Schizophrenia Research xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Genetic assessment of additional endophenotypes from the Consortium on the Genetics of Schizophrenia Family Study

Tiffany A. Greenwood [a,*], Laura C. Lazzeroni [b], Monica E. Calkins [c], Robert Freedman [d], Michael F. Green [e,f], Raquel E. Gur [c], Ruben C. Gur [c], Gregory A. Light [a,g], Keith H. Nuechterlein [e], Ann Olincy [d], Allen D. Radant [h,i], Larry J. Seidman [j,k], Larry J. Siever [l,m], Jeremy M. Silverman [l,m], William S. Stone [j,k], Catherine A. Sugar [n], Neal R. Swerdlow [a], Debby W. Tsuang [h,i], Ming T. Tsuang [a,o,p], Bruce I. Turetsky [c], David L. Braff [a,g]

[a] Department of Psychiatry, University of California San Diego, La Jolla, CA, United States
[b] Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA, United States
[c] Department of Psychiatry, University of Pennsylvania, Philadelphia, PA, United States
[d] Department of Psychiatry, University of Colorado Health Sciences Center, Denver, CO, United States
[e] Department of Psychiatry and Biobehavioral Sciences, University of California Los Angeles, Los Angeles, CA, United States
[f] VA Greater Los Angeles Healthcare System, Los Angeles, CA, United States
[g] VISN-22 Mental Illness, Research, Education and Clinical Center (MIRECC), VA San Diego Healthcare System, United States
[h] Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA, United States
[i] VA Puget Sound Health Care System, Seattle, WA, United States
[j] Department of Psychiatry, Harvard Medical School, Boston, MA, United States
[k] Massachusetts Mental Health Center Public Psychiatry Division of the Beth Israel Deaconess Medical Center, Boston, MA, United States
[l] Department of Psychiatry, The Mount Sinai School of Medicine, New York, NY, United States
[m] James J. Peters VA Medical Center, New York, NY, United States
[n] Department of Biostatistics, University of California Los Angeles School of Public Health, Los Angeles, CA, United States
[o] Center for Behavioral Genomics, Institute for Genomic Medicine, University of California San Diego, La Jolla, CA, United States
[p] Harvard Institute of Psychiatric Epidemiology and Genetics, Boston, MA, United States

ARTICLE INFO

Article history:
Received 17 July 2015
Received in revised form 6 November 2015
Accepted 10 November 2015
Available online xxxx

Keywords:
Endophenotype
Genetics
Schizophrenia
Association
Linkage
Heritability

ABSTRACT

The Consortium on the Genetics of Schizophrenia Family Study (COGS-1) has previously reported our efforts to characterize the genetic architecture of 12 primary endophenotypes for schizophrenia. We now report the characterization of 13 additional measures derived from the same endophenotype test paradigms in the COGS-1 families. Nine of the measures were found to discriminate between schizophrenia patients and controls, were significantly heritable (31 to 62%), and were sufficiently independent of previously assessed endophenotypes, demonstrating utility as additional endophenotypes. Genotyping via a custom array of 1536 SNPs from 94 candidate genes identified associations for *CTNNA2*, *ERBB4*, *GRID1*, *GRID2*, *GRIK3*, *GRIK4*, *GRIN2B*, *NOS1AP*, *NRG1*, and *RELN* across multiple endophenotypes. An experiment-wide p value of 0.003 suggested that the associations across all SNPs and endophenotypes collectively exceeded chance. Linkage analyses performed using a genome-wide SNP array further identified significant or suggestive linkage for six of the candidate endophenotypes, with several genes of interest located beneath the linkage peaks (e.g., *CSMD1*, *DISC1*, *DLGAP2*, *GRIK2*, *GRIN3A*, and *SLC6A3*). While the partial convergence of the association and linkage likely reflects differences in density of gene coverage provided by the distinct genotyping platforms, it is also likely an indication of the differential contribution of rare and common variants for some genes and methodological differences in detection ability. Still, many of the genes implicated by COGS through endophenotypes have been identified by independent studies of common, rare, and *de novo* variation in schizophrenia, all converging on a functional genetic network related to glutamatergic neurotransmission that warrants further investigation.

1. Introduction

Schizophrenia is a severe psychotic disorder with a lifetime prevalence of approximately 1% and an estimated heritability of 60–80% (Karayiorgou and Gogos, 1997; Sullivan, 2005; Wray and Gottesman, 2012). The genetic heterogeneity and polygenicity associated with

* Corresponding author at: University of California San Diego, Department of Psychiatry, 9500 Gilman Drive, MC 0689, La Jolla, CA 92093, United States.
E-mail address: tgreenwood@ucsd.edu (T.A. Greenwood).

http://dx.doi.org/10.1016/j.schres.2015.11.008
0920-9964/

schizophrenia are substantial and have hindered many attempts to confirm initial candidate gene associations and to replicate linkage regions across studies (Baron, 2001; Gogos and Gerber, 2006; Harrison and Weinberger, 2005; Lewis et al., 2003; Owen et al., 2004). Increasingly large genome-wide association studies (GWAS) have begun to provide insight into common genetic variants associated with schizophrenia risk, yet the neurobiological significance of these variants remains largely unexplored (O'Donovan et al., 2008; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Shi et al., 2009). While most common and rare variants confer small increases in risk for schizophrenia, it is likely that risk variants will cluster within a limited number of pathways (Purcell et al., 2014; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014).

Schizophrenia is a profoundly clinically heterogeneous disorder with patients exhibiting a broad range of neurobiological deficits and symptom severity, which has further complicated efforts to identify genetic risk variants. Recent studies have demonstrated that employing more specific phenotype definitions in genetic studies of complex diseases, including schizophrenia, is even more important than large sample sizes for detecting true genetic associations (Liang and Greenwood, 2015; Manchia et al., 2013). The use of endophenotypes as objective measurements related to specific neurobiological functions may be particularly useful in reducing the heterogeneity associated with the considerably more subjective diagnosis, facilitating the detection of risk variants and aberrant molecular pathways (Braff et al., 2007; Gottesman and Gould, 2003; Insel and Cuthbert, 2009). Many endophenotypes are also amenable to human neuroimaging and translational animal model studies, allowing for direct evaluations of neural circuit dysfunctions and neurobiological substrates (Swerdlow et al., 2008; Young et al., 2013).

The Consortium on the Genetics of Schizophrenia Family Study (COGS-1) previously reported significant heritability for 12 endophenotypes for schizophrenia, with candidate gene association and genome-wide linkage analyses that demonstrate their utility for resolving the genetic architecture of schizophrenia (Greenwood et al., 2007; Greenwood et al., 2011; Greenwood et al., 2013c). Other analyses of the COGS-1 sample suggested additional measures for several endophenotype domains that may provide complementary information (Horan et al., 2008; Olincy et al., 2010; Stone et al., 2011; Swerdlow et al., 2007; Turetsky et al., 2008), yet these measures have remained uncharacterized for their genetic contributions in this sample. We now report the significant heritability of nine new candidate endophenotypes derived from the same original endophenotype test paradigms that provide complementary information. These measures include pulse-alone startle magnitude, P50 conditioning amplitude, N100 conditioning amplitude, Degraded-Stimulus Continuous Performance Test (DS-CPT) hit rate, CPT Identical Pairs (CPT-IP) 3-digit d', Letter-Number Span (LNS) forward, California Verbal Learning Test, Second Edition, (CVLT-II) list B and delayed recall, and Logical Memory Stories total recall. For these measures, we also evaluated association using the COGS SNP Chip, a custom array that incorporates common variants in genes involved in pathways hypothesized to underlie schizophrenia risk, and linkage using a genome-wide SNP linkage panel to assess the joint impact of rare and common variation on the candidate endophenotypes.

2. Methods

Ascertainment, genotyping, and analysis methods are provided in brief below with full methods available in the Supplement and elsewhere (Calkins et al., 2007; Greenwood et al., 2011; Greenwood et al., 2013c).

2.1. Subjects

Families were ascertained at seven sites through probands who met DSM-IV-TR criteria for schizophrenia (American Psychiatric Association, 2000). Each family minimally consisted of a proband with schizophrenia, an unaffected sibling, and both parents. Unrelated community comparison subjects without personal or family history of psychosis were also recruited. Only those without history of any Axis I or Cluster A personality disorder were considered as controls here. All subjects underwent a standardized clinical assessment using the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994). Details of the ascertainment, diagnostic, and screening procedures are provided elsewhere (Calkins et al., 2007). Written informed consent was obtained for each subject per local IRB protocols. The final COGS-1 dataset of 296 families consisted of 1364 subjects, 1004 of whom were characterized for the endophenotype paradigms. While most families (62%) consisted of the minimum discordant sibling pair and both parents, the remaining 38% represented larger families. The majority of subjects (89%) were confirmed to be of European ancestry.

2.2. Neurophysiological and neurocognitive measures

Detailed descriptions of the rationale and assessment procedures for all COGS-1 test paradigms and the heritability assessments of the 12 primary endophenotypes have been published (Greenwood et al., 2007; Gur et al., 2007; Turetsky et al., 2007). The two neurophysiological and three neurocognitive test paradigms administered yielded various quantitative measures in addition to the primary endophenotypes, from which 13 measures were selected for further validation as candidate endophenotypes as described below. These measures had previously shown promise as endophenotypes in COGS-1, and most have also demonstrated good test-retest reliability in an independent sample (Light et al., 2012).

While prepulse inhibition of startle at 60 ms was our primary endophenotype, we assessed pulse-alone startle magnitude on non-prepulse trials and both the difference and percent startle habituation from the first to final block of testing as additional measures (Swerdlow et al., 2007). The primary endophenotype of P50 suppression was the difference in amplitudes of the event-related potentials generated in response to the conditioning (S1) and test stimuli, and the S1 amplitude was considered an additional measure (Olincy et al., 2010). N100 amplitude was also derived from the P50 paradigm and measured as the minimum trough occurring 75–125 ms post-stimulus. Only the N100 conditioning (C1) amplitude was considered based on initial investigations in a subset of this sample (Turetsky et al., 2008).

We used two forms of the CPT to measure sustained, focused attention, one with a high perceptual load (DS-CPT) (Nuechterlein et al., 1983) and one with a working-memory load (CPT-IP) (Cornblatt et al., 1988). For the DS-CPT, the primary endophenotype was a signal/noise discrimination index (d') derived from correct target detections (hit rate) and incorrect responses to nontargets, and hit rate was considered an additional measure. For the CPT-IP, 3-digit d' was considered an additional measure. The LNS was used to assess working memory with the primary endophenotype considered as the correct reordering of intermixed numbers and letters and a simple repetition in the order dictated (forward) considered as an additional measure (Horan et al., 2008). We used the CVLT-II to assess verbal learning and memory (Stone et al., 2011), and considered the immediate recall of items from list A summed over 5 trials (list A total score) as the primary endophenotype. Additional measures included list B immediate recall, the free recall of list A after a 20-minute delay, and recall of list A items via sematic and serial clustering. The Logical Memory Test from the Wechsler Memory Scale was added midway through the COGS-1 study as an verbal learning and memory task, and total story recall was considered an additional measure (Wechsler, 1997).

Please cite this article as: Greenwood, T.A., et al., Genetic assessment of additional endophenotypes from the Consortium on the Genetics of Schizophrenia Family Study, Schizophr. Res. (2015), http://dx.doi.org/10.1016/j.schres.2015.11.008