ANXA7, *PPP3CB*, *DNAJC9*, and *ZMYND17* Genes at Chromosome 10q22 Associated with the Subgroup of Schizophrenia with Deficits in Attention and Executive Function

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Background: A genome scan of Taiwanese schizophrenia families suggested linkage to chromosome 10q22.3. We aimed to find the candidate genes in this region.

Methods: A total of 476 schizophrenia families were included. Hierarchical clustering method was used for clustering families to homogeneous subgroups according to their performances of sustained attention and executive function. Association analysis was performed using family-based association testing and TRANSMIT. Candidate associated regions were identified using the longest significance run method. The relative messenger RNA expression level was determined using real-time reverse transcriptase polymerase chain reaction.

Results: First, we genotyped 18 microsatellite markers between D10S1432 and D10S1239. The maximum nonparametric linkage score was 2.79 on D10S195. Through family clustering, we found the maximum nonparametric linkage score was 3.70 on D10S195 in the family cluster with deficits in attention and executive function. Second, we genotyped 79 single nucleotide polymorphisms between D10S1432 and D10S580 in 90 attention deficit and execution deficit families. Association analysis indicated significant transmission distortion for nine single nucleotide polymorphisms. Using the longest significance run method, we identified a 427-kilobase region as a significant candidate region, which encompasses nine genes. Third, we studied messenger RNA expression of these nine genes in Epstein-Barr virus-transformed lymphoblastic cells. In schizophrenic patients, there was significantly lower expression of *ANXA7*, *PPP3CB*, and *DNAJC9* and significantly higher expression of *ZMYND17*.

Conclusions: *ANXA7*, *PPP3CB*, *DNAJC9*, and *ZMYND17* genes are potential candidate genes for schizophrenia, especially in patients with deficits in sustained attention and executive function. The responsible functional variants remained to be clarified.

Key Words: *ANXA7*, chromosome 10, endophenotype, fine mapping study, *PPP3CB*, schizophrenia

Schizophrenia is a neuropsychiatric illness having a strong genetic component and high heritability (1). Many researchers favor a multilocus model, in which epistasis of several genes leads to the disorder (2). However, genome-wide linkage analyses of schizophrenia have produced conflicting results (3). The reasons for these inconsistencies likely stem from the complex etiology of schizophrenia. Thus, one combination of genes may be responsible for schizophrenia in some families and other combinations may explain it in other families. Linkage studies may have unwittingly combined families of different genetic etiologies in the same test group, creating further confusion.

Using endophenotypes to refine phenotypes has been advocated for tackling the genetic heterogeneity of schizophrenia (4). Some neurocognitive phenotypes, such as sustained attention deficits and executive dysfunction, are potential schizophrenia endophenotypes. Sustained attention deficits, measured by the Continuous Performance Test (CPT), are present in schizophrenic patients and nonpsychotic relatives of schizophrenic patients (5,6). The recurrence risk ratio for CPT performance deficits among parents or siblings as disease phenotype was higher than using schizophrenia alone (7). Executive functions, as measured by the Wisconsin Card Sorting Test (WCST), are impaired in schizophrenic patients, (8,9), in subjects with schizotypal features (10), and in their first-degree relatives (11). Thus, use of the CPT and WCST to define endophenotypes for schizophrenia might help resolve the variable results of schizophrenia linkage analyses.

Several studies have reported evidence of weak linkage of chromosome 10q to schizophrenia (12–16). A genome scan in Ashke-

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nazi Jews indicated significant linkage to chromosome 10q22.3 (17). Our previous genome scan of Taiwanese schizophrenic families also suggested linkage to this region (18). However, genome-wide scans of other ethnic groups and meta-analyses (3,19) found no association of this region.

Based on our previous finding that there is linkage to chromosome 10q22.3 in a Taiwanese schizophrenia sample (18), we aimed to identify candidate genes for schizophrenia in this chromosome region using the original sample. We hypothesized that evidence for linkage would be significant for specific neurocognitive subgroups of schizophrenia (as defined by the CPT and WCST performance) and that different candidate genes would be associated with the different subgroups of schizophrenia.

Methods and Materials

Subjects

The subjects for the fine-mapping study were recruited in the Taiwan Schizophrenia Linkage Study from 1998 to 2002. Detailed information has been published previously (18,20). Briefly, the families having at least two siblings with schizophrenia were recruited. All recruited subjects were interviewed using the Diagnostic Interview for Genetic Studies (DIGS) (21). Final diagnostic assessment was based on DSM-IV criteria. A total of 557 Taiwan Schizophrenia Linkage Study families underwent genome scans and 476 of these families took neuropsychological tests. Thus, we used 476 families to determine family clustering and for the fine-mapping study. The detail family information is listed in Table 1.

The subjects for the messenger RNA (mRNA) expression study were from another independent project. The Institutional Review Board of National Taiwan University Hospital approved this project. Written informed consent was obtained after the procedures had been fully explained. All patients and control subjects were interviewed using the DIGS (21). We recruited 85 patients meeting the DSM-IV diagnostic criteria for schizophrenia and 36 control subjects having no lifetime psychiatric disorders. All subjects took neuropsychological tests. The mean age of control subjects was 36.5 (± 10.3) years and 41.7% were male. The mean age of patients was 33.0 (± 9.1) years and 58.8% were male. A comparison of age and gender of patients and control subjects indicated no significant difference.

Table 1. Descriptive Information of the Families Who Were Given Neuropsychological Tests and Underwent Genotyping

Variable	*n*
Number of Families	476
Number of Genotyped Individuals	1955
Gender Distribution	
Men (55.4%)	1083
Women (44.6%)	872
Relationship to Proband	
Proband	476
Sibling	754
Father	319
Mother	406
Affected Individuals	1035
Affected Probands	476
Affected Parents	52
Affected Siblings	507
Families with Two Parents Genotyped	249
Families with One Parent Genotyped	227
Families with No Parents Genotyped	0
Number of Affected Sibling Pairs	
Independent	507
All possible	542

Neuropsychological Assessments

We used a CPT machine from Sunrise Systems version 2.20 (Pembroke, Massachusetts) to assess sustained attention. Each subject undertook two CPT sessions: the undegraded 1–9 task and the 25% degraded 1–9 task. We used sensitivity (d′) as the performance index, which indicates the ability to discriminate target stimuli from nontarget stimuli (22). The effect of age, education, and sex on the CPT performance was adjusted based on a community-based sample of 345 control subjects (21). The d′ was calculated as the *Z* scores that were adjusted for these demographic characteristics.

We employed a computerized version of the WCST (23). The indicators of WCST used in this study were perseverative errors and categories achieved. Previous studies found that these indicators were most likely to be endophenotypes of schizophrenia (8,9,11). The effect of age, education, and sex on the WCST performance was adjusted based on the results of 211 healthy control subjects (24). Performance on the WCST was calculated as the *Z* scores that were adjusted for these demographic features.

Genotyping Method

Microsatellite markers (short tandem repeat polymorphisms [STRPs]) were genotyped at deCODE Genetics Inc. All single nucleotide polymorphism (SNP) markers were genotyped using matrix-assisted laser desorption/ionization-times of flight mass spectrometry. The details are described in Supplement 1.

Epstein-Barr Virus-Transformed Lymphoblast Cell Culture and Real Time Reverse Transcriptase Polymerase Chain Reaction

The detail procedures of Epstein-Barr virus (EBV)-transformed lymphoblast cell culture are described in Supplement 1. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) was performed for studying the expression of target genes and a housekeeping gene (TATA-box binding protein [TBP]), using predesigned gene-specific TaqMan probes and primer sets (Applied Biosystems, Foster City, California). Real-time RT-PCR amplification was conducted using TaqMan One-Step RT-PCR Master Mix Reagent (Applied Biosystems) on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). Gene expression was quantified relative to TBP expression using Sequence Detector Software (Applied Biosystems) and the relative quantification method. The expression level of candidate genes compared with that of TBP was defined as $-\Delta$cycle threshold (CT) $= -$ [$CT_{target\ gene} - CT_{TBP}$]. The target gene mRNA/TBP mRNA ratio was calculated from $2^{-\Delta CT} \times K$, in which K is a constant.

Statistical Analysis

Linkage Analysis. We used the GENEFINDER (http://people.virginia.edu/~wc9c/genefinder/) (25) to determine 95% confidence intervals of the genomic regions from the previous linkage analysis (18). Nonparametric linkage analysis was performed with MERLIN (http://www.sph.umich.edu/csg/abecasis/Merlin/index.html) (26).

Patients and Family Clustering. We used cluster analysis method to obtain more homogeneous family subgroups. We took all affected siblings of the 476 families (a total of 817 individuals) for clustering, using four indicators: adjusted *Z* scores of d′ on the undegraded and degraded CPT and adjusted *Z* scores of perseverative errors and category achieved on the WCST. We measure dissimilarity between a pair of patients using the Euclidean distance between vectors of Pearson correlation coefficients with respect to all other patients. After defining the dissimilarity between patient pro-

files, we invoked an average-link algorithm (27,28) to build a dendrogram. The detail formulations for clustering are described in Supplement 1.

Association Analysis. We performed family-based association with TRANSMIT (http://www-gene.cimr.cam.ac.uk/clayton/software/) (29) and family-based association testing (FBAT; http://www.biostat.harvard.edu/~fbat/default.html) (30). Possible haplotype structure was predicted using the Haploview (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview) (31). We took two approaches for management of the problem of multiple comparisons. Firstly, we used the false discovery rate (FDR) method (32) as the conventional statistical correction on locus-specific multiple tests. Secondly, we used the region-specific testing procedure, the longest significance run (LSR) method (33), to estimate a region-specific *p* value, an index of the most likely genomic region with susceptible genes, as a complementary method for conventional statistical corrections on multiple tests. The proposed LSR method is a two-stage procedure. The first stage conducts conventional association tests. Based on the prespecified size of a given test, the *p* value of each test is converted into a zero/one indicator (1 for significance or 0 otherwise). In the second stage, this binary sequence is scanned for the longest region of consecutive 1's and the results determine whether the run is inordinately long or simply a random pattern (33).

Gene Expression Analysis. We compared the relative transcriptional levels of genes between patients and control subjects using independent *t* test and among the different genotypes using one-way analysis of variance and least significant difference method for post hoc comparison.

Results

First Stage STRP Fine Mapping

Based on the results of the previous linkage analysis (18), we used GENEFINDER to identify the linkage region with a 95% confidence interval from D10S1432 (93.97 cM in deCODE map; Chr10: 74,329,402) to D10S1239 (121.81 cM; Chr10:96,935,067) (Figure 1). Then, we genotyped 18 additional STRPs, with an average intermarker distance of 1.2 cM, for the 476 families. Combining the genotyping data of the six STRPs within this region in the original study (18), we found a maximal nonparametric linkage (NPL) score of 2.79 ($p = .002$) for marker D10S195 (94.72 cM). The detailed positions and NPL scores of all 24 markers are plotted in Figure 1 and listed in Table S1 in Supplement 1.

Family Clustering

After clustering, we derived the four largest clusters from the dendrogram by splitting tree nodes recursively. We named the four patient clusters after their neuropsychological performances, that is, attention deficit and execution deficit (ADED) ($n = 260$); attention deficit and execution nondeficit ($n = 199$); attention nondeficit and execution deficit ($n = 170$); and attention nondeficit and execution nondeficit ($n = 188$). These four clusters were clearly differentiated by the four neuropsychological indicators (Figure S1 in Supplement 1).

For further family clustering, we categorized each family into a cluster if all affected siblings of the family belonged to the same patient cluster. Table 2 provides details of the demographic data, symptom profiles, and neuropsychological performance indicators of the affected siblings of the four family clusters. The four family clusters were clearly differentiated by the four neuropsychological indicators.

Next, we performed linkage analysis for the four family clusters,

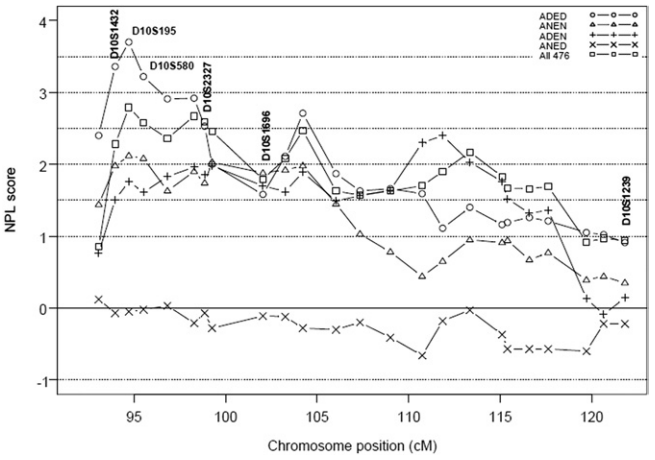

Figure 1. Nonparametric linkage plots of the 24 short tandem repeat polymorphism markers in the first stage of the fine-mapping study of all families and the four neurocognitive subgroups. All families ($n = 476$); families with attention deficit and execution deficit ($n = 90$); families with attention deficit and execution nondeficit ($n = 72$); families with attention nondeficit and execution deficit ($n = 53$); families with attention nondeficit and execution nondeficit ($n = 53$). ADED, families with attention deficit and execution deficit; ADEN, families with attention deficit and execution nondeficit; ANED, families with attention nondeficit and execution deficit; ANEN, families with attention nondeficit and execution nondeficit; NPL, nonparametric linkage.

respectively. The results showed that the maximal NPL score was 3.70 ($p = .00008$) in the ADED family cluster on marker D10S195. For the other three family clusters, the NPL scores for D10S195 were lower than that in the entire sample (Figure 1).

To avoid the random chance of selecting out small groups from a larger group, we performed a permutation test by random sampling of 90 families as the number of ADED family cluster and calculated the NPL scores of the random samples. We found the empirical *p* value of the NPL score of 3.70 on D10S195; after repeating 10,000 times sampling was significant ($p = .0023$).

Second Stage SNP Fine-Mapping Results

Next, we selected 79 haplotype tag SNPs from the HapMap (http://hapmap.ncbi.nlm.nih.gov/) (Chinese + Japanese) with the criteria of minor allele frequency above .1 and r^2 above .8. The 79 haplotype tag SNPs are located between D10S1432 (93.97 cM) and D10S580 (95.52 cM) (Figure 1), which were the flanking markers of D10S195, with NPL scores of 3.36 and 3.22 in the ADED family cluster, respectively. The average intermarker distance of the SNPs was 43 kilobase (kb). Then, we genotyped the 79 haplotype tag SNPs for the 90 ADED families.

All SNPs except two were compatible with Hardy-Weinberg equilibrium. Single point association analysis indicated that there were significant transmission distortions of nine SNPs (*p* value = .004 − .04), based on either FBAT or TRANSMIT. Of the nine SNPs, four (rs4492736, rs7087762, rs12644, and rs4746136) were adjacent to each other among the selected tag SNPs. Table 3 shows detailed results of single point association analysis of the nine significant SNPs and Figure 2 shows *p* values of the 79 tag SNPs.

There was no statistical significance of the nine SNPs after correcting for multiple comparisons using the FDR method (32). However, the single point association analysis (Figure 2) found a region in which the *p* values of the SNPs were either significant or borderline-significant. We employed the LSR method (33) to estimate a region-specific *p* value, thus providing an index of the most likely candidate genomic region. Based on a significance level of .05, all 79

Table 2. Demographic Data, Symptom Profiles, and Neuropsychological Performance Indicators of the Affected Siblings of Four Family Clusters

	ADED ($n = 90$) Mean (SD)	ADEN ($n = 72$) Mean (SD)	ANED ($n = 53$) Mean (SD)	ANEN ($n = 53$) Mean (SD)	Post Hoc Comparisons[a]
Age (years)[b]	34.8 (7.5)	37.2 (8.8)	33.7 (6.9)	33.1 (8.5)	2 > 3, 4
Gender (male/female)[c]	130/58	87/59	60/49	75/31	—
Education (years)[b]	10.0 (2.6)	8.1 (3.2)	12.1 (2.4)	10.5 (2.5)	3 > 1, 4 > 2
Age at Onset (years)	22.8 (6.1)	22.1 (6.8)	23.0 (5.7)	22.4 (6.2)	—
Mean Score of Negative Symptoms[b,d]	2.24 (.99)	2.61 (1.08)	1.92 (1.02)	1.90 (1.09)	2 > 1 > 3, 4
Mean Score of Positive Symptoms[d]	1.80 (1.02)	1.73 (.97)	1.62 (1.02)	1.56 (1.11)	—
Mean Score of Disorganizing Symptoms[b,d]	1.65 (.99)	1.93 (1.10)	1.47 (.96)	1.29 (.95)	2 > 3, 4; 1 > 4
Zd′[b]	−3.16 (1.87)	−3.60 (1.94)	−1.80 (1.75)	−1.07 (1.60)	3, 4 > 1, 2
Zmd′[b]	−3.42 (1.07)	−3.57 (.93)	−1.56 (1.40)	−1.08 (1.29)	3, 4 > 1, 2
zPE[b]	3.35 (1.67)	.31 (1.33)	1.61 (1.64)	.45 (1.16)	1 > 3 > 2, 4
zCAT[b]	−3.51 (.78)	−2.51 (.94)	−3.99 (.73)	−2.70 (.81)	2, 4 > 1 > 3

ADED, families with attention deficit and execution deficit; ADEN, families with attention deficit and execution nondeficit; ANED, families with attention nondeficit and execution deficit; ANEN, families with attention nondeficit and execution nondeficit; ANOVA, analysis of variance; CPT, Continuous Performance Test; zCAT, adjusted *Z* score of category achieved; Zd′, adjusted *Z* score of d′ of undegraded CPT; Zmd′, adjusted *Z* score of d′ of degraded CPT; zPE, adjusted *Z* score of perseverative error.

[a]Post hoc comparison by the Scheffe method. Group 1: ADED; Group 2: ADEN; Group 3: ANED; Group 4: ANEN.

[b]$p < .001$, by one-way ANOVA.

[c]$p < .05$, by chi-square test.

[d]Clinical symptoms were rated using the Schedule for Assessment of Negative Symptoms and the Schedule for Assessment of Positive Symptoms. Mean score of negative symptoms was the mean of all global scores of four negative symptom dimensions, including affective blunting, alogia, avolition-apathy, and anhedonia-asociality. Mean score of positive symptoms was the mean of the global scores of hallucination and delusion. Mean score of disorganizing symptoms was the mean of bizarre behavior and positive formal thought disorder.

p values from the FBAT were divided into 0 or 1, with *p* values lower than .05 classified as 1 and the others as 0. Then, all *0*'s and *1*'s were arranged sequentially according to the relative position of the 79 SNPs. We performed the LSR procedure and found the *p* value was significant for the region spanning four SNPs, rs4492736, rs7087762, rs12644, and rs4746136 ($p = .0048$).

Figure 2 shows the haploytpe structure of the 79 SNPs predicted by Haploview. There were 15 haplotype blocks in this region. The region with significant region-specific *p* values using LSR was located within block 2, which contained six SNPs. If more stringent criteria ($r^2 > .8$) were used for haplotype block formation, then block 2 contained three SNPs, rs7087762, rs12644, and rs4746136. We performed haplotype association tests for this block and found that the C-C-A haplotype of rs7087762-rs12644-rs4746136 was significantly overtransmitted to affected individuals ($p = .01$) and the T-T-G haplotype was undertransmitted ($p = .009$) (Table 4). Haplotype association analyses of the other 14 blocks yielded no statistically significant associations.

The candidate region clarified by LSR method spans a 427-kb region that encompasses nine genes: NUDT13, ECD, *DNAJC9*, MRPS16, TTC18, *ANXA7*, *ZMYND17*, *PPP3CB*, and USP54.

To identify genes with the most significant association, we genotyped one tag SNP in each gene in the original ADED families. Table 5 lists the association results of the 10 SNPs in the nine genes. Most of the associations were either significant or borderline-significant, with FBAT-determined *p* values ranging from .0097 to .057. This analysis did not identify the most significant gene.

Gene Expression Study

Next, we compared mRNA expression of the nine genes in the significant candidate region in EBV-transformed lymphoblastic cells of 85 schizophrenic patients and 36 normal control subjects. There were no significant differences of cell passage between patients and control subjects (5.50 ± 3.22 vs. 6.24 ± 3.17, $t = -1.16$, $df = 119$, $p = .247$). The results showed that in patients, expression of *ANXA7*, *PPP3CB*, and *DNAJC9* was significantly lower and expression of *ZMYND17* was significantly higher compared with control subjects. The mRNA levels of the other five genes were not signifi-

Table 3. Nine SNPs with Statistically Significant Results in Single Point Association Analysis with FBAT and TRANSMIT

dbSNP ID	Chromosome Position[a]	Gene[a]	Allele Type[b]	Minor Allele Frequency	Transmit			FBAT			Overtransmitted Allele
					n	Chi	*p*	*n*	*Z*	*p*	
rs4745707	74389968	*PLA2G12B*	G/C	.37	89	4.08	.043	25	2.05	.041	G
rs4492736	74544268	*NUDT13*	T/C	.31	89	2.34	.126	26	2.05	.04	T
rs7087762	74570127	*ECD*	C/T	.41	89	4.14	.042	31	2.59	.01	C
rs12644	74867057	*PPP3CB*	C/T	.39	89	4.37	.037	34	2.51	.012	C
rs4746136	74971000	*USP54*	A/G	.43	88	3.13	.077	32	2.53	.012	A
rs7899542	76338723	Intergenic region	C/T	.19	90	8.32	.004	21	2.38	.017	C
rs2395129	76482747	*DUPD1*	G/A	.15	90	4.10	.043	24	−.68	.494	A
rs10762656	76515470	Intergenic region	T/G	.17	90	4.12	.042	22	−1.35	.179	G
rs12782308	76840691	*ZNF503*	G/C	.21	90	5.28	.022	24	−2.29	.022	C

dbSNP, Single Nucleotide Polymorphism Database; FBAT, family-based association testing; NCBI, National Center for Biotechnology Information; SNP, single nucleotide polymorphism.

[a]Chromosome position and gene were determined from NCBI Reference Sequence Build 36.

[b]Second allele under oblique line (/) is the minor allele.

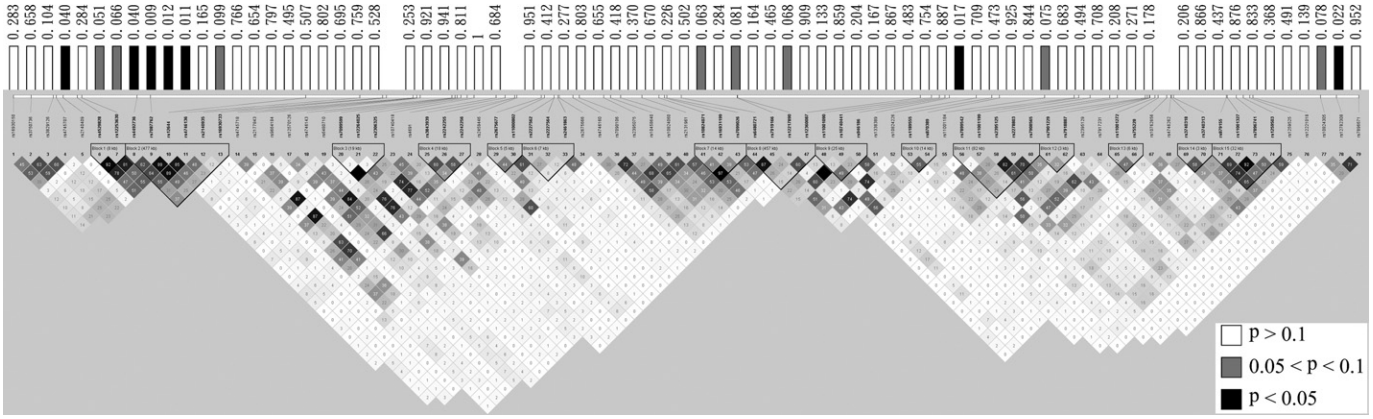

Figure 2. The *p* values and haplotype structures of the 79 haplotype tag single nucleotide polymorphisms (SNPs) between D10S1432 and D10S580 (analysis with family-based association testing and Haploview, respectively). The gray-scaled bar above each SNP represents the *p* value of that SNP; a black bar represents $p < .05$, a gray bar $.05 < p < .1$; and a white bar $p > .1$. The number inside each square represents the r^2 between a pair of SNPs.

cantly different between patients and control subjects (Figure 3). Then, we compared the expression of the four genes in patients carrying the risk allele of the tag SNP in each gene and those without and control subjects. The results showed that the level of *ANXA7* was significantly lower in patients carrying the C allele (risk allele) of rs12258241 (the tag SNP in *ANXA7*) than in those without this allele and control subjects. Expression of the other three genes was not significantly related to genotypes (Figure 3).

We compared the four neuropsychological indicators of patients with the risk haplotype (C-C-A) of rs7087762-rs12644-rs4746136 and those without this haplotype and found that patients with the risk haplotype had significantly poorer performance of d′ from the degraded CPT ($t = -2.01$, $p = .048$, df = 81) and categories achieved from the WCST ($t = -2.3$, $p = .025$, df = 68.54). There was a trend of poorer performance for patients with the risk haplotype on the d′ from the undegraded CPT and perseverative errors from the WCST, though not to significant levels.

Finally, we analyzed the correlation of the expression of the four genes with the four neuropsychological indicators among patients and found that the Pearson correlation coefficients for expression of *ANXA7* and perseverative errors and categories achieved of WCST were significant ($r = -.32, .26$; $p = .004$, $p = .021$, respectively, df = 79), as were those between the levels of *PPP3CB* and the same indicators of WCST ($r = -.33, .22$; $p = .004$, $p = .047$, respectively, df = 79). The correlations between expression of *DNAJC9* and *ZMYND17* and the four neuropsychological indicators were all nonsignificant, as were those between the levels of *ANXA7* and *PPP3CB* and the two CPT indicators.

Table 4. Haplotype Association Analysis of the Haplotype Block Consisting of rs7087762, rs12644, and rs4746136

	TRANSMIT ($n = 88$)			FBAT			
Haplotype	Haplotype Frequency	Chi	*p*	Haplotype Frequency	*n*	*Z*	*p*
CCA	.559	2.51	.11	.557	32	2.56	.01
TTG	.40	5.32	.02	.399	34	−2.59	.009
TCG	.021	.34	.56	.02	2	—	—
CCG	.015	.84	.36	.015	2	—	—
TCA	.003	1.02	.31	.006	0	—	—
CTG	.003	1.01	.32	.003	1	—	—

FBAT, family-based association testing.

Discussion

We found that there is a peak NPL score of 3.70 ($p = .00008$) on marker D10S195 in a subgroup of schizophrenic families of affected siblings who have concordant deficits in sustained attention and executive function (ADED). Fine mapping found a significant association between a 427-kb region that spanned four SNPs (rs4492736, rs7087762, rs12644, and rs4746136) and schizophrenia in ADED families. In this genomic region, mRNA expressions of *ANXA7*, *PPP3CB*, and *DNAJC9* in lymphocytes were significantly lower and that of *ZMYND17* was significantly higher in schizophrenic patients. Our research identified *ANXA7*, *PPP3CB*, *DNAJC9*, and *ZMYND17* as potential candidate genes for schizophrenia, especially in patients with deficits on sustained attention and executive function.

ANXA7 was originally described as a mediator of the aggregation of chromaffin granules and the fusion of membranes and phospholipids (34). Calcium, guanosine-5′-triphosphate, and protein kinase C are proposed to interact with *ANXA7* in driving the process of membrane fusion, which is required during exocytosis (35). Interestingly, Lidow (36) proposed that calcium signaling was involved in the pathophysiology of schizophrenia. Heterozygous murine knockouts for *ANXA7* gene spend much less time in the center of the cage than wild-type littermates (37). *PPP3CB* is the beta-isoform of the catalytic subunit of calcineurin, which plays an important role in central nervous system functions, including neurite extension, synaptic plasticity, learning, and memory (38,39). Previous studies have shown that the gamma isoform of the catalytic subunit of calcineurin (PPP3CC) is associated with schizophrenia in several ethnic groups (40,41) and also in our sample of Taiwanese patients (42). Two researchers found no significant association between *PPP3CB* and schizophrenia, but these studies did not examine specific neurocognitive subgroups (43,44). In a postmortem brain study, Eastwood *et al.* (45) found that the mRNA levels of all three isoforms and proteins of the catalytic subunit of calcineurin were significantly lower in the hippocampus of schizophrenia. A forebrain-specific calcineurin knockout mouse has neurobehavioral phenotypes strikingly similar to those described for schizophrenia (46). Taken together, the biological function of *ANXA7* and *PPP3CB* suggest that the two genes are relevant to the pathophysiology of schizophrenia.

DNAJC9 is a novel human-type C DnaJ/HSP40 protein, which may protect cells against stress in a cell culture study (47). *ZMYND17* is a novel protein that contains a myeloid translocation protein 8,

Table 5. Single Point Association Analysis of the Ten SNPs in the Nine Genes in the Significant Candidate Region (Analysis with FBAT and TRANSMIT)

dbSNP ID	Chromosome Position[a]	Gene[a]	Allele Type[b]	Minor Allele Frequency	TRANSMIT			FBAT			Overtransmitted Allele
					n	Chi	*p*	*n*	*Z*	*p*	
rs4492736	74544268	*NUDT13*	T/C	.31	89	2.34	.13	26	2.05	.04	T
rs7087762	74570127	*ECD*	C/T	.41	89	4.14	.042	31	2.59	.0097	C
rs7100458	74673099	*DNAJC9*	C/T	.27	89	4.13	.042	24	2.51	.012	C
rs2018198	74679113	*MRPS16*	T/A	.26	89	4.01	.045	23	2.28	.023	T
rs4294502	74705263	*TTC18*	T/C	.26	89	4.24	.039	23	2.44	.015	T
rs12258241	74830263	*ANXA7*	C/A	.27	89	3.65	.056	25	2.21	.027	C
rs1332178	74859592	*ZMYND17*	C/T	.26	89	3.51	.061	24	2.36	.019	C
rs12644	74867057	*PPP3CB* (3′ UTR)	C/T	.37	89	4.37	.037	34	2.51	.012	C
rs16930627	74883522	*PPP3CB*	T/C	.22	81	4.19	.041	22	1.91	.057	T
rs4746136	74971000	*USP54*	A/G	.43	89	3.13	.077	32	2.53	.012	A

dbSNP, Single Nucleotide Polymorphism Database; FBAT, family-based association testing; NCBI, National Center for Biotechnology Information; SNP, single nucleotide polymorphism; UTR, untranslated region.

[a]The chromosome position and gene was determined based upon the NCBI Reference Sequence Build 36.

[b]Second allele under oblique line (/) is the minor allele.

Nervy, and DEAF1 (MYND)-type zinc finger domain, but its function is unknown.

We found that lower expression of *ANXA7* was associated with the risk genotypes and that expression of *ANXA7* and *PPP3CB* were positively correlated to the WCST performance. There were no similar results for the other two genes. Considering the results of our gene expression study and the known biological functions of these four genes, we suggest that *ANXA7* and *PPP3CB* are the most likely candidate genes.

There are still debates about whether WCST is a suitable endophenotype for genetic study. To date, only one research group (48) has reported significant heritability of WCST, while three studies reported no evidence of heritability (49–51). In a high-risk sample of offspring of schizophrenic parents, WCST did not distinguish between subjects developing psychosis and those remaining healthy (11). We tried to use CPT performance alone as the indicators for clustering but found that the linkage scores were not significantly higher when using CPT alone than using both CPT and WCST (Table S2 in Supplement 1). The significance of familial aggregation of ADED cluster is the highest among the four family clusters (Table S3 in Supplement 1). Therefore, we suggested using both CPT and WCST performance as clustering indicators.

The statistical significance of the individual markers in this study was low when tested by FDR (32). Hence, the association results of individual markers were negative. However, the LSR method showed that the special nonrandom pattern in the 427-kb candidate region was indicative of disease genes in this region. Conventional statistical corrections on locus-specific multiple tests usually have lower statistical power as the number of markers increases. A simulation study indicated that the LSR method had much better statistical power and false discovery rate than the Bonferroni method or the FDR method, while all methods provided a low rate

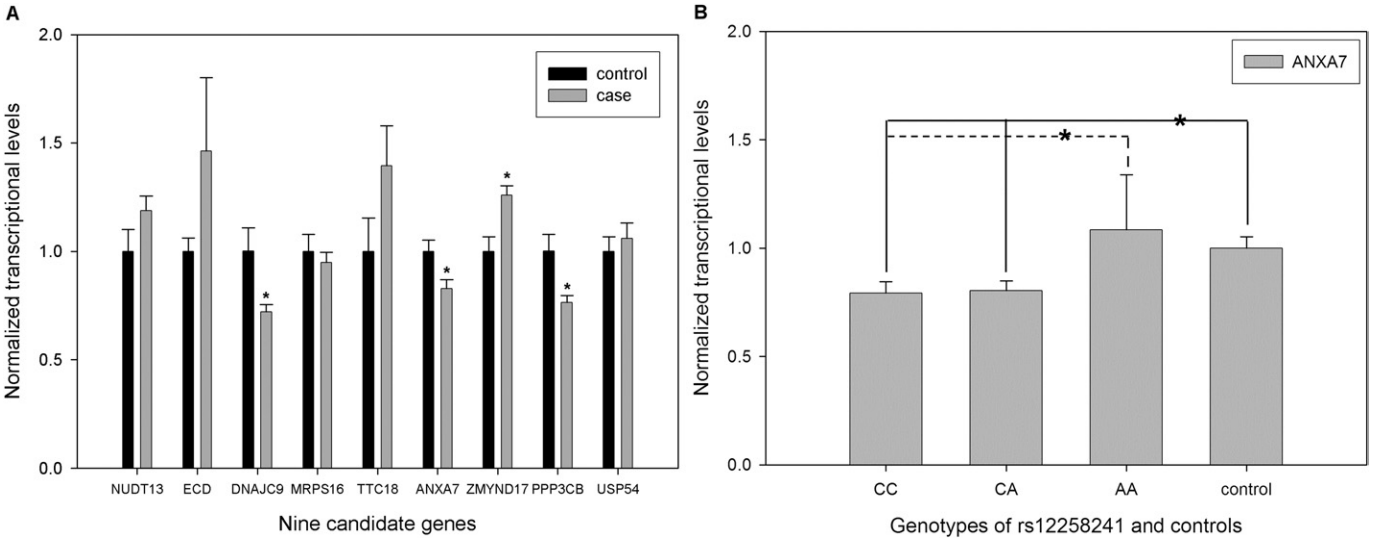

Figure 3. Gene expression study in the Epstein-Barr virus-transformed lymphoblastic cells of schizophrenic patients and control subjects. **(A)** Relative transcriptional levels of the nine candidate genes in the candidate genomic region (rs4492736 to rs4746136) in cases and control subjects. Data are normalized by the means of the control group and represented as mean ± SEM. *Significant difference ($p < .05$). The levels of *ANXA7* ($t = 2.37$, df = 119, $p = .019$), *PPP3CB* ($t = 3.34$, df = 119, $p = .001$), *DNAJC9* ($t = 2.45$, df = 42.16, $p = .019$), and *ZMYND17* ($t = 3.32$, df = 117, $p = .001$) are significantly different in cases and control subjects. **(B)** Comparison of the levels of *ANXA7* transcriptional activity revealed significant differences among patients with different genotypes and control subjects [$F(3,117) = 3.667$, $p = .014$, using one-way analysis of variance]. Patients with the CC ($n = 37$) or CA ($n = 39$) genotype have significantly lower transcriptional levels than patients with the AA genotype ($n = 9$) and control subject subjects ($n = 36$) using least significant difference method for post hoc comparison. *Significant difference ($p < .05$).

of false positives (33). The LSR method avoids the controversial multiple-test adjustment required for locus-specific association tests. Thus, this method has potential applications in exploratory data analysis and can be used to screen the most likely regions for further biological examination. Without further biological studies, the LSR method clarified only the most likely candidate region for disease genes but not the most potential candidate genes. It is different from conventional locus-specific association tests, which clarified the markers with significance working through corrections for multiple tests, indicating the most potential candidate genes. Following this concept, we used gene expression studies to identify four genes as potential candidate genes.

In this study, we only fine-mapped the genomic region with the highest potential for positive genes from D10S1432 to D10S580 (93.97 cM–95.52 cM; Chr10:74,329,402–77,728,892). Based on the results of GENEFINDER, the 95% confidence interval of the first-stage STRP genotyping results spanned around 10 cM, ranging from D10S1432 to D10S1696 (93.97 cM–102.04 cM; Chr10:74,329,402–83,221,057) (Figure 1). Therefore, we cannot rule out the possibility that the region from D10S580 to D10S1696 harbors other candidate genes for a subgroup of schizophrenia with neurocognitive deficits.

The fine-mapping study on chromosome 10q22-23 in Ashkenazi Jews recently found strong evidence of association using the delusion factor as the quantitative trait at three SNPs of NRG3 (52). We did not genotype the SNPs of NRG3 (Chr10: 83,625,077–84,735,341) in this study, because the gene is located out of the 95% confidence interval of the first-stage STRP genotyping results. The candidate genes we identified are also located out of the fine-mapping range of that study (Chr10:79,550,189–92,037,551) (52). The CPT performances in schizophrenia were reported associated with the negative and disorganizing symptom dimension but not associated with delusion/hallucination (53,54). Therefore, there might be two distinct loci for schizophrenia on chromosome 10q22-23, the proximal one, as identified in this study, specifically for the neurocognitive deficits, which may relate to the negative and disorganizing symptom dimension, and the distal one, as identified by Chen *et al.* (52), specifically for the delusion factor.

We studied gene expression in an independent sample, not in the original neurocognitive deficient subgroup used in the genetic association study. The findings of the expression study seemed to be present in the group of schizophrenia as a whole, not specific to the neurocognitive subgroup. However, we found that patients with the risk haplotype had poorer performance on cognitive tests and a positive correlation between expression of *ANXA7* and *PPP3CB* and the performance on the WCST. This suggests that the results of the gene expression study may apply to the same subgroup identified in the genetic association study.

We measured expression of the nine genes in peripheral cells because it is not possible to directly assess the impact of genetic variants in live human brain tissue. Previous research has shown that critical pathways in schizophrenia may be studied in peripheral tissue as part of a strategy of functional genomic convergence (55). This lends support for our use of EBV-transformed lymphocytes as a peripheral marker of schizophrenia. Lestou *et al.* (56) found that EBV integrated into the human genome of lymphoblastic cell lines in a nonrandom manner, most occurring at 1p31, 1q43, 2p22, 3q28, 4q13, 5p14, 5q12, and 11p15. This is far from 10q22, so host genes on 10q22 were unlikely to be affected by the integration of viral genes. A postmortem brain study indicated that expression of *PPP3CB* is lower in the hippocampus of schizophrenic patients (45). This is consistent with our study.

We need to interpret the results with caution for several reasons. First, we used two different analytic approaches (FBAT and TRANSMIT), but only the results of FBAT are apparently positive with LSR. Second, the SNPs bringing about significant LSR results are in high linkage disequilibrium (LD). Thus, the positive results of LSR could very well be the result of a false-positive SNP signal together with the SNPs in LD with it, though it can also be explained by the modest or minor effect of a disease allele with a few adjacent loci that are in LD, which is often observed in the identification of candidate regions of complex disorders. Third, in the expression study, with 10 SNPs and their combinations in haplotypes, multiple genes with expression data, and four neuropsychological indicators, the correlation matrix is quite large and the true significance of the reported correlations is not clear.

Based on the results of our genomic study of a specific neurocognitive subgroup of schizophrenia, we suggest that the pathophysiology of schizophrenia may be related to the reduced expression of *ANXA7*, *PPP3CB*, and *DNAJC9* and increased expression of *ZMYND17*, which suggest that the functional variants may be located in the regulatory elements of these genes.

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Supplementary material cited in this article is available online.

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