

Genome-Wide Association Study of Multiplex Schizophrenia Pedigrees

Douglas F. Levinson, M.D.
 Jianxin Shi, Ph.D.
 Kai Wang, Ph.D.
 Sang Oh, M.Sc.
 Brien Riley, Ph.D.
 Ann E. Pulver, Ph.D.
 Dieter B. Wildenauer, Ph.D.
 Claudine Laurent, M.D., Ph.D.
 Bryan J. Mowry, M.D.,
 F.R.A.N.Z.C.P.
 Pablo V. Gejman, M.D.
 Michael J. Owen, Ph.D.,
 F.R.C.Psych.
 Kenneth S. Kendler, M.D.
 Gerald Nestadt, M.D.
 Sibylle G. Schwab, Ph.D.
 Jacques Mallet, Ph.D.
 Deborah Nertney, B.Sc.
 Alan R. Sanders, M.D.
 Nigel M. Williams, Ph.D.
 Brandon Wormley, B.S.
 Virginia K. Lasseter, B.A.
 Margot Albus, M.D.
 Stephanie Godard-Bauché, M.S.
 Madeline Alexander, Ph.D.
 Jubao Duan, Ph.D.
 Michael C. O'Donovan, Ph.D.,
 F.R.C.Psych.
 Dermot Walsh, M.D.

Anthony O'Neill, M.D.
 George N. Papadimitriou, M.D.
 Dimitris Dikeos, M.D.
 Wolfgang Maier, M.D.
 Bernard Lerer, M.D.
 Dominique Campion, M.D.,
 Ph.D.
 David Cohen, M.D., Ph.D.
 Maurice Jay, M.D.
 Ayman Fanous, M.D.
 Peter Eichhammer, M.D.
 Jeremy M. Silverman, Ph.D.
 Nadine Norton, Ph.D.
 Nancy Zhang, Ph.D.
 Hakon Hakonarson, M.D., Ph.D.
 Cynthia Gao, M.S.
 Ami Citri, Ph.D.
 Mark Hansen, Ph.D.
 Stephan Ripke, M.D.
 The Schizophrenia Psychiatric
 GWAS Consortium
 Frank Dudbridge, Ph.D.
 Peter A. Holmans, Ph.D.

Objective: The authors used a genome-wide association study (GWAS) of multiply affected families to investigate the association of schizophrenia to common single-nucleotide polymorphisms (SNPs) and rare copy number variants (CNVs).

Method: The family sample included 2,461 individuals from 631 pedigrees (581

in the primary European-ancestry analyses). Association was tested for single SNPs and genetic pathways. Polygenic scores based on family study results were used to predict case-control status in the Schizophrenia Psychiatric GWAS Consortium (PGC) data set, and consistency of direction of effect with the family study was determined for top SNPs in the PGC GWAS analysis. Within-family segregation was examined for schizophrenia-associated rare CNVs.

Results: No genome-wide significant associations were observed for single SNPs or for pathways. PGC case and control subjects had significantly different genome-wide polygenic scores (computed by weighting their genotypes by log-odds ratios from the family study) (best $p=10^{-17}$, explaining 0.4% of the variance). Family study and PGC analyses had consistent directions for 37 of the 58 independent best PGC SNPs ($p=0.024$). The overall frequency of CNVs in regions with reported associations with schizophrenia (chromosomes 1q21.1, 15q13.3, 16p11.2, and 22q11.2 and the neurexin-1 gene [NRXN1]) was similar to previous case-control studies. NRXN1 deletions and 16p11.2 duplications (both of which were transmitted from parents) and 22q11.2 deletions (de novo in four cases) did not segregate with schizophrenia in families.

Conclusions: Many common SNPs are likely to contribute to schizophrenia risk, with substantial overlap in genetic risk factors between multiply affected families and cases in large case-control studies. Our findings are consistent with a role for specific CNVs in disease pathogenesis, but the partial segregation of some CNVs with schizophrenia suggests that researchers should exercise caution in using them for predictive genetic testing until their effects in diverse populations have been fully studied.

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We report here on the first genome-wide association study (GWAS) in families with multiple members with schizophrenia. Significant associations of single-nucleotide polymorphisms (SNPs) can suggest new

disease susceptibility mechanisms. For schizophrenia, large GWAS analyses of common SNPs have found associations in the major histocompatibility complex (MHC, chromosome 6) (1–3) and several specific genes

TABLE 1. Genotyped Individuals, Families, and Ancestries^a

Site	Affected and Unaffected Subjects and Ancestry								Total
	Genotyped Affected				Genotyped Unaffected				
	European		Other		European		Other		
	N	Male	N	Male	N	Male	N	Male	
Australia/United States	128	0.63	2	0.50	107	0.49	1	0.00	238
Cardiff	43	0.70	2	0.50	30	0.33	2	0.50	77
Johns Hopkins	282	0.64	6	0.67	261	0.44	3	0.33	552
Illinois/NorthShore	118	0.74	2	1.00	92	0.48	1	0.00	213
Paris	53	0.57	99	0.62	37	0.54	83	0.36	272
VCU/Ireland	399	0.67	0	—	286	0.43	0	—	685
Western Australia/Germany	195	0.52	28	0.57	177	0.47	24	0.50	424
Total	1,218	0.64	139	0.61	990	0.45	114	0.39	2,461

^a Shown are the number of genotyped affected and unaffected subjects and the proportion of males in each group, and the number of families with each genotypic ancestry (Admix1, Admix2, and Admix3 are admixed European/African families with increasing proportions of African admixture, as shown in Figure S2 in the online data supplement; Malabar refers to La Réunion families of South Indian origin). Subjects were drawn from seven samples previously collected for linkage studies. All families were multiplex (had at least two narrow-diagnosis cases ascertained), but some had only one affected case in the nuclear family that was selected as informative, or because DNA was not available or failed quality control for some cases. Based on power analyses of simulated data to optimize power in relation to cost, analyses included nuclear families with one case and at least one parent, or with two or more cases and zero to two genotyped parents; plus unaffected siblings where available (up to two with no genotyped parents and one with one genotyped parent). The number of affected cases available and selected for genotyping was 1 (102 families), 2 (391 families), 3 (104 families), 4 (20 families), 5 (7 families), 6 (3 families), or 7 (4 families). The primary analysis included the European-ancestry families. Most of the non-European families were from La Réunion Island (Paris VI site) and were included because of large size. VCU=Virginia Commonwealth University.

(3–5). The Psychiatric GWAS Consortium (PGC) analyzed 21,856 individuals from 17 GWAS samples and then added data from an additional 29,839 individuals (including the present data set) for the most promising findings. The results strongly supported association in seven genes or regions between genes, including the MHC (6). The present study was designed before the typical effect sizes of common SNPs on disease risks became clear (e.g., odds ratios of only 1.1–1.2, conferring a 10%–20% increase in risk), and our sample is now known to be underpowered. However, we can address whether SNPs with larger effects might be “enriched” in families with multiple cases.

The PGC analysis (6) also confirmed a previous finding (1) that is interpreted as suggesting a polygenic effect of many common SNPs on schizophrenia susceptibility, based on the ability of association test results for many SNPs in one data set to predict case versus control status in a second data set. In the present study, we evaluated whether common risk SNPs in multiply affected families are likely to overlap with those in unrelated cases by testing whether our family study results can predict case-control status in the large PGC data set. We also explored whether any known functional gene pathways are enriched for modestly significant SNP associations. In single-SNP, polygenic, and pathway analyses, family data provide some protection against spurious associations due to case-control differences in ancestral backgrounds, because counts of SNP alleles that are transmitted from parents to ill offspring are contrasted with counts of the alleles that parents did not transmit.

GWAS analyses have also shown that rare chromosomal deletions of chromosomes 1q21.1, 15q13.3, and 22q11.2

and of exons of the neurexin-1 gene (NRXN1) and duplications of 16p11.2 (collectively present in around 1.25% of cases) each produce significant eightfold or greater increases in risk; notably, each has also been reported in autism, mental retardation, and epilepsy (7). We determined the frequency of these copy number variants (CNVs) in our families and examined how well they correlate (segregate) with disease in families, which has implications for diagnostic testing. We also identified new “candidate” CNVs.

Method

Subjects

The sample (Table 1) includes seven subsamples that were recruited for linkage studies (8–15) and subsequently combined (16–19), excluding families from the National Institute of Mental Health’s Schizophrenia Genetics Initiative because a previous GWAS studied the probands (2). Briefly, family members gave informed consent and were diagnosed using semistructured interviews, psychiatric records, and informant reports. Case subjects had DSM-III-R diagnoses of schizophrenia or schizoaffective disorder (probands had schizophrenia), which cosegregate in families (20) and are difficult to differentiate reliably (21). These families were originally ascertained because the constellation of affected relatives was informative for linkage studies, and all families had at least two directly evaluated narrow-diagnosis cases. For some families, only one affected case subject was included in this analysis, either because there was only one case subject in the nuclear family who met inclusion criteria or because DNA was not available for GWAS genotyping or the specimen failed quality control filters. Families were analyzed here if they had DNA available for one affected offspring plus one or both parents, for two affected siblings and at least one parent or one unaffected sibling, or for three or more affected siblings.

TABLE 1. (Continued)

European	Families					Total
	Mediterranean	Admix1	Admix2	Admix3	Malabar	
57	1	0	0	0	0	58
21	0	0	0	0	0	21
122	1	0	0	0	0	123
53	1	0	0	0	0	54
21	0	11	11	5	8	56
216	0	0	0	0	0	216
91	12	0	0	0	0	103
581	15	11	11	5	8	631

Some families included more than one sibship that met these criteria. Based on an analysis of power versus cost (not shown), we included all available parents plus two unaffected siblings (if available) if no parents were genotyped, or one unaffected sibling if one parent was genotyped.

Genotyping, SNP Quality Control, and Genotypic Ancestry

Genotyping was performed with the Illumina 610-Quad array (at Illumina, Inc., La Jolla, Calif., for families and at the Children's Hospital of Philadelphia [by H.H.] for control subjects; see p. 18 of the online data supplement for discussion of the CNV case-control analysis), and genotypes were called with the BeadStudio software package (Illumina, Inc.). HG18 genomic locations are reported. Based on principal components analysis (22) of 55,010 autosomal SNPs with low pairwise linkage disequilibrium (LD), families were divided into six ancestry groups (Table 1; see also Figure S1 in the data supplement that accompanies the online edition of this article): European, Mediterranean (primarily Sephardic Jewish), and four with varying degrees of African or South Indian admixture (Réunion Island). Because somewhat different genetic architecture has been observed for schizophrenia in European- and African-origin samples in previous single-SNP (2) and polygenic (1) GWAS results, separate analyses were carried out for the European-ancestry group and for the six ancestry groups combined.

Exclusion criteria for SNPs were as follows: third allele observed; pseudo-autosomal or mitochondrial; minor allele frequency <1% (in European-ancestry group or all founders); call rate <98.8%; $p < 0.0001$ for deviation from Hardy-Weinberg expectation (in unrelated unaffected individuals); GenCall10 quality score <0.55; and more than four Mendelian inconsistencies for parent-child pairs and more than seven for parent-parent-child trios. Genotypes were removed for the family for SNPs with Mendelian inconsistencies and for males for chromosome X SNPs called as heterozygous. There were 576,976 autosomal and 15,146 chromosome X SNPs before quality control analysis (QC), and 531,195/12,936 for European-ancestry and 528,297/13,202 for all analyses after QC.

DNA sample exclusion criteria were as follows: duplicates of another sample; genotypically inconsistent with known gender or family structure; >104 parent-child or >199 parent-parent-child Mendelian inconsistencies; call rate <98%; or mean heterozygosity inconsistent with ancestry subgroup. Chromosome X data were excluded if genotypic gender was ambiguous (possible cell culture artifact) but autosomal QC was acceptable.

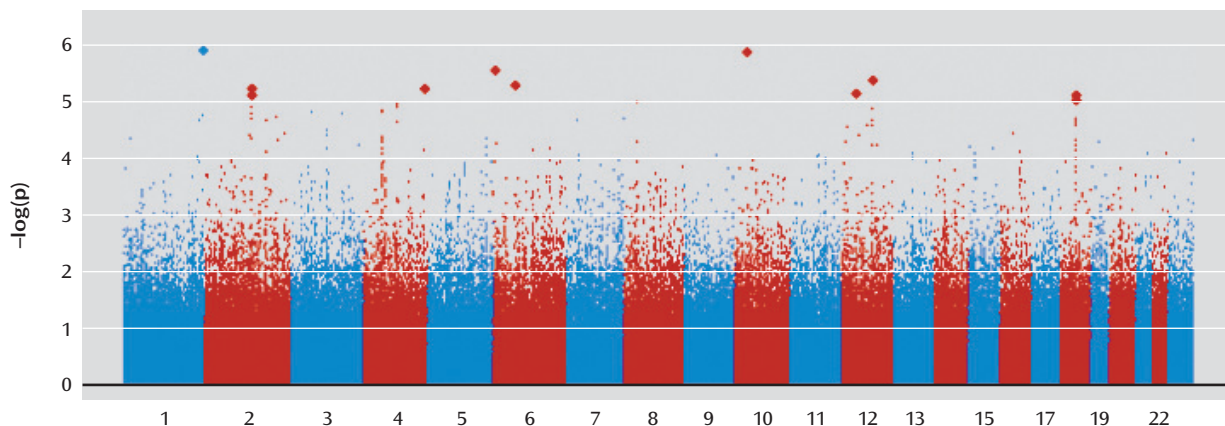
Statistical Analyses of Genetic Association to SNPs

Family-based association tests were performed using TRANSMIT, version 2.5.4 (23), for autosomal SNPs. TRANSMIT was selected because it is fast and can handle any constellation of genotyped relatives. However, it is not recommended for chromosome X, so UNPHASED, version 3.1.5 (24), modified for consistency with TRANSMIT in handling ungenotyped individuals, was used for that chromosome. These programs test whether each SNP allele is transmitted more or less often than chance expectation. Because they use data set allele frequencies as well as the family's data to estimate nontransmitted alleles of ungenotyped parents, analyses were performed separately for each of the six ancestry subgroups. European-ancestry and all-family results are reported (with the latter combining observed and expected transmission counts across groups). Autosomal odds ratios were estimated by subtracting an estimate of the number of homozygous parents (allele frequency squared, times the number of parents) from the total number of transmissions of each allele to obtain transmissions from heterozygous parents (expected to be 50% for each allele by chance), and computing the ratio of counts for the two alleles. Genomic control lambda was computed as the median chi-square value divided by the expected value (0.456).

Two previous studies noted that TRANSMIT can sometimes inflate type I error (25, 26). One of the studies (26) is difficult to generalize because it used TRANSMIT's bootstrapping routine to compute p values, which can produce discrete distributions in small samples (37 pedigrees in that study). For the robust variance estimator used here to compute p values, Martin et al. (25) previously clarified that the problem was seen in larger samples when only two affected siblings could be genotyped, in the presence of linkage, and for recessive inheritance with much larger effect sizes than are observed in any GWAS of schizophrenia. We excluded sibling-pair-only families. Also, we initially evaluated TRANSMIT's type I error rate in 5,000 replicates of our European-ancestry pedigrees for each of a range of minor allele frequencies and linkage models (up to a value of 2 for the relative risk to siblings versus population risk, much stronger than is realistic for schizophrenia) and observed no inflation of type I error rate at nominal significance levels of 0.05–0.001. Finally, our quantile-quantile plots (see Figure S2 in the online data supplement) demonstrate that no substantial inflation occurred.

To estimate power, genotypes were simulated for European-ancestry families under a range of genetic models, and each replicate was analyzed with TRANSMIT. The sample was well powered (>80%) to detect genome-wide significant association for additive allelic relative risks of approximately 1.5 (25%–50%

FIGURE 1. GWAS Results for 583 European-Ancestry Families^a



^a Each dot represents the $-\log(p)$ value for one of the 544,131 autosomal and X chromosome SNPs included in the European-ancestry analysis. Chromosome numbers are shown on the x-axis.

allele frequencies), but not in the range of 1.1–1.2 (1%–2% power to detect genome-wide significant effects).

We performed ALIGATOR (27) analyses of whether gene pathways contained SNPs with low p values more often than would be expected by chance given the observed distribution of SNP p values, for the GO, KEGG, MGI, PANTHER, BioCarta, and Reactome databases plus two locally curated pathways (see p. 12 in the online data supplement).

We used polygenic score tests (1) to evaluate the hypothesis of multiple common risk SNPs, using 112,869 post-QC autosomal SNPs with limited pairwise LD ($r^2 < 0.25$) that were also available for the PGC phase 1 European-ancestry data set of 9,394 cases and 12,462 controls (using data that were either genotyped or imputed [28] based on HapMap 3 reference haplotypes with information content > 0.9). A reference allele for each SNP was assigned a weight equal to the log-odds ratio for association in the family study. For each PGC subject, the observed reference alleles were weighted and summed. The significance of the PGC case-control score difference was analyzed by logistic regression (using the R package), corrected for seven ancestry-based principal component scores as covariates. The proportion of variance explained (R^2) by the polygenic scores was computed by subtracting the Nagelkerke R^2 attributable to ancestry covariates alone from the R^2 for polygenic scores plus covariates. The analysis was repeated 10 times, starting with only the SNPs with the best 0.01% of p values in the family data, and finally including all SNPs (see Figure 2 legend for details).

Finally, the 58 independent ($r^2 < 0.2$) SNPs with the best p values in the phase 1 PGC GWAS (which did not include the present families) were selected for analysis of consistency of direction of effect in the family study (6). These were drawn from the 81 SNPs with $p < 2 \times 10^{-5}$, including only the best SNP from the extended MHC region that contained most of the significant SNPs but is characterized by extensive LD. For SNPs not genotyped here, we selected a nearby proxy (highest r^2 with the PGC SNP). After inverting the family study odds ratios when necessary because of differences in chromosomal strand and/or test allele, we determined the number of SNPs with the same direction (both odds ratios < 1 or both > 1) in the two analyses and computed a binomial test of the probability of observing at least that many consistencies, given the chance expectation of 50% consistency of direction of effect.

CNV Analysis

Data are presented here for segregation of previously identified schizophrenia-associated CNVs within families (chromosomes 1q21.1, 15q13.3, 16p11.2, and 22q11.2 and NRXN1) (7, 29–31). An exploratory case-control analysis to identify new candidate CNVs was also carried out (for the methods and results, see p. 18 of the online data supplement). CNVs spanning three or more probes were called with the PennCNV software program (32). Subjects were excluded if they had ≥ 50 CNV calls or if the standard deviation of the $\log(R)$ ratio (a normalized expression of relative probe intensity for a given subject, which is related to copy number) was > 0.4 (indicating increased signal variability across all probes). CNVs were merged if two or more adjacent deletions or duplications had different estimated copy numbers (0 and 1 for deletions, 3 and 4 for duplications) or if a segment with an estimated copy number of 2 contained $< 30\%$ of the probes in a CNV formed by merging it with two surrounding deletions or duplications (and these merger rules were also applied to chains of such events). For subjects with one of the schizophrenia-associated CNVs and for all of their family members, CNV data for that region were visualized by plotting $\log(R)$ ratio and B-allele frequency (the proportion of intensity detected for a designated test allele) and by computing and visualizing point-by-point estimates of copy number using a second algorithm (33). In all cases, the PennCNV call for these large CNVs was confirmed by these additional steps. For the five selected CNV regions, we then examined evidence for transmission within families and for segregation with schizophrenia.

Results

Association of Common SNPs

For European-ancestry families (Figure 1), lambda (the median chi-square divided by the expected median in null data, 0.456) was 1.025 (see Figure S2 in the online data supplement), indicating minimal technical or ancestry-related artifact. Table 2 lists results for genes with at least one SNP with $p < 0.0001$ within the gene or within 50 kb of it. (See Table S1 in the online data supplement for details of nongenic regions meeting this criterion.) The all-family analysis produced similar results (see Figures S2

TABLE 2. SNP Association Results in European-Ancestry Families^a

SNP	LOC	Allele 1 Total Counts				Allele 2 Total Counts			
		A1	Frq	T	NT	A2	Frq	T	NT
rs12210050	chr6:420489	T	0.23	565	459	C	0.77	1763	1869
rs12426725	chr12:80367259	A	0.15	308	411	G	0.85	2030	1927
rs1170612	chr2:124699526	T	0.22	593	481	C	0.78	1745	1857
rs16934812	chr12:29763585	G	0.13	331	248	T	0.87	2003	2086
rs12511372	chr4:45811189	G	0.50	1229	1106	A	0.50	1107	1230
rs3197999	chr3:49696536	T	0.30	747	628	C	0.70	1587	1706
rs4716801	chr7:157381124	G	0.46	1151	1022	A	0.54	1185	1314
rs7805806	chr7:20693853	G	0.12	334	250	A	0.88	2004	2088
rs12239401	chr1:235261146	T	0.44	953	1080	C	0.56	1383	1256
rs6433323	chr2:172581306	G	0.38	963	842	A	0.62	1375	1496
rs1037231	chr3:85845797	A	0.42	913	1038	G	0.58	1421	1296
rs3892156	chr16:48877496	A	0.25	659	552	G	0.75	1669	1776
rs2396465	chr2:228234344	G	0.10	267	198	A	0.90	2071	2140
rs12565770	chr1:19427647	A	0.12	229	312	G	0.88	2109	2026
rs1851185	chr2:212235974	T	0.24	593	490	C	0.76	1745	1848
rs12321966	chr12:8592432	T	0.09	264	196	G	0.91	2072	2140
rs4805453	chr19:34814743	C	0.42	1050	927	T	0.58	1286	1409
rs6901207	chr6:3798905	G	0.44	1110	995	A	0.56	1228	1343
rs6443997	chr3:186016225	A	0.06	106	156	G	0.94	2232	2182
rs795955	chr12:77160181	T	0.40	883	993	C	0.60	1455	1345
rs10507070	chr12:94873188	A	0.17	451	359	G	0.83	1885	1977
rs7179849	chr15:22589304	T	0.18	383	475	C	0.82	1955	1863
rs7180015	chr15:85305969	G	0.09	162	226	A	0.91	2176	2112
rs1782	chr6:90124434	C	0.12	311	240	T	0.88	2017	2088
rs2362643	chr16:68503033	G	0.32	815	701	A	0.68	1523	1637
rs2211871	chr21:38744520	G	0.09	242	179	T	0.91	2094	2157
rs4925449	chr22:47486086	A	0.08	170	237	G	0.92	2166	2099
rs175	chr7:25000316	C	0.47	1142	1031	A	0.53	1194	1305
rs10760120	chr9:99908721	G	0.47	1024	1143	A	0.53	1308	1189
rs10489577	chr1:231021449	C	0.04	103	61	T	0.96	2235	2277
rs921383	chr11:77388489	A	0.47	1172	1057	G	0.53	1162	1277

^a Shown are association test results for 583 European-ancestry families for single-nucleotide polymorphisms (SNPs) with $p < 10^{-4}$ that are within 50 kb of a RefSeq gene. The bp distances are given for upstream (positive number) or downstream (negative number) of the transcribed region of the closest gene; if no number follows the gene name, the SNP is within the transcribed region. For each gene, only the SNP with the lowest p value is shown, along with the number of SNPs with $p < 10^{-4}$ (SNPs column). Frq=frequency. T=transmitted from parents; NT=nontransmitted (counts estimated by TRANSMIT). All locations are according to the HG18 genome build. SNPs with $p < 1.0E-05$ were also observed in nongenic regions, including chr1:242457187, chr4:183374392, chr6:33959151, chr10:21607571, and chr18:40344665 (see Table S1 in the online data supplement).

and S3 and Table S2 in the online data supplement). No SNP achieved genome-wide significance ($p < 5 \times 10^{-8}$) in either analysis.

In polygenic score analyses (Figure 2), family-based results significantly predicted PGC case-control status for all thresholds, with the lowest p value of 1×10^{-17} (explaining 0.4% of the variance) achieved for 34,937 SNPs with $p < 0.2$ in the family study.

PGC and family study odds ratios were in the same direction for 37 of the 58 tested SNPs (one-sided binomial $p = 0.024$) (see Table S4 in the online data supplement), or 29/45 after excluding proxy SNPs with $r^2 < 0.8$ ($p = 0.036$).

ALIGATOR analyses (see Tables S5 and S6 in the online data supplement) did not detect significant pathway

effects (single pathways or excess of number of pathways) after correction for multiple testing.

Previously Documented CNV Regions

Figure 3 illustrates eight pedigrees with CNVs with previous significant evidence for association with schizophrenia (7). We observed 1q21.1 and 15q13.3 duplications segregating with schizophrenia in offspring, but only the reciprocal deletions have been strongly associated in these regions, with weaker evidence for 1q21.1 duplications (7). One of two affected offspring had an exonic NRXN1 deletion, but not the unaffected father (the mother was unavailable). For 16p11.2, duplications were observed in an unaffected mother and two of three affected children.

TABLE 2. (Continued)

T From Heterozygous Parents						
A1	A2	Odds Ratio	p	SNPs	Genes (Within 50 kb)	
445.1	371.7	1.20	2.9E-06	1	EXOC2,9648	
254.1	347.9	0.73	4.2E-06	4	PPFIA2	
475.1	339.3	1.40	5.9E-06	7	CNTNAP5	
294.0	219.6	1.34	7.2E-06	1	TMTC1	
643.8	524.2	1.23	1.4E-05	11	GABRG1 (and GABRA2 in the SNP cluster)	
539.6	437.1	1.23	1.6E-05	1	BSN,12550; APEH,598; MST1; RNF123,-5457; AMIGO3,33432; GMPPB,37399; IHPK1,40195	
652.2	509.0	1.28	2.1E-05	1	PTPRN2	
298.9	203.8	1.47	2.2E-05	1	ABCB5	
495.2	657.5	0.75	2.2E-05	1	RYR2,-11178	
623.6	479.2	1.30	2.3E-05	2	HAT1,24460; MAP1D	
506.2	629.0	0.80	3.3E-05	4	CADM2,-12524	
509.5	371.3	1.37	3.7E-05	1	ADCY7,-1827; BRD7,32945	
244.6	168.1	1.46	3.8E-05	1	DKFZp547H025,-28212; SLC19A3,23825	
197.6	281.8	0.70	4.5E-05	1	UBR4,-18314; KIAA0090; MRTO4,-23014; AFAR3,37415	
462.8	380.3	1.22	4.8E-05	1	ERBB4	
243.5	153.6	1.58	5.1E-05	1	CLEC4D,26205; CLEC4E,-7607	
635.7	503.2	1.26	5.3E-05	1	POP4,16196; PLEKHF1,-33423	
650.3	503.8	1.29	5.6E-05	1	FAM50B,2355	
98.0	159.2	0.62	6.1E-05	1	VPS8	
503.7	621.1	0.81	6.2E-05	1	NAV3,29260	
381.6	285.1	1.34	6.3E-05	1	CCDC38,-12629; AMDHD1; HAL,18084; LTA4H,45553	
305.7	389.8	0.78	6.6E-05	1	SNRPN,-30582	
144.4	225.8	0.64	7.1E-05	1	AGBL1	
279.7	197.7	1.41	7.7E-05	1	GABRR2,-42748; UBE2J1,-5096; RRAGD,9878	
578.2	436.4	1.32	7.8E-05	1	WWP2; LOC348174,-39277	
222.4	166.6	1.33	8.3E-05	2	ERG	
153.4	207.6	0.74	8.8E-05	1	FAM19A5	
635.4	527.1	1.21	9.2E-05	1	OSBPL3,-14031	
514.3	646.8	0.80	9.3E-05	1	NANS,23543; TRIM14; CORO2A,17575	
100.0	62.3	1.60	9.5E-05	1	KIAA1383,8734	
656.4	506.4	1.30	9.8E-05	1	INTS4,-5124; KCTD14,15919	

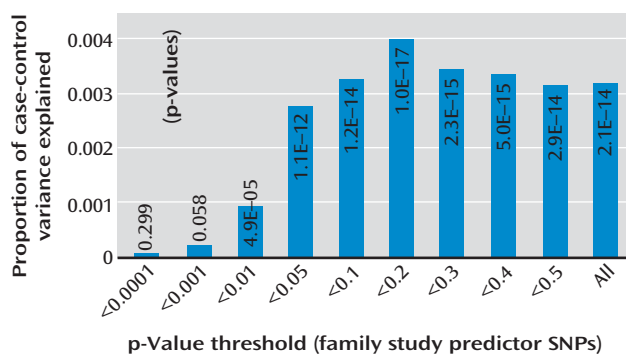
The recruiting site reported a duplication in an unaffected sibling (not genotyped here) (34). It is unlikely that the affected father, who was deceased, carried the same rare CNV. Four cases had 22q11.2 deletions (three typical 3 Mb and one proximal 1.5 Mb), all de novo. Excluding the 15q duplication, these CNVs were seen in seven of 633 families (1.1%), compared with 1.3% of cases in a recent meta-analysis (7). No large 3q29 deletions or exonic VIPR2 duplications were observed (7).

Discussion

Our results suggest that there is substantial overlap between the common SNPs that confer schizophrenia risk in multiply affected families and in unrelated cases, based on the highly significant polygenic score analysis: when association test results from the family study were used to weight the genotypes of PGC subjects, the resulting polygenic scores significantly differentiated case subjects

from control subjects. Note that this result does not prove that there are *no* genetic effects that are individually stronger or more prevalent in multiply affected families.

It has been proposed that this cross-study consistency is due to a large number (perhaps many hundreds) of risk SNPs in the genome (1, 35). In very large samples, the best results will contain some true associations; for example, in the PGC two-stage analysis of single SNPs, seven chromosomal regions ultimately produced highly significant results, drawn from 58 independent SNPs in the best 53 regions of association in stage 1 (6) (most of them with consistent directions of effect in the family sample). Here, with a small predicting sample, the polygenic score analysis became significant as the proportion of best SNPs included in the analysis increased from 0.1% to 1%, but it was most significant using the best 20%, and in the PGC analysis (with a much larger predicting sample), significance continued to improve when all independent SNPs

FIGURE 2. Prediction of Psychiatric GWAS Consortium Case-Control Status^a

^a Each bar shows the proportion of variance explained (R^2) in analyses using polygenic scores (1), computed based on association test results from this family-based study, to predict the case-control status of 9,394 schizophrenia case subjects and 12,462 control subjects from the Psychiatric GWAS Consortium (PGC) GWAS (6). A subset of 112,869 family study SNPs was selected for which PGC had data (genotyped or imputed from HapMap 3 information with information content >0.9), with minor allele frequency $>2\%$ in both data sets, and correlation (r^2) between SNPs <0.25 . Shown below each bar is the proportion of the SNPs (rank-ordered by family study p value) used in that analysis. For each PGC subject, a polygenic score was computed by multiplying (for each SNP) the family study association test result ($\log[\text{odds ratio}]$) by the subject's genotype (how many of the designated test alleles the subject carried) and then summing these products across SNPs. The p value shown within each bar is from a logistic regression of PGC case-control status predicted by polygenic scores plus seven ancestry-based covariates. The R^2 is the difference between Nagelkerke's R^2 for prediction using scores and covariates minus the R^2 for covariates alone. (See Table S3 in the online data supplement for additional details.) The best prediction was observed when SNPs with the best 20% of p values were included. The prediction is highly significant, although with a very small proportion of total variance explained.

were included. This suggests that risk SNPs are distributed across the range of p values (or odds ratios), because most of them gave quite small individual effects. Polygenic score analysis cannot currently determine *which* SNPs are truly involved in risk. Here, network-based analyses did not further define the polygenic effect, and it is likely that an increased understanding of gene and protein functions and interactions will be needed to accomplish this.

The actual proportion of variance in PGC case-control status that could be explained was quite low (0.4%). The variance that can be explained by this type of cross-data set analysis is limited by the need to use only independent SNPs in the analysis, by the fact that GWAS assays do not provide information about all common SNPs, and by loss of information as a result of differences in genotyping methods and ancestral backgrounds of samples. Other forms of analysis suggest that common SNPs actually explain around 20%–30% of the genetic variance for schizophrenia (1, 36). Polygenic score analyses of case-control samples have predicted larger amounts of variance as the predicting sample size has increased, from around 4% with prediction and test samples with approximately

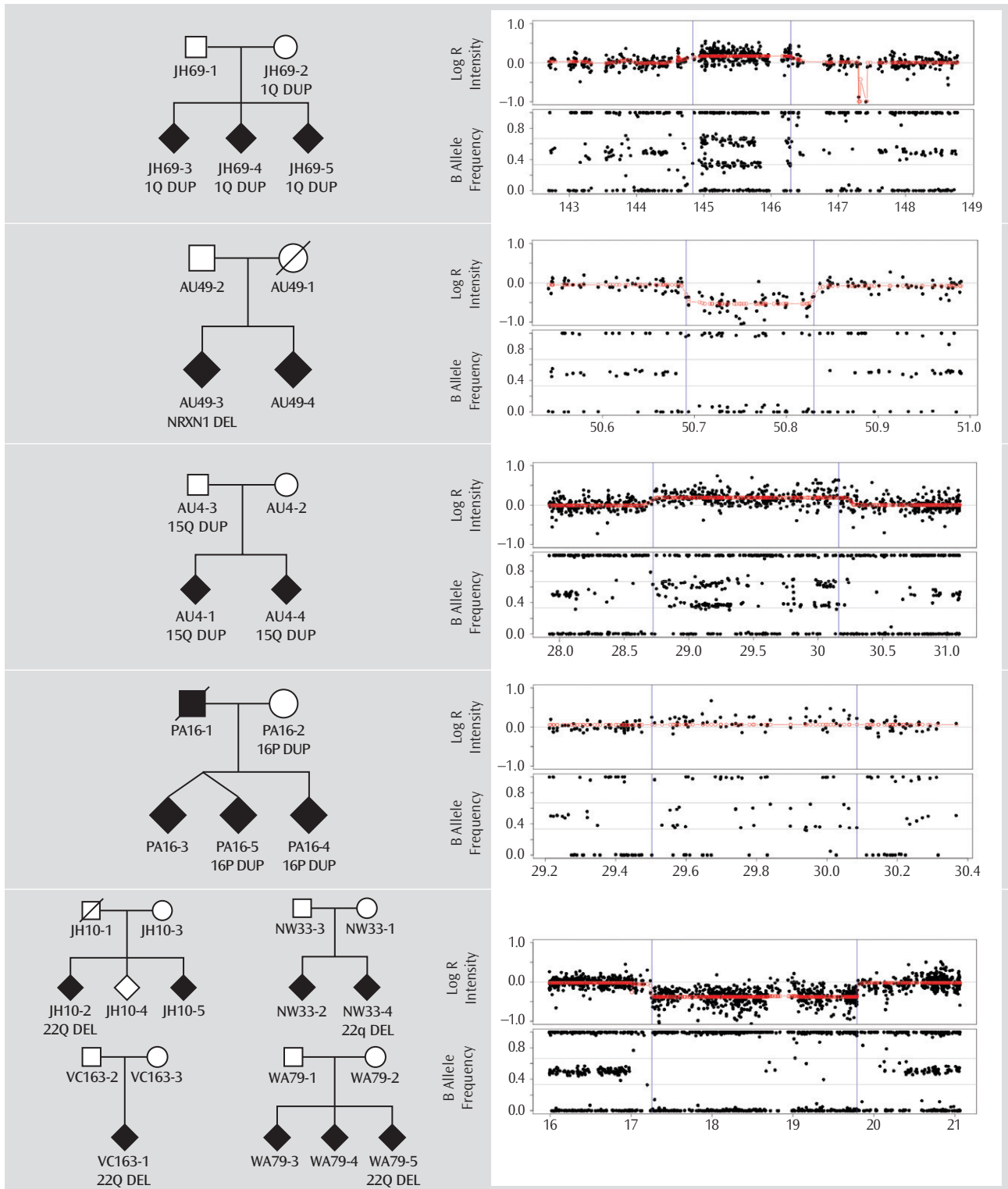
3,000 cases (1) to approximately 7% with a larger predicting sample (around 6,500 cases) and a test sample of approximately 3,000 cases. Here, we used the smaller family sample for prediction to the larger PGC case-control sample, because there is no current method for computing polygenic scores for individual subjects based on family data with some parental genotypes inferred rather than directly observed. Therefore, while our results demonstrate a highly significant overlap in common risk SNPs in these families and the PGC case sample, we cannot determine whether there is any reduction in overlap in multiplex families compared with unrelated cases.

It has been suggested that this polygenic signal could be due in part to weak correlations between common SNPs and nearby rare SNPs or structural variants with larger effects on risk (37). Most evidence does not favor this hypothesis (35); for example, we have not found single families with significant linkage signals that might be produced by rare, heritable large-effect variants. The next generation of sequencing-based studies might shed more light on the genetic effects of various types of sequence and structural variants across the full range of frequencies.

We did not observe larger effect sizes of single SNPs in these multiply affected families than have been reported in case-control samples (www.genome.gov/gwastudies, accessed May 7, 2011). Because exonic deletions in *NRXN1* are the only single-gene mutations shown to be associated with large increases in schizophrenia risk (approximately eightfold) (7), we were interested to note that several SNPs with low p values were in or near genes with related functions involving brain development and neuronal cell adhesion and signaling (*CNTNAP5*, *CADM2*, *ERRB4*, *PPFIA2*, *PTPRN2*, *CLEC4D/E*, *AMIGO3*, and *CNTN5* for all ancestries). However, we did not detect statistically significant evidence for association of any defined pathway after correcting for multiple testing of pathways. This could be due to lack of statistical power from the relatively small sample size or because the pathophysiological mechanisms underlying schizophrenia risk are not adequately captured by current pathway definitions.

Five rare CNVs are strongly associated with schizophrenia, and three of them (16p11.2 duplications, 22q11.2 deletions, and *NRXN1* exonic deletions) were observed here, along with duplications that are reciprocal to associated deletions of 1q21.1 and 15q13.3; there is some evidence for association of 1q21.1 duplications, but not for 15q13.3 duplications (7). The total frequency of these CNVs (excluding the 15q13.3 duplication) was similar to that observed in previously reported case samples. The family data provide several insights. First, the possibility of a de novo (nontransmitted) 22q11.2 deletion should not be ignored in multiply affected families—indeed, the prevalence of these deletions was similar to that reported in large samples with primarily nonfamilial cases (7). There must have been other genetic or nongenetic risk factors in these families, but it is not known whether their effects were

FIGURE 3. Families With CNVs in Regions Previously Shown to Be Associated With Schizophrenia^a



^a Shown are the eight pedigrees with carriers of CNVs in five chromosomal regions with well-documented association of CNVs to schizophrenia, including 1q21.1 (typical HG18 boundaries 144.6–146.3 Mb), NRXN1 (interrupting exons of the gene, which lies on chromosome 2, 50–51.1 Mb), 15q13.3 (28.7–30.3 Mb), 16p11.2 (29.5–30.1 Mb), and 22q11.2 (17.1–20.2 Mb), or less commonly, a proximal 1.5 Mb deletion, as observed in individual NW33-4). Only genotyped offspring and their parents are shown, but all families were multiply affected. An illustrative example of each CNV is shown: the top plot shows the log(R) intensity (also known as log[R] ratio) for each probe location, with point-by-point estimates (in red) of changes in copy number (up for duplications, down for deletions) using a second algorithm (33). The bottom plot shows the B-allele frequency, i.e., where copy number=2, the designated “B” allele has 0%, 50%, or 100% of the total fluorescent intensity, but when copy number=1, only values of 0% or 100% are seen, while with copy number=3, some alleles have 33% or 67% of the total intensity, producing a distinctive pattern as shown. (Family IDs are masked.)

limited to the siblings without a 22q11.2 deletion or whether they also influenced the emergence of the schizophrenia phenotype in the carrier, given that schizophrenia develops in only ~30% of 22q11.2 carriers. Second, two transmitted CNVs (16p11.2 duplications and a NRXN1 deletion) failed to segregate perfectly with schizophrenia within the family, suggesting again that other risk factors were present.

Conclusions

This GWAS of multiply affected families produced significant support for a polygenic model that posits that multiple common SNPs confer part of the genetic risk of schizophrenia, with a significant overlap between common risk SNPs in multiply affected families and samples of unrelated case subjects. Significant association was not detected for any single SNP, which is consistent with the relatively small sample size, but for the most significant SNPs in the large PGC GWAS analysis, the direction of effect was the same in both samples for a significant excess of SNPs. Several of the “top SNPs” in the family study were in genes related to neurodevelopment, but no statistically significant evidence was observed for association of currently defined gene pathways. Rare CNVs were observed in regions with strong previously documented association with schizophrenia, but with variable patterns of segregation. This should serve as a reminder that we still know relatively little about the distribution of these CNVs in the entire population (e.g., in individuals with no or only mild cognitive problems) or about the reasons for the emergence of schizophrenia in only a minority of carriers, so great caution is required in genetic counseling and prediagnosis.

Received Sep. 23, 2011; revision received April 17, 2012; accepted May 07, 2012 (doi: 10.1176/appi.ajp.2012.11091423). From the Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, Calif.; Departments of Psychiatry and Preventive Medicine, University of Southern California, Los Angeles; Departments of Psychiatry and Human Genetics, Virginia Commonwealth University, Richmond; Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore; Department of Psychiatry, University of Western Australia, Perth; NorthShore University Healthcare Research Institute, Evanston, Ill.; Department of Psychiatry, University of Chicago, Chicago; Queensland Brain Institute, University of Queensland, Brisbane, Australia; Queensland Centre for Mental Health Research, Brisbane; Department of Child and Adolescent Psychiatry, Faculty of Medicine, Université Pierre et Marie Curie, Paris; Department of Psychiatry and Psychotherapy, University of Erlangen-Nuremberg, Erlangen, Germany; ICM Institute, Hôpital de la Pitié Salpêtrière, Paris; State Mental Hospital, Haar, Germany; Institut National de la Santé et de la Recherche Médicale (INSERM), Hôpital de la Pitié-Salpêtrière, Paris; Department of Psychiatry, University of Bonn, Bonn, Germany; Department of Psychiatry, University of Athens Medical School, Athens, Greece; the Health Research Board, Dublin, Ireland; Queens University, Belfast, Northern Ireland; Department of Psychiatry, Hadassah-Hebrew University Medical Center, Jerusalem; INSERM, Unité 614, Institut Hospitalo-Universitaire de Recherche Biomédicale, Rouen, France; Department of Research, Centre Hospitalier de Saint Etienne du Rouvray, Rouen; Mental Health Service Line, Washington VA Medical Center, Washington, D.C.; Department of

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Participants in the Schizophrenia Psychiatric GWAS Consortium are listed in the online data supplement (starting on p. S28).

Address correspondence to Dr. Levinson (dflev@stanford.edu).

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DNA samples were collected by older studies without consent for repository deposition and are not publicly available.

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