Article

Genome-Wide Linkage Analyses of 12 Endophenotypes for Schizophrenia From the Consortium on the Genetics of Schizophrenia

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Objective: The Consortium on the Genetics of Schizophrenia has undertaken a large multisite study to characterize 12 neurophysiological and neurocognitive endophenotypic measures as a step toward understanding the complex genetic basis of schizophrenia. The authors previously demonstrated the heritability of these endophenotypes; in the present study, genetic linkage was evaluated.

Method: Each family consisted of a proband with schizophrenia, at least one unaffected sibling, and both parents. A total of 1,286 participants from 296 families were genotyped in two phases, and 1,004 individuals were also assessed for the endophenotypes. Linkage analyses of the 6,055 single-nucleotide polymorphisms that were successfully assayed, 5,760 of which were common to both phases, were conducted using both variance components and pedigree-wide regression methods.

Results: Linkage analyses of the 12 endophenotypes collectively identified one region meeting genome-wide significance criteria, with a LOD (log of odds) score of 4.0 on chromosome 3p14 for the antisaccade task, and another region on 1p36 nearly meeting genome-wide significance, with a LOD score of 3.5 for emotion recognition. Chromosomal regions meeting genome-wide suggestive criteria with LOD scores >2.2 were identified for spatial processing (2p25 and 16q23), sensorimotor dexterity (2q24 and 2q32), prepulse inhibition (5p15), the California Verbal Learning Test (8q24), the degradedstimulus Continuous Performance Test (10q26), face memory (10q26 and 12p12), and the Letter-Number Span (14q23).

Conclusions: Twelve regions meeting genome-wide significant and suggestive criteria for previously identified heritable, schizophrenia-related endophenotypes were observed, and several genes of potential neurobiological interest were identified. Replication and further genomic studies are needed to assess the biological significance of these results.

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Schizophrenia is a severe psychiatric disorder with a lifetime prevalence of approximately 1% (1). Like other common, complex disorders, schizophrenia is a multifactorial polygenic disorder that reflects the combined influence of both genetic and nongenetic factors (1, 2). Epidemiological studies indicate a heritability of up to 80% for schizophrenia, reflecting a strong genetic influence (3). Several candidate genes have been proposed but as yet remain unconfirmed as harboring causal mutations (4, 5). Linkage studies of schizophrenia have implicated many chromosomal regions (6), yet the identification of causative

genes within the linked regions has proven difficult. This difficulty in translating linkage findings to causal genes may be due to several factors, including the modest nature of the linkage signals and the broad genetic regions they encompass, genetic heterogeneity, polygenic inheritance, and environmental influences associated with schizophrenia. Additionally, schizophrenia is a highly heterogeneous disorder, with patients exhibiting a broad range of deficits and symptom severity subsumed under a single categorical diagnosis, labeled by Bleuler as the "group of schizophrenias" (7).

TABLE 1. Des	cription of 12 Ne	urophysiologica	I and Neurocogr	itive Endophenotypes
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Endophenotype	Abbreviation	Description	Measure		
Prepulse inhibition (14–16)	PPI	Inhibition of the startle reflex in response to a weak prestimulus with a 60-ms interval	Percent inhibition		
P50 suppression (17–19)	P50	Suppression of the P50 event-related potential in response to a conditioning stimulus	Difference between the test and conditioning amplitudes		
Antisaccade task (20, 21)	AS	Test of oculomotor inhibition in which subjects respond to a peripheral target by looking in the opposite direction with equal magnitude	Ratio of correct antisaccades to total saccades		
Degraded-stimulus Continuous Performance Test (22, 23)	DS-CPT	Measure of sustained attention involving target stimulus identification	Correct target detections and incorrect responses to nontargets (d')		
California Verbal Learning Test, 2nd edition (24, 25)	CVLT-II	A verbal memory task involving recall of 16 verbally presented items	Total recall score summed over five trials		
Letter-Number Span (26–28)	LNS	A working memory task involving recall of intermixed numbers and letters	Correct reordering		
Abstraction and mental flexibility (29, 30)	ABF	The subject is presented with four objects and must choose the one that does not belong	Efficiency score ^a		
Face memory (29, 30)	FMEM	Recognition of 20 target faces among distracter faces	Efficiency score ^a		
Spatial memory (29, 30)	SMEM	Uses Euclidean shapes as a recognition paradigm identical to face memory	Efficiency score ^a		
Spatial processing (29, 30)	SPA	Two lines are presented at an angle, and corresponding lines must be identified on a simultaneously presented array	Efficiency score ^a		
Sensorimotor dexterity (29, 30)	S-M	The mouse is used to quickly click on a target that gets increasingly smaller	Efficiency score ^a		
Emotion recognition (29, 30)	EMO	The identification of a variety of facial expressions of emotion	Efficiency score ^a		

^a A modified version of the University of Pennsylvania Computerized Neurocognitive Battery was used to evaluate the "efficiency" of the endophenotype, calculated as accuracy/log₁₀ (speed) and expressed as standard equivalents (Z score).

One way to dissect the underlying genetics and neural circuit abnormalities of a complex disorder like schizophrenia is through the use of endophenotypes known or likely to represent the subclinical pathology of the disease. Endophenotypes are discrete, genetically determined disease-related phenotypes with demonstrated reliability, stability, and heritability (8–10). An advantage of using endophenotypes is that they relate to specific neurobiological functions and substrates associated with the disease, which may make them more useful for gene identification than the more subjective diagnosis of schizophrenia.

Investigations of schizophrenia and other common disorders have recently moved toward genome-wide association studies (GWAS), which have greater power to detect weak associations to common variants. However, a considerable proportion of the observed heritability is not detectable in these large studies of unrelated patients and comparison subjects (11, 12). One likely source of this "missing heritability" is allelic heterogeneity, which may substantially reduce the power of GWAS to detect causative genes because the overall genetic effect is divided among multiple variants, some of which may be rare and not well captured by common tag single-nucleotide polymorphisms (SNPs). Linkage, however, can detect the aggregate effects of multiple rare and common variants within a susceptibility gene or region, even with different mutations conferring risk in different families. Linkage data may also be used to weight evidence for association and potentially increase the power of GWAS (13). Thus, linkage studies and family-based samples continue to be relevant in the age of rapidly advancing technologies.

The Consortium on the Genetics of Schizophrenia (COGS) focuses on investigating endophenotypes as a strategy for dissecting the genetic architecture of schizophrenia and filling in the "gene to phene" gap (8–10). Twelve heritable neurophysiological and neurocognitive endophenotypes that are characteristically impaired in schizophrenia patients were chosen for the COGS study: prepulse inhibition of the startle response (14-16), P50 event-related potential suppression (17-19), the antisaccade task for eye movements (20, 21), the Continuous Performance Test (degradedstimulus version) (22, 23), the California Verbal Learning Test, 2nd edition (24, 25), the Letter-Number Span (26-28), and six measures from the University of Pennsylvania Computerized Neurocognitive Battery (abstraction and mental flexibility [29, 30], face memory [29, 30], spatial memory [29, 30], spatial processing [29, 30], sensorimotor dexterity [29, 30], and emotion recognition [29, 30]). Deficits in all endophenotypes have been demonstrated not only in patients with schizophrenia but also in their clinically unaffected relatives, suggesting that these deficits reflect part of the heritable risk for the illness. Complete reviews of each endophenotype in the COGS study, including the rationale for selection and data regarding stability, reliability, and heritability, have been reported elsewhere

	N	Pairs ^b		Residual			Covariate (p)			
Endophenotype ^a		Sibling	Parent-Offspring	Heritability (h ² _r) ^c	SE	р	Age	e Sex S		Variance ^d
PPI	701	428	308	0.29	0.09	0.0004	n.s.	< 0.0001	n.s.	0.04
P50	568	248	197	0.20	0.10	0.019	n.s.	n.s.	n.s.	
AS	825	516	391	0.36	0.07	< 0.0001	< 0.0001	n.s.	n.s.	0.03
DS-CPT	881	555	449	0.34	0.06	< 0.0001	< 0.0001	n.s.	0.005	0.04
CVLT-II	949	634	500	0.26	0.06	< 0.0001	< 0.0001	< 0.0001	0.005	0.15
LNS	951	638	492	0.34	0.06	< 0.0001	< 0.0001	n.s.	n.s.	0.07
ABF	888	533	473	0.25	0.06	0.0001	< 0.0001	n.s.	n.s.	0.17
FMEM	912	569	490	0.30	0.06	< 0.0001	< 0.0001	0.010	n.s.	0.12
SMEM	900	557	479	0.34	0.06	< 0.0001	< 0.0001	n.s.	n.s.	0.10
SPA	837	486	421	0.52	0.06	< 0.0001	< 0.0001	< 0.0001	n.s.	0.22
S-M	898	561	463	0.38	0.06	< 0.0001	< 0.0001	n.s.	n.s.	0.21
EMO	896	556	475	0.16	0.05	0.002	< 0.0001	0.0001	n.s.	0.17

TABLE 2. Heritability Estimates Observed for the 12 Endophenotypes in the 296 Families

^a The 12 endophenotypes are described in Table 1.

^b Data indicate the number of informative pairs.

^c Data represent the residual heritability after adjustment for significant covariates.

^d The proportion of the trait variance explained by all significant covariates.

(31–33). We previously reported evidence of significant heritability for these 12 endophenotypes in a subset of 183 COGS families and have demonstrated association with several carefully chosen candidate genes in 130 of these families (34, 35). In the present study, we report the results of a genome-wide SNP linkage scan for these endophenotypes in the complete COGS sample of 296 families.

Method

Participants

Families were ascertained at seven sites through the identification of probands who met DSM-IV-TR criteria for schizophrenia as determined by administration of the Diagnostic Interview for Genetic Studies and the Family Interview for Genetic Studies (36-38). Each family consisted of a proband with schizophrenia, at least one unaffected sibling, and both parents, with blood samples required for all participants and endophenotypes required for each proband and unaffected sibling. Unlike studies that focus exclusively on affected sibling pairs or large families with multiple affected members, this type of ascertainment strategy provides greater potential for phenotypic contrasts between and among siblings. Additional affected and unaffected siblings were included whenever possible, and families missing one or both parents were accepted if one or two additional siblings were available. Blood was collected at the time of assessment and sent to the Rutgers University Cell and DNA Repository for cell line maintenance and DNA isolation. The ascertainment and screening procedures and inclusion and exclusion criteria have been discussed in detail elsewhere (31). After participants received a detailed description of the study procedure, they provided written informed consent per local institutional review board protocols.

Phenotyped individuals ranged in age from 18 to 65 years old and received urine toxicology screening for drugs of abuse prior to assessment (negative screens were required). The three neurophysiological and nine neurocognitive endophenotypes are summarized in Table 1; a more detailed description of the assessment procedure for each endophenotype is available elsewhere (31–33). The 296 families comprised 1,364 participants, with an average family size of 4.6 members (range: 4–14 members). The majority of the families (62%) consisted of a single sibling pair discordant for schizophrenia, with sibships of three accounting for 26% of families and larger sibships accounting for 12%. A total of 1,286 individuals had DNA available for genotyping, and of these, 1,004 were assessed for the 12 endophenotypes. Of the 710 sibling pairs in the sample, 16 were concordant for schizophrenia, 464 were discordant, and 230 were unaffected with an average of 523 informative pairs for each endophenotype (Table 2). Of the 1,526 parent-offspring pairs in the sample, an average of 428 pairs were informative for each endophenotype (Table 2). We note that there were variable rates of data loss across the endophenotypes due to variances in the completion rate by participants, the difficulty of measurement, and the quality-control processes required.

Genotyping and Data Cleaning

Genotyping was performed in two phases by the Center for Inherited Disease Research. Initial genotyping with a microsatellite panel allowed for the elimination of errors due to sample handling or nonpaternity. The first phase of genotyping included 198 families (N=891) and was performed using the Illumina Infinium HumanLinkage-12 panel (Illumina, San Diego) containing 6,090 SNP markers across the genome, of which 6,001 SNPs were successfully assayed with a reproducibility rate of 99.997% as determined from 60 blind duplicates. The second phase of genotyping included 98 families (N=395) and was performed using the Illumina Infinium HumanLinkage-24 panel containing 5,913 SNPs across the genome, 5,724 of which were successfully assayed with a reproducibility rate of 100.000% as determined from 36 blind duplicates. All SNPs were evaluated by the Center for Inherited Disease Research for clustering, call rate, replicate errors, and intensity using Illumina GenomeStudio (Illumina, San Diego) and were excluded as necessary based on internal quality-control criteria. Participants were also excluded for poor genotyping performance across all SNPs (N=11). Of the successfully genotyped SNPs, 5,670 were common between the two platforms, with 331 SNPs unique to the HumanLinkage-12 panel and 54 SNPs unique to the HumanLinkage-24 panel.

Genotypes causing Mendelian inconsistencies were identified using PedCheck (39) and removed from all individuals in the family for a sporadic error rate estimation of 0.01%. MERLIN (multipoint engine for rapid likelihood inference) (40) was used to identify and remove an additional 64 unlikely genotypes. All SNPs were ordered on the physical map according to Genome Build 36 (National Center for Biotechnology Information, Bethesda, Md.), and the deCODE genetic map (deCODE Genetics, Reykjavik, Iceland) was used to estimate genetic map distances (41). The final SNPs had an average physical spacing of 512 kb and an average genetic spacing of 0.65 cM.

Statistical Analyses

Heritability analyses were conducted using SOLAR 4.3.1 (sequential oligogenic linkage analysis routines) to evaluate potential covariates for linkage (42). The revised heritability estimates for the 296 families, as listed in Table 2, approximate those we previously reported in 183 COGS families, with all but emotion recognition within one standard error of the previous estimate (34). Bivariate environmental (ρ_E) and genetic (ρ_G) correlation estimates were also computed (see Table S1 in the data supplement that accompanies the online edition of this article) to verify our previous findings and to inform the multivariate linkage analyses (34, 43). Details of these analyses are summarized in the online data supplement.

PEDSTATS (44) was used to identify 43 markers that deviated from Hardy-Weinberg equilibrium in the parents (p \leq 0.001). Since departures from equilibrium can occur for numerous reasons, including association between marker alleles and disease susceptibility, we report only results that included all markers, noting that the exclusion of these markers for regions with LOD (log of odds ratio) scores >2.2 had a negligible effect on the results.

The variance component method implemented in SOLAR was used as our primary method for the quantitative trait linkage analyses. Two-point and multipoint LOD scores were calculated for each endophenotype using normalized trait values, a correction for ascertainment bias, and covariate adjustment as appropriate (42, 45). Simulation analyses were performed using 10,000 replicates to permit the estimation of empirical LOD scores for each endophenotype individually (46). For comparison, the model-free pedigree-wide regression method implemented in MERLIN was used to compute multipoint LOD scores for each autosome (47). This method has been shown to be robust regarding issues involving incomplete marker informativity and is appropriate for selected samples, allowing for the specification of population-based parameters (47-49). Variance component models in MERLIN were used for multipoint analysis of the X chromosome data, since neither the regression algorithm nor SOLAR permits multipoint analyses of X chromosome data. For all analyses, multipoint identical-by-descent matrices were generated using the respective program at a 1-cM resolution, which is slightly larger than the average spacing between SNPs. Since linkage analysis of tightly linked loci can inflate LOD scores, we required that the r² value between markers be less than 0.05. For regions in which coincident linkage signals were observed for multiple, genetically correlated endophenotypes, multivariate linkage analyses were conducted using SOLAR. This sample has 80% power to detect a locus explaining 35%-40% of the trait variance across endophenotypes (excluding P50 suppression) with a LOD score of 2.2.

Results

We performed a genome-wide linkage scan for each endophenotype using variance components methods as the primary analysis. Regression-based methods were then used to confirm and extend the results. As shown in Figures 1 and 2 and summarized in Table 3, these analyses have collectively identified several linkage regions meeting at least suggestive evidence of linkage across the 12 endophenotypes, according to the criteria established by Lander and Kruglyak (50). A summary of all multipoint LOD scores >1.0 with the corresponding empirical p values is provided in Table S2 in the online data supplement, and a complete listing of all multipoint LOD scores is available in Table S3 in the data supplement.

Significant evidence for linkage (LOD score >3.6) was observed for the antisaccade task on chromosome 3p14, with a variance components LOD score of 4.0. While the regression LOD score for this region only reached suggestive evidence for linkage, with a peak LOD score of 2.4, simulation analyses indicated an empirical p value <0.0001. No other endophenotype displayed linkage to this region with a LOD score >1.0 (see Table S2 in the online data supplement). Several neuronally expressed genes are located beneath this linkage peak, including ataxin 7 (ATXN7), which encodes a protein involved in chromatin remodeling and plays a role in transcriptional regulation. A polyglutamine repeat expansion in this gene is implicated in spinocerebellar ataxia type 7, which also presents with retinal degeneration and visual loss, dementia, hypoacusia, severe hypotonia, and auditory hallucinations (51). The ATXN1 gene on chromosome 6p22, which causes spinocerebellar ataxia type 1 through a similar mechanism, has also been investigated as a candidate gene with associations to schizophrenia (52, 53).

Another region nearly reaching genome-wide significance under the variance components model was chromosome 1p36, which produced a LOD score of 3.5 for emotion recognition. The regression LOD score for this region only reached 2.5, yet simulation analyses indicated an empirical p value <0.0001. The Letter-Number Span also revealed modest evidence for linkage to this region, with a LOD score of 1.6 (see Table S2 in the online data supplement). Several genes are located beneath this peak, including the serotonin receptor 6 gene (*HTR6*), which functions in the modulation of cholinergic and dopaminergic neurotransmission, plays a role in spatial learning and memory, and has a high affinity for several conventional and atypical antipsychotics (54).

Suggestive evidence for linkage (LOD score >2.2) under the variance components model was observed for prepulse inhibition on chromosome 5p15, with a peak LOD score of 2.5 (regression LOD score=2.4); for face memory on chromosome 10q26, with a peak LOD score of 2.3 (regression LOD score=2.4); and for spatial processing on chromosome 16q23, with a peak LOD score of 2.6 (regression LOD score=2.5). The regression method identified several additional regions meeting suggestive evidence for linkage, most of which displayed at least some evidence for linkage, with LOD scores >1.0 in the variance components analysis. These regions included 2p25 (spatial processing), 2q24 and 2q32 (sensorimotor dexterity), 8q24 (California Verbal Learning Test), 10q26 (degraded-stimulus



FIGURE 1. Results of the Genome-Wide Single-Nucleotide Polymorphism Linkage Scan in the 296 Families for Each of the Six Primary Neurophysiological and Neurocognitive Endophenotypes^a

^a Results for the following analyses are shown: the variance components multipoint (red), the pedigree-wide regression multipoint (blue), and the variance components two-point (gray). Log of odds (LOD) scores are indicated on the y-axis, along with the name of the corresponding endophenotype. Chromosomes are aligned along the x-axis end to end with the p-terminus on the left and locations indicated at the top of the graph. Dashed horizontal lines indicate genome-wide significant and suggestive LOD scores of 3.6 and 2.2, respectively. LNS=Letter-Number Span; CVLT-II=California Verbal Learning Test, 2nd edition; DS-CPT=degraded-stimulus Continuous Performance Test; AS=antisaccade task; P50=P50 suppression; PPI=prepulse inhibition.

Continuous Performance Test), 12p12 (face memory), and 14q23 (Letter-Number Span).

Several genes of potential interest were identified beneath the suggestive peaks. The gene encoding the zinc finger protein 804A (*ZNF804A*) lies beneath the linkage peak for sensorimotor dexterity on chromosome 2q32. This gene has shown strong evidence for association with schizophrenia in several large GWAS (55–57). Although the region on chromosome 5p15 with linkage to prepulse inhibition is very gene-dense, one gene of particular note, the dopamine transporter gene (*SLC6A3/DAT*), lies beneath the peak. This gene has shown evidence of association with prepulse inhibition and startle habituation (58), schizophrenia (59), and several of our neurocognitive endophenotypes (35). Prepulse-inhibition deficits have also been reported in *DAT* knockout mice (60). Finally,



FIGURE 2. Results of the Genome-Wide Single-Nucleotide Polymorphism Linkage Scan in the 296 Families for Each of the Six Endophenotypes From the University of Pennsylvania Computerized Neurocognitive Battery^a

^a Results for the following analyses are shown: the variance components multipoint (red), the pedigree-wide regression multipoint (blue), and the variance components two-point (gray). Log of odds (LOD) scores are indicated on the y-axis, along with the name of the corresponding endophenotype. Chromosomes are aligned along the x-axis end to end with the p-terminus on the left and locations indicated at the top of the graph. Dashed horizontal lines indicate genome-wide significant and suggestive LOD scores of 3.6 and 2.2, respectively. EMO=emotion recognition; S-M=sensorimotor dexterity; SPA=spatial processing; SMEM=spatial memory; FMEM=face memory; ABF=abstraction and mental flexibility.

the glutamate (N-methyl-D-aspartic acid) receptor 2B gene (*GRIN2B*) lies beneath the linkage peak for face memory on chromosome 12p12. Several studies have found evidence for association of *GRIN2B* with schizophrenia (58, 61–63), and we previously reported associations with several of our neurocognitive endophenotypes (35, 58).

We also identified many regions of coincident linkage in which at least two endophenotypes produced modest evidence for linkage (LOD score >1.0), including the 1p36, 10q26, and 12p12–13 regions described above (see Table S2 in the online data supplement). Two regions in particular on 10q26 and 17p13 revealed linkage to multiple endophenotypes, some of which were genetically correlated (see Table S1 in the data supplement). A multivariate linkage analysis combining the antisaccade task, degraded-stimulus Continuous Performance Test, face memory, and spatial

			SOLAR ^b				MERLIN ^d	
Chromosome	Endophenotype ^a	Location (cM)	Peak LOD	Interval (cM/Mb) ^c	Location (cM)	Peak LOD	Interval (cM/Mb) ^c	Nearby Genes ^e
1p36	EMO	38	3.5	31–48/17.3–29.5	37	2.5	28–43 / 15.6–23.7	PAX7, UBR4, ALDH4A1, NBL1, HTR6, EPHA8, EPHB2
2p25	SPA				17	2.5	14-20 / 6.0-8.1	none
2q24	S-M	164	1.4		168	2.8	167–171 / 158.6–166.6	GALNT13, BAZ2B
2q32	S-M	188	1.5		190	2.7	187–192/182.9–192.1	ZNF804A
3p14	AS	87	4.0	83-95/61.0-70.5	88	2.4	83-94/61.0-69.7	SYNPR, ATXN7, PRICKLE2, MITF
5p15	PPI	0.6	2.5	0-12/0.7-4.6	0.6	2.4	0-10/0.7-3.8	SLC6A3 (DAT)
8q24	CVLT-II	153	1.1		136	2.4	133-140/127.8-131.4	POU5F1, MYC
10q26	DS-CPT	153	1.9		155	2.4	147-162/123.5-130.0	FANK1, ADAM12
10q26	FMEM	168	2.3	159–179/129.5–134.9	171	2.4	161-179/130.0-134.9	MGMT, TCERG1L
12p12	FMEM				34	2.8	30-39/12.9-20.2	GRIN2B, LMO3
14q23	LNS	65	2.0		61	2.5	60-67/58.9-67.9	SYNE2, GPHN, ESR2
16q23	SPA	102	2.6	96–112/77.4–82.0	105	2.5	98–111/77.7–81.9	WWOX, GAN, PLCG2

TABLE 3. Summary of All Chromosomal Regions With Log of Odds (LOD) Scores Reaching at Least Suggestive Evidence for Linkage

^a The 12 endophenotypes are described in Table 1.

^b Data represent analyses conducted using SOLAR 4.3.1 (sequential oligogenic linkage analysis routines).

^c Genetic and physical boundaries of LOD scores within one unit of the maximum (1-LOD interval); cM=centimorgan, Mb=megabase pairs.

^d Data represent analyses conducted using MERLIN (multipoint engine for rapid likelihood inference).

^e Genes within the 1-LOD interval prioritized by proximity to single-nucleotide polymorphisms with two-point LOD scores >1.5.

memory produced a LOD score of 2.1 for 10q26. However, face memory alone produced suggestive LOD scores of 2.2 and 2.4 with the two linkage methods, and the degradedstimulus Continuous Performance Test also produced a suggestive LOD score of 2.4 with the regression method. It is thus likely that these linkages represent distinct signals despite their close proximity. A similar analysis was conducted for chromosome 17, combining the degradedstimulus Continuous Performance Test, face memory, spatial memory, and sensorimotor dexterity. While individual endophenotypes produced LOD scores of 1.3-1.8 for 17p13 under the variance components model, a suggestive LOD score of 2.2 was observed through their joint analysis, which may indicate the presence of a gene in this region that is involved in some aspect of neurocognition or one that generally contributes to schizophrenia susceptibility. Notably, the YWHAE gene lies below this peak and encodes 14-3-3epsilon, a member of a highly conserved family of proteins involved in a wide range of signaling pathways. YWHAE is a binding partner of DISC1 and has been proposed as a susceptibility gene for schizophrenia (64, 65).

Discussion

Investigations of endophenotypes that reflect aspects of the brain pathology involved in schizophrenia may facilitate the identification of genes contributing to schizophrenia susceptibility (8–10). Genome-wide linkage analyses of 12 heritable neurocognitive and neurophysiological endophenotypes collectively identified 12 regions displaying genome-wide significant or suggestive evidence for linkage using two complementary linkage analysis methods. Several genes of potential interest are located beneath these linkage peaks, including *HTR6* on chromosome 1p36 (emotion recognition), *ZNF804A* on 2q32 (sensorimotor dexterity), *ATXN7* on 3p14 (the antisaccade task), *DAT* on 5p15 (prepulse inhibition), *GRIN2B* on 12p12 (face memory), and *YWHAE* on 17p13 (multivariate cognitive phenotype).

We did not find evidence for linkage of the COGS endophenotypes to some of the prominent linkage regions for schizophrenia, such as 1q21-22, 4q31, 5q22-31, 6p22-24, 8p21-22, 9q21-22, and 10p11-15. However, we did find at least modest evidence to support linkage with LOD scores >1 observed for at least one endophenotype to other linkage regions identified for schizophrenia (6), such as 1q32-41, 5p13-14, 6q21-22, 13q14-32, 15q13-15, 22q11-13, and Xp11 (see Table S3 in the online data supplement). A SNP linkage study of schizophrenia, conducted by Holmans et al. (66), identified additional suggestive linkage regions on chromosomes 8q24, 9q34, and 12q24, while another study found suggestive evidence for linkage of schizophrenia covaried for positive symptom dimensions on chromosomes 2q32, 10q26, and 20q12 (67). In our study, we also observed suggestive evidence for linkage to 2q32 (sensorimotor dexterity), 8q24 (the California Verbal Learning Test), and 10q26 (the degradedstimulus Continuous Performance Test and face memory).

Few studies investigating schizophrenia endophenotypes through genome-wide analyses have been published to date. One study of several measures of neurocognition in schizophrenia identified a linkage peak that reached genome-wide significance on chromosome 12q24 for undegraded Continuous Performance Test hit rate (68). We identified modest evidence for linkage to this region for one of our neurocognitive endophenotypes (emotion recognition). In another study of multigenerational schizophrenia families with theoretically greater genetic loading for both schizophrenia and associated endophenotype deficits, significant evidence of linkage was observed for schizophrenia on 19q13 and abstraction and mental flexibility on 5q15 (69). Modest evidence for linkage was observed in our sample for neurocognitive endophenotypes in these regions, including abstraction and mental flexibility on 5q15.

We also identified several regions of overlapping linkage signals of two or more endophenotypes, some of which have featured prominently in previous linkage studies of schizophrenia, such as 6q21-22, 15q13-15, and 22q11-13 (70-77). The 15q13-15 region was identified as a susceptibility locus for schizophrenia through a linkage study of P50 suppression and contains the alpha-7 nicotinic acetylcholine receptor gene (CHRNA7), a candidate gene for schizophrenia (18). Deletions within the 22q11 region have been associated with schizophrenia, and two prominent candidate genes, catechol-O-methyltransferase (COMT) and proline dehydrogenase (PRODH), are located in this region (78). A meta-analysis combining the results of several linkage studies confirmed this region as a valid linkage region for both schizophrenia and bipolar disorder that likely contains one or more susceptibility genes (79), and evidence of linkage for a composite inhibitory phenotype combining P50 suppression and antisaccade was observed for 22q11-12 (80). Finally, recent studies suggest that rare deletions in the 15q13 and 22q11 regions predispose to schizophrenia (81-84). Thus, these regions may warrant further investigation in other samples.

Recent GWAS have identified several risk genes associated with schizophrenia at genome-wide significance levels in very large samples. These include microRNA 137 (MIR137) on chromosome 1p21, ZNF804A on 2q32, the major histocompatibility complex region on 6p21-22, CUB and sushi multiple domains 1 (CSMD1) on 8p23, the neurogranin gene (NRGN) on 11q24, and transcription factor 4 (TCF4) on 18q21 (55-57, 85, 86). The voltagedependent L-type calcium channel alpha-1C (CACNA1C) on chromosome 12p13 also reached genome-wide significance in a joint analysis with bipolar disorder (85). Other than the aforementioned suggestive evidence of linkage for sensorimotor dexterity to the 2q32 region containing ZNF804A, we observed only modest evidence of linkage for the 8p23 (the California Verbal Learning Test), 12p13-12 (abstraction and mental flexibility, sensorimotor dexterity), and 18q21 (P50 suppression) regions.

One might expect that the endophenotypes with the highest heritabilities would produce the strongest genetic signals, yet, as our results demonstrate, this is not always the case. In our study, antisaccade had one of the highest heritabilities, at 36%, and produced the only genome-wide significant linkage signal, whereas spatial memory did not produce a linkage signal meeting genome-wide suggestive criteria, despite having a comparable heritability of 34%. The second strongest linkage signal was observed for emotion recognition, the endophenotype with the lowest heritability in this study at 16%, although we do note that emotion recognition demonstrated a much higher heritability of 32% in our previous analysis of 183 families (34). Although it is possible that the genetic signal from the original families is strong enough to influence the overall linkage signal for emotion recognition, these results may simply illustrate that heritability estimates are not perfect predictors of the potential "mapability" of the underlying genetic variants. An endophenotype with a relatively low but significant heritability may exhibit large effects of a small number of genes, which would facilitate mapping. Alternatively, an endophenotype may be highly heritable but also highly polygenic, similar to schizophrenia itself, which would significantly complicate gene mapping by producing low-level signals across a multitude of genomic regions. For example, a recent study evaluating the variance in liability explained by the identified variants for 10 complex diseases, including schizophrenia, found that each associated SNP explained a median variance of only 0.25% (87). While endophenotypes provide reliable measures of specific neurophysiological and neurocognitive processes that are deficient in schizophrenia, they may not exhibit simpler genetic architectures (88).

There are several limitations or caveats applicable to this study. First, the COGS family ascertainment strategy focused on the recruitment of siblings discordant for schizophrenia to increase variation in the endophenotypic values. As a result, only 16 affected sibling pairs and eight affected parents exist in the sample, too few to reliably assess linkage to schizophrenia or determine whether the genomic regions identified by the endophenotypes have an effect on schizophrenia susceptibility. We were also unable to assess the degree of genetic correlation between the endophenotypes and schizophrenia. Additionally, this ascertainment scheme may result in the underestimation of endophenotype heritabilities and genetic correlations to the extent that they are correlated with schizophrenia. Second, with a linkage study of 12 endophenotypes, multiple comparisons are an issue. It is difficult to determine the appropriate correction in this case, since most of the endophenotypes are significantly correlated with each other (34). Finally, our sample of predominantly small nuclear families lacks sufficient power to reliably detect loci with smaller effects in a linkage analysis. The relatively low power of this study is independent of the heritabilities of these endophenotypes and in part reflects variance in the available data across endophenotypes. Yet, we successfully identified several genomic regions that warrant further investigation, despite being underpowered.

We embarked on this endophenotype strategy to provide a platform to dissect the polygenic basis of schizophrenia

susceptibility and to identify therapeutic molecular targets for the treatment of schizophrenia. In this context, endophenotypes were used as a complementary strategy to augment the dissection of the clinical and genetic heterogeneity of schizophrenia, as we and other investigators have discussed (8-10). The linkage analyses of the 12 endophenotypes described in this study represent a first step toward this goal. While the absence of significant linkage signals for all endophenotypes may be an issue of power deficiencies, it is also likely a reflection of the genetic complexity of the endophenotypes. For example, abnormalities in at least 20 different brain regions have been identified in various schizophrenia cohorts, five of which fall within regions known to regulate mammalian prepulse inhibition (89, 90). Deficient prepulse inhibition in schizophrenia might arise from any of these neural abnormalities, which almost certainly reflect a heterogeneous group of genetic determinants. Thus, while the biological basis of these endophenotypes may be simpler than that of schizophrenia per se, they nonetheless remain complex and appear to be highly polygenic. Conceivably, a refinement of these endophenotypes may probe a more specific physiology and thereby be sensitive to a more pure genetic signal. Despite these complexities, we have identified several regions meeting the standard genome-wide significant and suggestive criteria that provide further support for several existing schizophrenia candidate genes and chromosomal regions. The extent to which these regions harbor specific mutations that are involved in schizophrenia susceptibility, or the cognitive and neurophysiological processes tapped by our endophenotypes, remains a topic for future discussion to be informed by ongoing assessments of copy number variation burden and methylation events, as well as future sequencing efforts. While schizophrenia and its treatment will not be easily resolved, the use of interlocking genomic and endophenotype approaches offers much hope for the future (91).

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