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Deep Resequencing and Association Analysis of Schizophrenia Candidate Genes

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In 2005, we selected 10 genes for which there was reasonable evidence for involvement in the etiology of schizophrenia (*COMT, DAOA, DISC1, DRD2, DRD3, DTNBP1, HTR2A, NRG1, SLC6A3, SLC6A4,* Table S1)[1]. Although these genes have not received support from far larger and comprehensive subsequent studies, and may not contain etiological common variation[2], it is possible that they contain uncommon variation of etiological importance. To test this hypothesis, we conducted a multistage resequencing study.

In Stage 1, we used Sanger methods to sequence the exons, 5′ and 3′ UTRs, splice sites, promoters and conserved intronic regions of these 10 genes in 727 cases with schizophrenia from CATIE[3] and 733 controls of European (EUR) and African (AFR) ancestry. In Stage 2, we validated single nucleotide variants (SNVs) using Roche 454 sequencing in the same samples. In Stage 3, we genotyped prioritized SNVs in independent samples (Supplemental Material and Figure S1).

In Stage 1, Sanger sequencing identified 782 variants, including 587 novel SNVs not found in dbSNP132 (Tables S2–S4). As expected, the number of novel variants discovered per individual was higher in those of AFR (1.46) than EUR ancestry (0.95) but cases and controls did not differ (EUR cases/controls: 0.920/0.980; AFR cases/controls: 1.492/1.430). The numbers of SNVs per gene were also similar although *DISC1* showed a non-significant excess in cases (EUR cases/controls: 0.138/0.119; AFR cases/controls: 0.243/0.167) mostly due to novel variants in AFR subjects (cases/controls: 0.173/0.099). Three unrelated cases, but zero controls, were found to each have a single novel nonsense mutation: two for *DISC1* (truncating only the “Es” splice variants) and one for *SLC6A4* (Tables S2–S4). The ratio of nonsynonymous to synonomous variants was similar in cases and controls (1.25 vs 1.16).

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In Stage 2, we prioritized 254 of the 782 variants for technical replication since they met at least one of the following criteria: 1) novel nonsense, missense or splice site variant, 2) novel intronic variant in ≥1 EUR case, 3) novel variant with an odds ratio >2 in the EUR cohort, 4) dbSNP nonsense, missense or splice site variant in ≥1 EUR case. Validation sequencing by Roche 454 revealed 225 true variants and 29 false positives (accuracy rate of 89%, Figure S2).

In Stage 3, we selected 92 of the 225 SNVs (Table S5) from Stage 2 for genotyping in an independent sample of 2,191 cases and 2,659 controls (EUR and AFR). We included: 1) all novel nonsense, missense or splice site variants seen in ≥1 case, 2) all variants seen in >1 case, 3) three nonsense variants observed in one case each. After genotyping 92 SNVs in the replication samples, 29 were monomorphic (22 of these were seen in only one case in Stage 1), six had low quality genotypes, and 57 SNVs were tested for association with schizophrenia (logistic regression, separately for EUR and AFR subjects). Table 1 lists the SNVs with the smallest p-value in each gene (complete results in Table S6). No gene contained a SNV reaching criteria for genome-wide significance ($p < 5 \times 10^{-8}$). We then tested the aggregate effects of uncommon variants within a gene for 35 non-intronic SNVs with MAF <0.01 (Table 1). No gene was significant following correction for multiple testing. For example, of the 20 uncommon variants in *DISC1* (Figure S3), there were 32 minor alleles in EUR cases and 40 in EUR controls.

Thus, multistage resequencing of ten schizophrenia candidate genes did not yield support for uncommon exonic variation. This result is consistent with common variation results that do not, to date, provide support for these genes despite a sample size of 21,856 individuals. The replication sample had 80% power to detect a genotypic relative risk of 3.2 in the EUR cohort and 5.1 in the AFR cohort, with MAF of 0.01 and significance level of $P = 5\times10^{-8}$. For a relaxed threshold P = 0.001, there would have been >99% power to detect genotypic relative risk of 2.9 in the EUR cohort and 4.6 in the AFR cohort.

The Stage 1 and 2 results hinted that *DISC1* might contain an excess of uncommon variants, and *DISC1* was thus the main focus in Stage 3 replication. However, there was no evidence in our results to support the hypothesis that *DISC1* contains uncommon variants of relevance to schizophrenia as no single-SNV or aggregate test approach was even of nominal significance. Although *DISC1* nonsense variants were present in two Stage 1 cases and 0 controls, no additional cases or controls in the larger Stage 3 sample had those particular nonsense mutations.

DISC1 has been the focus of dozens of genetic studies[4]. However, a recent comprehensive meta-analysis of common variation did not support its role in schizophrenia susceptibility[5]. To our knowledge, four groups have sequenced *DISC1* in cases with schizophrenia[6–9], and all had discovery samples far smaller than reported here (34, 90, 198 and 288 cases). Of the association results in these studies, none met criteria for genome-wide significance. Only one study had a replication component, and the initial finding did not replicate[9]. Therefore, despite employing a replication sample three times the size of the discovery sample, *DISC1* was not found to contain common or uncommon variants individually or in aggregate associated with schizophrenia.

The results of this study suggest that classical schizophrenia candidate genes do not harbor uncommon coding region variation of etiological importance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Summary of Stage 3 replication genotyping results.

	Single-marker analysis (57 SNVs)			Gene-based analysis (35 SNVs)				
Gene	# SNVs	EUR (minimum p-value)	AFR (minimum p-value)	# uncommon variants	EUR (# of case/ control alternative allele)	EUR p-values (CALPHA/VT)	AFR (# of case/ control alternative allele)	AFR p-values (CALPHA/VT)
COMT	4	0.037	0.128	3	8/1	0.02/0.003	0/3	0.29/0.71
DAOA	5	0.039	0.121	0	-	-	-	-
DISC1	22	0.099	0.081	20	32/40	0.67/0.33	39/32	0.27/0.08
DRD3	3	0.375	0.264	0	-	-	-	-
DTNBP1	5	0.390	0.106	5	18/19	0.32/0.72	7/4	0.34/0.16
HTR2A	3	0.037	0.380	1	3/13	0.03/0.7	0/1	0.71/0.71
NRG1	8	0.119	0.017	1	4/1	0.14/0.1	159/185	0.34/0.15
SLC6A3	4	0.339	0.326	2	4/4	0.6/0.8	0/1	0.76/0.67
SLC6A4	3	0.418	0.310	3	10/9	0.45/0.25	1/1	0.4/0.36

Single-marker and aggregate analyses of EUR and AFR samples. Logistic regression was performed on 57 SNVs. Gene-based analysis was performed on 35 exonic SNVs.