identified genes mapping to those loci that have epigenetic functions, and tested the risk alleles defining those loci for association with cognitive deficits. We developed a list of 350 genes with epigenetic functions and cross-referenced this with the GWAS loci. This identified eight candidate genes: BCL11B, CHD7, EP300, EPC2, GATAD2A, KDM3B, RERE, SATB2.Usinga dataset of Irish psychosis cases and controls (n ¼ 1235), the schizophrenia risk SNPs at these loci were tested for effects on IQ, working memory, episodic memory, and attention. Strongest associations were for rs6984242 with both measures of IQ (P ¼ 0.001) and episodic memory (P ¼ 0.007). We link rs6984242 to CHD7 via a long range eQTL. These associations were not replicated in independent samples. Our study highlights that a number of genes mapping to risk loci for schizophrenia may function as epigenetic

INTRODUCTION

Epigenetic mechanisms are heritable and have an important role in the dynamic regulation of various genomic functions, including gene expression. This is largely achieved through the covalent

modification of DNA and histones. Epigenetic chromatin modifications establish and maintain gene expression programs to help orchestrate brain development, adult neurogenesis, and synaptic plasticity [Qureshi and Mehler, 2013], processes that when perturbed are thought to contribute to the pathophysiology of schizophrenia [Liu et al., 2014; Wockner et al., 2014]. Recent exome sequencing [McCarthy et al., 2014; Takata et al., 2014] has identified rare mutations in genes associated with epigenetic chromatin remodeling in schizophrenia. Pathway analysis of genome-wide association study (GWAS) data have supported a role for epigenetic mechanisms (histone methylation) in major psychiatric disorders [O'Dushlaine, 2015].

A core feature of schizophrenia is cognitive dysfunction in the form of impairments in memory, attention and IQ. This has a genetic basis and decline in cognitive performance occurs years prior to onset of illness as the brain is still developing [Kahn and Keefe, 2013]. For genetic disorders where cognitive impairment is more severe than in schizophrenia such as intellectual disability, there are a disproportionally high number of genes involved in the epigenetic regulation of gene transcription [Kleefstra et al., 2014]. Loss-of-function (LoF) mutations in the MECP2 gene (encoding a methyl-DNA binding protein) causing Rett syndrome is the classic example of an "epigenetic gene" being mutated to cause a disorder with severe cognitive deficits. There are currently >50 genes identified that function in different epigenetic mechanisms and are mutated in cognitive disorders [Kleefstra et al., 2014]. Evidence now supports some shared genetic aetiology between schizophrenia and intellectual disability [Fromer et al., 2014]. Therefore, it is pertinent to ask if an overlapping phenotypic characteristic of schizophrenia and intellectual disability (cognitive deficits) could be due to genetic variability in a shared pathobiology (epigenetic mechanisms).

A meta-analysis of over 50 GWAS studies performed by the Psychiatric Genomics Consortium (PGC) has culminated in the identification of 108 risk loci for schizophrenia that contain some 350 genes [Schizophrenia Working Group of the Psychiatric Genomics, 2014]. Our study asks: do any genes at these new schizophrenia risk loci regulate epigenetic mechanisms, specifically as chromatin modulators of gene expression? For those loci containing those genes, we next ask whether they independently associate with cognitive functions? Our overall hypothesis is that GWAS-identified risk genes for schizophrenia, which regulate epigenetic mechanisms, are associated with deficits in cognitive function.

MATERIALS AND METHODS

Chromatin Modulators

We prepared a list of genes encoding the major classes of chromatin modulators which encompasses epigenetic regulators of gene expression (Table I). Relevant genes were collected from two of the main online gene databases; Gene Cards (http://www. genecards.org/) and the Gene Ontology Consortium (http://geneontology.org/) as follows: Searches of these databases were conducted using specific keywords to distinguish the type of chromatin modulation that was being investigated. For example, in the case of Genecards, a human gene database, we used the advanced search option (http://www.genecards.org/Search) using terms such as "histone AND acetyltransferase" to search the summaries section. In the case of Gene Ontology we searched the ontology database (http://amigo2.berkeleybop.org/amigo/search/ontology) using terms such as "histone acetylation" giving the branch "GO:0016573 histone acetylation" under inferred tree view, following the "link to all genes and gene products annotated," filtering with the term "Homo sapiens" returns a list of relevant genes. In addition to using these two databases, in the case of histone ubiquitination we have included the full list of E1 ubiquitin activating enzymes, and E2 ubiquitin conjugating enzymes as it is not always known which of these coordinates with the E3

ubiquitin ligase enzyme to mediate the modification. This list was selected from the list compiled by Van Wijk and Timmers [2010]. The two lists for the same chromatin modulation were combined, duplicate entries were removed and each gene was then manually queried for legitimate inclusion. At this point an associated list was appended onto the list of known modulators, this includes factors that exist in complex with the enzyme in question, and are required for its activity (Supplementary Table SI). Also shown in the list are the target residues/histone of the enzyme where known, and a brief statement of evidence as taken from Genecards' summary section, which summarizes gene function from Entrez Gene, UniProtKB (UniprotKB/Swiss-Prot/UniprotKB/TrEMBL), Tocris Bioscience, PharmGKB, and Gene Wiki. These genes were assembled into a final list of 350 unique chromatin-modulating genes (Supplementary Table SI), which from here on we refer to as "epigenetic genes." The epigenetic gene list (n ¼ 350) was cross-referenced with genes located in 108 chromosomal regions associated with schizophrenia risk (n ¼ 350) [Schizophrenia Working Group of the Psychiatric Genomics, 2014] to identify candidate genes for this study.

TABLE I. Post-Translational Modification Categories for Epigenetic Gene List

Writers	Erasers
DNA methyltransferases	DNA demethylases
Histone methyltransferases	Histone demethylases
Histone ubiquityltransferases	Histone deubiqutinases
Ubiquitin-like histone modifiers	
Histone acetyltransferases	Histone deacetylases
Histone kinases	Histone phosphatases
Chromatin remodelers	
Histone chaperones	

Study Participants and Neuropsychological

Testing

This study used an Irish dataset of broad psychosis cases (n ¼ 890) and controls (n ¼ 330) who had completed tests in five main areas of cognition and for whom GWAS data were available. Cases were clinically stable patients with a DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th Edition) diagnosis of schizophrenia (n ¼ 559), schizoaffective disorder (n ¼ 111), bipolar disorder (n ¼ 148), major depressive disorder with psychotic features (n ¼ 38), or psychosis not otherwise specified (n ¼ 34) recruited from five sites across Ireland (Table II). Inclusion criteria required that participants were clinically stable at the time of neuropsychlogical testing, were aged 18–65 years, had no history of comorbid psychiatric disorder, no reported substance abuse in the preceding 6 months, no prior head injury with loss of consciousness, and no history of seizures. Diagnosis was confirmed by trained psychiatrists using the Structured Clinical Interview for DSM-IV-TR [First et al., 2002]. A more detailed description of sample ascertainment has previously been published [Cummings et al., 2013]. Healthy control participants were recruited via online and poster

advertising. They were aged 18–65 years, with no reported history of substance abuse in the preceding 6 months, no prior head injury with loss of consciousness, no history of seizures, and no personal history of psychosis or in their first-degree relatives. All assessments were conducted in accordance with the relevant ethics committees' approval from each participating site. All participants had four grandparents born in Ireland and provided written informed consent.

Participants completed a neuropsychological assessment battery designed to target the cognitive deficits observed in schizophrenia, this battery included general cognitive function, episodic memory, working memory, and attentional control. General cognitive functioning (IQ) was measured using selected subtests (Vocabulary, Similarities, Block Design, and Matrix Reasoning) from the Wechsler Adult Intelligence Scale (WAIS); [Weschler, 1997a, 2001] which assessed verbal, performance, and full-scale IQ as well as the Wechsler Test of Adult Reading (WTAR). Episodic memory was assessed using the logical memory (LM) 1 and 2 tasks as well as the "Faces 1 and 2" task subtests from the Wechsler Memory Scale (WMS) III [Weschler, 1997b] as well as the Paired-Associates Learning task (PAL). Working memory was assessed using the spatial working memory task (SWM) from the Cambridge Neuropsychological Test Automated Battery (CANTAB) [Robbins et al., 1994] and letter-number sequencing from the WMS III [Weschler, 1997b]. Attentional control was assessed using the Continuous Performance Task (CPT)—Identical Pairs version, and the Sustained Attention to Response Task [Cornblatt et al., 1988; Robertson, 1994].

Genotyping and Statistical Analysis

Genotyping was conducted on DNA extracted from blood or saliva from patient and control participants. SNP data were obtained from two different sites; a recent GWAS using the Affymetrix SNP Array 6.0 platform, conducted as part of the Wellcome Trust Case Control Consortium 2 [Irish Schizophrenia Genomics Consortium and the Wellcome Trust Case Control Consortium 2, 2012] and a collaborative GWAS with Cardiff University using an Illumina Human Core Exome (þcustom) SNP array. Full GWAS data on these samples included recently completed imputation using 1000 Genomes Phase I data and IMPUTE2 [Howie et al., 2009] to give ⊡10 million SNPs genome-wide per sample. Only samples that had passed the quality control filtering were imputed using the 1000 Genomes reference panel. Imputed data were converted to best guess genotypes using PLINK [Purcell et al., 2007], which was also used to extract data on the index SNP at each gene from GWAS data and combine it in Statistical Package for the Social Sciences (SPSS) 21, IBM) with data from an extensive neuropsychological battery. Statistical analysis was carried out in SPSS using a linear-based model of regression. Because of differences in cognitive scores between male and female participants and between older and younger individuals, gender and the age at the time of assessment were used as co-variate measures to determine a general genotype effect across all cases and controls. Cognitive test scores for each genotype group were compared using a three group analysis, that is, homozygous non-risk versus heterozygous versus homozygous risk genotype groups. R^2 is a measure of how close the data fits to the regression line. R^2 change values were used in this analysis which identifies changes in the original R^2 based on the linear contribution of variables added into the regression model. Regarding power in this sample, which in itself is too small for GWAS, to detect for example, differences in Performance IQ for a SNP where the mean difference in scores between risk allele carriers and non-carriers was 0.5 standard deviations (SD), our sample had 87% power at a ¼ 0.05. For a mean difference of 0.33 or 0.75 SD, the power was 59% and 99%, respectively for the same type I error rate.

TABLE II. Participant Demographics and Neurocognitive and Clinical Measures

Characteristic	Patients		Healthy participants (n = 330)
	Psychosis broad (n = 890)	Psychosis narrow (n = 670)	
Psychosis subtype (n)			
Schizophrenia	559	559	NA
Schizoaffective disorder	111	111	
Bipolar disorder	148	NA	
Major depressive disorder	34	NA	
Psychosis not otherwise specified	38	NA	
Male: Female ratio	1.94:1.0	2.4:1.0	1.0:1.2
Age, mean (S.D.)	42.93 [12.42]	42.28 [12.48]	35.81 [12.66]
Age at onset, mean (S.D.)	23.78 [8.28]	23.23 [7.68]	NA
Cognitive full scale IQ, mean (S.D.)	92.04 [19.21]	90.29 [18.08]	119.81 [15.57]

NA, not applicable.

Interaction Analysis

Online tools and resources including STRING (http://string-db. org/) and the ingenuity pathway analysis (IPA) database (http://www.ingenuity.com/) were used to identify direct (physical bind-ing) and indirect (functional) interacting partners of each candidate gene based on genomic and co-expression data as well as experimental data from literature searches. Gene IDs from each interaction network were compiled into lists and cross-referenced with the epigenetic gene list (n ¼ 350) and the GWAS schizophrenia gene list (n ¼ 350) to determine if any interacting partners had epigenetic functions and were associated with schizophrenia risk. Among the candidate genes, three pairs of genes were identified as interacting partners: SATB2 and BCL11B, EP300 and BCL11B, and CHD7 and GATAD2A. All three combinations were taken forward for neuropsychological interaction analysis using the same Irish dataset. Interaction analysis was carried out in SPSS based on a linear model of regression using up to five genotype groups where study participants were assigned to groups based on the number of schizophrenia risk alleles they carried at the two SNPs from a pair of interacting genes. Gender and age at time of assessment were used as co-variate measures in this analysis.

UK Replication Sample

About 772 individuals with schizophrenia or schizoaffective dis-order—depressive subtype were recruited from community mental health teams in Wales and England. The Schedule for Clinical Assessment in Neuropsychiatry (SCAN) interview was conducted followed by case note review and consensus diagnosis according to DSM-IV criteria. Individuals were assessed using the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Consensus Cognitive Battery (MCCB). The MCCB is a 10 test cognitive battery consisting of seven cognitive domains: speed of processing, attention and vigilance, working memory, verbal learning, visual learning, reasoning and problem solving, and social cognition [Nuechterlein et al., 2008]. These domains were chosen as primary cognitive outcomes given that the MATRICS tests were selected on the basis of the domains they measured, and the fact that this approach minimized measurement error as some domains are assessed by more than one test. Allinterviewers were trained in the use of the SCAN interview and MCCB. The UK Multicentre Research Ethics Committee (MREC) approved the study and all participants provided valid informed consent. These samples were genotyped at the Broad Institute, Massachusetts on the Illumina HumanOmniExpressExome-8v1. QC procedures followed those of the PGC [Schizophrenia Working Group of the Psychiatric Genomics, 2014]. Imputation was

carried out by the PGC using IMPUTE2 and for this analysis we converted imputed to best guess genotypes using PLINK [Purcell et al., 2007; Howie et al., 2009].

German Replication Sample

Patients with a DSM-IV diagnosis of schizophrenia were ascertained from mental health services in the Munich area. Exclusion criteria were a history of head injury or neurological disease. Patients, ages 18–80 years, were interviewed with the Structured Clinical Interview for DSM-IV (SCID), and the interviews were rated by psychiatrists or psychologists. Healthy comparison sub-jects of German descent, ages 18–80 years, were randomly selected from population registers from the Munich area. Participants underwent an extensive screening process to exclude those with neurological or psychotic disorders and those who had first-degree relatives with psychotic disorders. In the case of participants older than 60 years, the Mini-Mental State Examination was employed to exclude individuals with possible cognitive impairment [Walters et al., 2013]. In this sample, IQ was measured by the full German Wechsler Adult Intelligence Scale—Revised (WAIS-R). Episodic memory was assessed using the immediate and delayed logical memory tests from the German Wechsler Memory Scale—Revised (WMS-R) [H€arting et al., 2000]. For Full Scale IQ and Verbal IQ, data were available for 355 patients and 2,147 healthy comparison subjects. For Episodic Memory, data were available for 362 patients and 558 healthy comparison subjects. GWAS data were available for these samples. Individuals were genotyped on different plat-forms and imputed in six batches. Quality control and imputation of batches 1 (Human610-Quadv1_B, Human660W-Quad_v1_A, Human610-Quadv1_B), 2 (HumanHap 300 v1.0.0), and 3 (Affymetrix 6.0) were performed in the framework of the PGC2 schizophrenia meta-analysis [Schizophrenia Working Group of the Psychiatric Genomics, 2014]. Batches 4 (HumanHap 300 v1.0.0), 5 (Illumina Human OmniExpress 12v1.0), and 6 (Illumina Omni1-Quad) were imputed following the same QC and imputation protocols with minor adaptations. Sample call-rate was adjusted to account for smaller sample sizes with a minimum threshold of 96%. After imputation the batches were combined and all markers that differed more than 0.1 in minor allele frequency between any of the batches were excluded. Association analysis was done with PLINK using a linear regression model corrected for age, sex, education, affection status and batch.

RESULTS

The PGC GWAS identified SNPs associated with SZ in 108 loci that contain 350 genes [Schizophrenia Working Group of the Psychiatric Genomics, 2014]. Cross referencing our list of chromatin modulators with the genes in the GWAS schizophrenia risk loci identified a shortlist of 17 epigenetic genes in schizophrenia risk regions. As some of the risk loci include large numbers of genes, we systematically reviewed each GWAS results plot to conservatively reduce our gene list to seven genes (BCL11B, EP300, EPC2, GATAD2A, KDM3B, RERE, and SATB2), where the associated SNP is located within or close to the gene location. Three of these genes, BCL11B, EPC2, and SATB2, are the only gene within their associated locus. For the remaining four genes, positional and linkage disequilibrium evidence from the relevant plots was strong enough to suggest that they could be affected by the risk SNPs at these loci.

We also considered the possibility that a risk SNP may affect a gene outside its risk region. In the schizophrenia GWAS it is worth noting that there are 21 loci that do not contain any genes. To identify such distantly affected genes, we took advantage of a recent genome-wide brain expression quantitative trait loci (eQTL) study [Kim et al., 2014]. This study reports results for eQTL analysis between SNPs and the expression of genes within 1 Mb of the SNP (i.e., a 2 Mb window). We extracted the eQTL results for all 128 schizophrenia index risk SNPs reported by the PGC and found

that 59 SNPs had an eQTL at nominal P < 0.05. For each of these SNPs we calculated the number of genes within the 2 Mb window and Bonferroni corrected for that number of genes at each SNP [Dunn, 1961]. This identified 16 unique SNPs with significant eQTL results that totalled 21 genes (Supplementary Table SII). We cross-referenced these 21 genes with our epigenetic gene list and identified one extra gene for study, CHD7. For each candidate gene, the highest associated SNP (the index SNP from the GWAS region containing that gene) was used for analysis of neuropsychological variables.

The final eight genes (with corresponding index SNPs) for analysis are listed here including brief details of function and descriptions of any cognitive disorders caused by these genes:

(1)   BCL11B (rs2693698) encodes B-Cell CLL/Lymphoma 11B, a protein associated with the Mi-2/nucleosome remodelling and deacetylase complex (NuRD) [Cismasiu et al., 2005].

(2)   CHD7 (rs6984242) encoding chromodomain helicase DNA binding protein 7, is an ATP-dependent chromatin remodeller. De novo mutations in CHD7 cause Charge syndrome, a disorder characterized by abnormal ear formation, hearing loss, cleft palate, coloboma, genital hypoplasia, delayed development, and intellectual disability [Lalani et al., 2006]. De novo mutations in CHD7 have also been found in cases of autism [O'Roak et al., 2012b; Jiang et al., 2013] and CHD7 has been shown to interact directly and indirectly with CHD8, a confirmed risk gene for autism [Batsukh et al., 2010; Wilkinson et al., 2015].

(3)   EP300 (rs9607782) encodes the histone acetyltransferase, E1A Binding Protein P300. De novo mutations within the EP300 gene cause rare cases of Rubinstein–Taybi syndrome, a disorder characterized by intellectual disability and developmental abnormalities [Negri et al., 2014].

(4)   EPC2 (rs200327371) encodes Enhancer Of Polycomb Homo-log 2, protein associated with a chromatin repressive complex. Microdeletions within the 2q23.1 region containing EPC2 are associated with intellectual disability, seizures, microcephaly, development delay, hypotonia, and behavioral features similar to autism or Angelman syndrome [Van Bon et al., 2010].

(5)   KDM3B (rs10043984), Lysine (K)-Specific Demethylase 3B is required for normal spermatogenesis and sexual behaviors in male mice [Liu et al., 2015] and encodes an enzyme that removes a key transcriptional repressive modification from chromatin [Kim et al., 2012]. Levels of this histone modification correlates with symptom severity in schizophrenia in a sex-specific manner [Chase et al., 2015].

(6)   GATAD2A (rs2905426) is a transcriptional repressor and subunit of the NuRD complex [Wade et al., 1999; Brackertz et al., 2002].

(7)   RERE (rs34269918) is a transcriptional co-repressor that binds chromatin and in mouse is involved in cerebellar development [Kim and Scott, 2014].

(8)   SATB2 (rs6704641) encodes a protein involved in chromatin remodelling. De novo structural and point mutations in SATB2 result in SATB2 haploinsufficiency and a defined SATB2 phenotype with developmental delay, mild to severe intellectual disability, behavioral problems and abnormal craniofacial features, specifically a cleft palate [Docker et al., 2014].

Each of the eight schizophrenia risk variants was analyzed against four areas of cognitive function (IQ, working memory, episodic memory, and attentional control). To maximize power, this was done in the combined sample of psychosis cases and controls. Table III highlights all nominally significant

associations detected. The strongest results were for rs6984242 (CHD7) where one association survives Bonferroni correction for the four cognitive domains tested and the eight variants tested (corrected P-value threshold ¼ 0.002). For that association, the schizophrenia risk allele (G) was associated with lower Verbal IQ (P ¼ 0.001) where an R2 change value of 0.010 indicates that 1.0% of the variance in test performance is explained by the genotype. The same variant was associated with two other measures of IQ (Full Scale IQ, P ¼ 0.007, and WTAR, P ¼ 0.003), as well as four measures of episodic memory function with the strongest result for the Faces task, delayed condition (P ¼ 0.007).

Of the remaining seven variants, four variants (at EP300, GATAD2A, KDM3B, and RERE) had nominally significant associations with at least one cognitive task (Table III). Of note, the risk allele for rs2905426 (GATAD2A, which interacts with CHD7) was also associated with lower Full Scale IQ (P ¼ 0.016). To follow this up we performed an interaction analysis using the data for the variants at CHD7 and GATAD2A. In this regression analysis participants were categorized into four groups on the basis of the number of risk alleles they carried (0, 1, 2, or 3/4 [few participants carried four risk alleles so these individuals were grouped with those that carried three risk alleles]). Association was found with four measures of IQ (Table IV): Full Scale IQ (P ¼ 0.001), Verbal IQ (P ¼ 0.002), WTAR (P ¼ 0.005), and Performance IQ (P ¼ 0.016). For Full Scale IQ, the R2 change value for the 2-SNP analysis was 0.013 whereas the R2 change value for rs6984242 (CHD7) on its own was 0.008. For Verbal IQ, the R2 change value for both the

2-SNP and single SNP analysis is 0.010 and the range of mean scores for the 2-SNP and single SNP analysis largely overlap. For other nominally significant associations from the 2-SNP analysis, the results do not differ much from the single SNP analysis. Thus, there is no strong evidence from this analysis that the effect from a genetic interaction between these two variants is contributing more significantly to poorer cognitive performance than the individual variants on their own. Interaction analysis using the two other pairs of interacting partners identified (SATB2 and BCL11B,and EP300 and BCL11B) did not detect any significant associations.

We sought independent replication of our Bonferroni-corrected significant association between Verbal IQ and rs6984242 (CHD7). In doing these analyses, we also sought independent support for the associations we detected between this SNP and Full Scale IQ and with Episodic Memory. We used two independent datasets. The UK dataset is based on the MATRICS battery and so composite scores were used for cognitive domains. Similar to the Irish dataset, the German dataset used the WAIS to measure IQ and the WMS to measure Episodic Memory. rs6984242 did not show association with IQ or Episodic Memory in either dataset (Tables V and VI).

Gene/variant (risk allele)	Cognitive domain	Neuropsychological variable	Genotype (sample N)	Mean score (SD)	P-value (r^2 change)
CHD7/rs6984242 (G)	IQ	WTAR	A/A (290)	36.67 (10.38)	0.003 (0.011)
			G/A (385)	35.57 (10.25)	
			G/G (134)	33.91 (11.19)	
CHD7/rs6984242 (G)	IQ	Verbal IQ	A/A (334)	99.40 (22.29)	0.001 (0.010)
			G/A (441)	98.43 (22.34)	
			G/G (164)	93.65 (21.87)	
CHD7/rs6984242 (G)	IQ	Full scale IQ	A/A (277)	100.69 (22.38)	0.007 (0.008)
			G/A (373)	99.06 (22.17)	
			G/G (126)	96.25 (22.69)	
CHD7/rs6984242 (G)	Episodic memory	"Faces 2" task	A/A (232)	36.74 (5.50)	0.007 (0.010)
			G/A (312)	36.19 (5.21)	
			G/G (97)	35.44 (5.91)	
CHD7/rs6984242 (G)	Episodic memory	Logical memory 1	A/A (343)	32.66 (15.17)	0.029 (0.005)
			G/A (454)	32.39 (14.71)	
			G/G (170)	29.74 (14.92)	
CHD7/rs6984242 (G)	Episodic memory	Logical memory 2	A/A (341)	19.03 (11.41)	0.023 (0.005)
			G/A (452)	18.67 (10.75)	
			G/G (167)	17.06 (10.71)	
CHD7/rs6984242 (G)	Episodic memory	PAL (stages completed)	A/A (52)	−0.53 (1.84)	0.034 (0.03)
			G/A (76)	−0.72 (2.65)	
			G/G (16)	−2.22 (3.97)	
EP300/rs9607782 (A)	Episodic memory	Logical memory 2	T/T (465)	19.33 (11.11)	0.038 (0.004)
			A/T (379)	18.03 (10.93)	
			A/A (64)	17.19 (10.92)	
GATAD2A/rs2905426 (G)	IQ	Performance	T/T (318)	99.00 (22.36)	0.042 (0.005)
			G/T (329)	97.45 (22.59)	
			G/G (113)	95.25 (22.299)	
GATAD2A/rs2905426 (G)	IQ	Full scale	T/T (315)	100.44 (22.38)	0.016 (0.007)
			G/T (325)	99.94 (22.19)	
			G/G (113)	95.49 (22.12)	
KDM3B/rs10043984 (T)	Attention	3-digit D'Prime	T/T (208)	1.16 (.98)	0.048 (0.008)
			T/C (159)	1.17 (.85)	
			C/C (37)	1.26 (.99)	
RERE/rs34269918 (InsA)	Attention	3-digit D'Prime	G/G (150)	1.29 (0.96)	0.03 (0.011)
			GA/G (188)	1.21 (0.93)	
			GA/GA (59)	1.08 (0.88)	

DISCUSSION

This study developed a list of 350 genes that function as chromatin modulators of gene expression. These genes were investigated for a role in cognitive deficits in schizophrenia by first determining which of these genes are present at loci that are genome-wide significant for schizophrenia from the largest GWAS to date. The eight candidate schizophrenia risk genes identified had various epigenetic functions and a number are the site of causative mutations for rare cognitive disorders, for example, Charge syndrome (CHD7), Rubinstein–Taybi syndrome (EP300) and SATB2-associated syndrome. Five of the eight risk SNPs in question (rs6984242, rs2905426, rs9607782, rs10043984, and rs34269918) were found to be associated with poorer performance in tests from three of the four cognitive domains; IQ, episodic memory, and attention. For seven of the eight candidate genes, the schizophrenia risk SNP is located within or near the gene of interest. The impact of these variants on gene function is not certain. For three genes, there is brain eQTL data that identifies the potential functional impact of SNPs on gene expression. Significant eQTLs for rs9607782 and EP300, for rs6984242 and CHD7, and for rs2905426 and GATAD2A are reported in the Kim et al. [2014] brain eQTL data that included 424 brain samples. Finally, our extended analysis of this eQTL dataset identifies CHD7 as a putative schizophrenia risk gene for the first time. The index SNP in this instance is rs6984242 and the association signal from the GWAS maps to chr8:60,475,469–60,954,469(hg19), with the SNP located 891 Kb upstream of the first exon of CHD7. There are only four genes within

the 2 Mb region that centres on rs6984242 and CHD7 is the only gene with a significant eQTL involving this SNP in the Kim et al. dataset. In an independent but much smaller brain eQTL dataset (n ¼ 134; www.braineac.org) the rs6984242-CHD7 eQTL is not supported by data from the 10 different brain regions analyzed. CHD7 belongs to the chromodomain helicase DNA (CHD) binding family of proteins, where it is a member of subgroup III (along with CHD6, CHD8, and CHD9) based on sequence similarities and additional functional domains [Marfella and Imbalzano, 2007]. CHD genes are members of the SNF2 super-family of ATP-dependent chromatin remodelers [Flaus et al., 2006; Marfella and Imbalzano, 2007] and function as part of large multisubunit complexes to regulate gene transcription, DNA repair, replication, and recombination. CHD7 interacts directly and indirectly with CHD8 [Batsukh et al., 2010], a gene that is recurrently mutated in autism [O'Roak et al., 2012a]. As brain eQTL and related functional datasets increase in size and power, we will seek more definite evidence to associate CHD7 with schizophrenia risk via rs6984242.

TABLE IV. Regression Analysis of rs6984242 (CHD7)—rs2905426 (GATAD2A) Interaction				
Cognitive domain	Neuropsychological variable	Genotype group (N)	Mean (SD)	P-value (r^2 change)
IQ	WTAR	0 (114)	36.38 (10.15)	0.005 (0.01)
		1 (284)	36.11 (10.91)	
		2 (261)	36.36 (9.89)	
		3/4 (119)	32.48 (10.34)	
IQ	Verbal	0 (133)	99.96 (22.22)	0.002 (0.01)
		1 (316)	99.22 (22.37)	
		2 (314)	97.58 (22.81)	
		3/4 (142)	94.18 (20.83)	
IQ	Performance	0 (108)	101.07 (23.14)	0.016 (0.007)
		1 (276)	97.71 (22.22)	
		2 (258)	97.06 (22.78)	
		3/4 (117)	96.19 (22.22)	
IQ	Full scale	0 (108)	102.83 (22.81)	0.001 (0.013)
		1 (271)	99.63 (21.93)	
		2 (256)	98.83 (22.34)	
		3/4 (117)	95.56 (22.08)	
Episodic memory	LM1	0 (138)	32.85 (15.00)	0.049 (0.004)
		1 (323)	32.39 (15.15)	
		2 (319)	32.35 (15.15)	
		3/4 (145)	30.13 (14.20)	
Episodic memory	LM2	0 (137)	19.32 (11.23)	0.018 (0.005)
		1 (322)	19.07 (11.31)	
		2 (315)	18.45 (10.92)	
		3/4 (145)	17.30 (10.32)	
Attention	CPT-28	0 (64)	2.10 (1.20)	0.009 (0.015)
		1 (141)	2.12 (1.13)	
		2 (150)	1.93 (1.18)	
		3/4 (73)	1.78 (1.09)	

It is rs6984242 that provides the strongest evidence of association where the schizophrenia risk allele (G) was associated with lower Verbal IQ (P ¼ 0.001) and explains 1.0% of the variance in test performance in our sample. This variant was also associated with other measures of IQ and episodic memory (P < 0.01). A combined analysis of rs6984242 with rs2905426 (at GATAD2A, known to interact with CHD7) also revealed a number of associations with IQ but the effect sizes did not point to an epistatic interaction between the two variants. Our analyses combined psychosis cases and healthy participants together to maximize sample size and power. When, in post hoc analyses, these samples were considered separately, the association between the risk allele at rs6984242 was not

significant in the healthy sample, but was marginally stronger in the case sample than for the sample as a whole (e.g., WTAR [P ¼ 0.004; R2 ¼ 0.013], Verbal IQ [P ¼ 0.002; R2 ¼ 0.012], Full Scale IQ ([P ¼ 0.003; R2 ¼ 0.014], Faces 2 [P ¼ 0.009; R2 ¼ 0.013]).

Our attempts to replicate significant results were conservatively restricted to following up the associations between rs6984242 and IQ and episodic memory. We did not uncover independent data to support our associations in UK and German samples or in a combined meta-analysis (data not shown). Similarly, restricting the replication analyses to case-only samples did not uncover significant results. There are a number of caveats to consider that could explain the non-replication. For the UK sample, comparable measures of general cognitive ability and memory were not available. In the German sample, where the same measures were available, the samples consisted mainly of healthy participants, for whom the association between rs6984242 and cognition was weaker in the Irish data. Finally, the modest though significant amount of variance associated with rs6984242, which was estimated as being around 1%, may also suggest that the samples included here, though large for these types of studies, lacked power to detect association.

Overall our study finds up to eight genes that function as chromatin modulators of gene expression and that map to loci that are associated with schizophrenia in the largest GWAS published to date. Rare mutations in a number of genes cause rare cognitive disorders. We found strong association between rs6984242, which we link to CHD7 via an eQTL, and measures of IQ and episodic memory in our dataset. However, we did not find replicating evidence of association between this variant in independent datasets. Further eQTL, functional and behavioral studies will be required both to confirm a link between rs6984242 and CHD7, thereby implicating this gene in schizophrenia biology, and to establish an association between this variant and cognition.

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