

ANK3 as a Risk Gene for Schizophrenia: New Data in Han Chinese and Meta Analysis

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Histological and neuroimaging evidence supports the hypothesis that neuronal disconnectivity may be involved in the pathogenesis of schizophrenia. A genome-wide association study (GWAS) showed a single nucleotide polymorphism (SNP), rs10761482 in ankyrin 3 (*ANK3*), a major neuron-enriched gene, was associated with schizophrenia although inconsistent results had been reported. Two meta analyses reported another SNP rs10994336 in *ANK3* gene confers risk to bipolar disorder (BD). Due to evidence of genetic overlap between schizophrenia and BD, we investigated common findings by analyzing the association of *ANK3* polymorphisms (rs10761482, rs10994336, and two missenses, rs3808942 and rs3808943) with schizophrenia, using the Han Chinese population. A total of 516 schizophrenia cases, 400 controls, and 81 trios of early onset schizophrenia were recruited for association studies. Furthermore, the published datasets were combined with our results to determine the effect of the loci on schizophrenia. Our association study showed the frequencies of C allele of rs10761482 and T allele of rs10994336 were higher in patients than in controls. Furthermore, allele condition analyses indicated the association signal observed at rs10761482 and rs10994336 was independent. A haplotype analysis revealed the rs10761482–rs3808942–rs3808943 haplotype was associated with schizophrenia. The frequency of the T–T–T haplotype was higher in patients than in controls. In the transmission disequilibrium test analysis, the C allele of rs10761482 and the rs10761482–rs3808942–rs3808943 haplotype were preferentially transmitted in the trios. Meta analysis incorporating previous and current studies also showed rs10761482 and rs10994336 were associated with schizophrenia. We conclude that *ANK3* gene has a major influence on susceptibility to schizophrenia across populations.

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Key words: case–control; trios of early-onset schizophrenia; association; SNP

INTRODUCTION

Schizophrenia is a devastating mental disorder which affects approximately 1% of the general population worldwide. Evidence

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from family, adoption, and twins studies supported high heritability in the development of schizophrenia (~80%). However, its exact etiology and genetic mechanisms are still unknown. One of the possible reasons for this situation is that until now association studies have mostly focused on a small group of candidate genes which are mainly involved in monoaminergic neurotransmission [Li et al., 2012; Nothdurfter et al., 2012]. But the etiology of this disorder might lie elsewhere. One plausible pathway could be genes involved in brain connectivity.

Functional abnormalities of neuronal connectivity have been reported in patients with schizophrenia [Yu et al., 2011]. Histological and neuroimaging studies have led to the hypothesis that dysfunctional myelination and neurons may be involved in the pathogenesis of schizophrenia. Magnetic transfer imaging, which is considered to be a useful technique to measure myelin in vivo,

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demonstrated decreased myelin or axonal membrane integrity in the temporal lobes of patients with schizophrenia [Foong et al., 2001]. In addition, ultrastructural alterations of myelin sheath lamellae have been described in the frontal cortex in schizophrenia subjects, and the loss of a correlation between neuron density and axon number have been found in the corpus callosum in schizophrenia [Bernstein et al., 2009; Simper et al., 2011]. Therefore, it implies that there may be pathological damage of brain connectivity in schizophrenia.

Gene expression analyses using DNA micro-array also supports the above hypothesis. Decreased expression of myelin- and neuron-related genes in schizophrenic patients has been identified [Tkachev et al., 2003; Cruz et al., 2009]. Among these genes, the expression of *ANK3*, a major neuron-enriched gene involved in the anchoring of voltage-gated sodium ion channels to the Nodes of Ranvier and maintenance of the junction between the axolemma and myelin loops [Poliak and Peles, 2003; Konrad and Winterer, 2008], was significantly decreased in the brains of patients with schizophrenia. It is possible that genetic variations affecting the expression of *ANK3* gene may also contribute to the susceptibility of individuals to schizophrenia. Recently, a GWAS analysis and a replication study have shown the association between one genetic variant, rs10761482 at *ANK3* locus and schizophrenia in Norwegian samples [Athanasou et al., 2010]. However, another association study conducted using German samples failed to confirm a genetic association between the rs10761482 and schizophrenia [Gella et al., 2011]. Interestingly, two recent meta analyses have reported that another variant, rs10994336 at *ANK3* locus, confers risk to bipolar disorder in US and UK samples [Ferreira et al., 2008; Schulze et al., 2009]. Due to evidence of genetic overlap between schizophrenia and bipolar disorder [Moskvina et al., 2009; Williams et al., 2011], two associations between rs10994336 and schizophrenia were later conducted in two independent cohorts of German and Nordic samples respectively [Gella et al., 2011; Tesli et al., 2011]. However, in the two studies, rs10994336 was found not to be associated with schizophrenia. Given the above controversial results on the association of *ANK3* gene with schizophrenia, its contribution to the etiology of the disorder requires further clarification.

Therefore, in order to further examine the association of *ANK3* with schizophrenia and to enhance the potential power for detecting the association, we conducted a case-control study followed by a family study, using schizophrenia samples from the Han Chinese population. In addition, we performed a meta analysis of the SNPs in the gene examined in the published studies and the current studies.

MATERIALS AND METHODS

Subjects

The case-control samples included 516 schizophrenia cases (351 males, 165 females; mean age = 41.6 ± 15.1 years) and 400 healthy controls (224 males, 176 females; mean age = 31.5 ± 8.5 years). The family samples consisted of 81 early-onset schizophrenia (EOS) probands and their biological parents. Age at the onset of schizophrenia was defined as the age when positive symptoms (either delusions or hallucinations), first became apparent based on inter-

view and supplemental clinical information obtained from medical records and family informants [Frangou et al., 2008]. EOS was defined as schizophrenia with onset before the age of 18 according to the previous literature [Kumra and Charles Schulz, 2008]. In the family sample, probands were composed of 43 males and 38 females (mean age = 19.1 ± 4.5 years; mean age of first episode = 14.1 ± 3.1 years, range from 6 to 18 years). All patients were diagnosed by at least two experienced senior psychiatrists according to Diagnostic and Statistical Manual of Mental Disorders, the fourth version (DSM-IV) criteria for schizophrenia on the basis of unstructured interviews and information from medical records. If these patients were diagnosed as schizophrenia, the diagnoses must be rechecked and validated by a deputy chief physician who reviewed the psychiatric case records and interviewed with patients within 6 months according to the Shanghai mental health ordinance approved by the 35th meeting of the 11th Shanghai Municipal People's Congress Standing Committee in 2001. Control subjects were recruited from hospital staff and volunteers who self-reported that they were free from severe physical diseases, as well as individual and family history of mental illness during the brief interviews by senior psychiatrists.

All subjects were of Han Chinese origin from the same geographical area. The present study was approved by the Ethics Committee of Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine and informed consent was obtained from all subjects.

SNPs Selection

In the Han Chinese samples we studied the following *ANK3* SNPs: rs10761482 (C/T) located in the first intron of *ANK3*; rs10994336 (C/T) located 30 kb upstream of *ANK3*; missense rs3808942 (C/T) and missense rs3808943 (C/T), both about 1 kb distal from the promoter region of *ANK3*. Locations are as shown in the Table I and location assignment is based on RefSeq NM_020987 of the UCSC Genome Browser (<http://genome.ucsc.edu>).

Genotyping

Genomic DNA was extracted directly from peripheral blood using Tiangen DNA isolation kits (Tiangen Biotech Inc., Beijing, China). All of the four SNPs were individually genotyped using a TaqMan SNP genotyping assay on an ABI PRISM 7900 sequence detection system instrument (Applied Biosystems, Foster City, CA). PCR was performed following the standard protocol with 5 μ l reaction volumes for each well in a 384-well plate and contained 5 ng of DNA. Thermal cycler conditions were 95°C for 10 min, 45 cycles of 92°C for 15 sec, and 60°C for 1 min. The SDS version 2.0 software (Applied Biosystems) was used for genotypic identification.

For quality control purposes, all genotypes were called blind to the case or control status in the genotyping process. One percent of the samples were repeated for the genotyping assay and the results were 100% concordant. Genotyping call rates ranged from 97% (controls) to 99% (cases) for rs3808942, rs3808943, and rs10761482, and from 98% (cases) to 99% (controls) for rs10994336. For family-based study, genotyping call rates ranged from 98% for rs3808942, rs3808943 and rs10761482, to 99% for rs10994336.

TABLE I. Genotype and Allele Distributions for SNPs in the ANK3 Gene Between Patients With Schizophrenia and Controls in a Chinese Population

SNP	SCZ						CON						MAF			OR (95% CI)		
	Position	M/M	M/m	m/m	M/M	M/m	M/m	M/m	M/M	M/m	m/m	SCZ	CON	FDR adjusted	P-value ^a		P-value ^b	FDR adjusted
rs10761482	1062085337	C/T	0.67	0.31	0.02	0.60	0.33	0.07	0.60	0.33	0.07	0.18	0.24	0.011	0.002	0.007	0.014	1.45 (1.15–1.82) ^c
rs10994336	1062179812	C/T	0.44	0.47	0.09	0.54	0.39	0.07	0.54	0.39	0.07	0.33	0.26	0.0008	0.001	0.0001	0.0004	1.40 (1.14–1.72) ^d
rs3808942	1062150848	T/C	0.54	0.40	0.06	0.59	0.34	0.07	0.59	0.34	0.07	0.26	0.23	0.360	0.153	0.563	0.563	1.17 (0.94–1.45) ^c
rs3808943	1062151015	C/T	0.62	0.32	0.06	0.56	0.35	0.09	0.56	0.35	0.09	0.22	0.26	0.440	0.039	0.324	0.432	1.26 (1.01–1.56) ^c

SCZ, patients with schizophrenia; CON, healthy controls; M, major allele; m, minor allele; FDR, false discovery rate; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.

^aP-values are not corrected for age, sex, and multiple test.

^bP-values are adjusted for age and sex using Plink.

^cTo ease comparisons, the OR (95% CI) is calculated with respect to C-allele and T-allele, respectively.

^dTo ease comparisons, the OR (95% CI) is calculated with respect to C-allele and T-allele, respectively.

Statistical Analysis

The statistic power of our sample size was calculated by the G*Power program based on Cohen's method [Faul et al., 2007]. Our sample size had >80% power in the case–control-based samples to detect a significant ($\alpha < 0.05$) association for genotypes, alleles and haplotypes when an effect size index of 0.1 (corresponding to a “weak” gene effect) was used.

Deviation of the genotype counts from the Hardy–Weinberg equilibrium (HWE) was tested using online software SHEsis (<http://analysis.bio-x.cn>). No deviations from HWE were detected for the four SNPs (all $P > 0.05$) both in case and control groups. Genotype and allele frequencies were estimated in case–control samples and family samples using UNPHASED (v.3.10) program (<http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphase>) [Dudbridge, 2008]. The adjustment for age and gender factors in the case–control samples was executed using PLINK (v.1.03) (<http://pngu.mgh.harvard.edu/~purcell/plink/>). A false discovery rate (FDR) controlling procedure was carried out to control false positive results [Benjamini et al., 2001].

The pairwise linkage disequilibrium (LD) analysis was applied to detect the inter-marker relationship in case–control and parents subjects, using D' values. The LD blocks were identified using the solid spine of LD method, with extended spine if $D' > 0.5$ in Haploview (version 4.1) [Barrett et al., 2005]. Haplotypic association analysis was performed for common haplotypes (frequency ≥ 0.01), and individual and global haplotypic P -values were calculated in UNPHASED program. To further explore the individual contribution of each marker to schizophrenia susceptibility in our case–control samples, we tested for independent effects of each SNP and potential interaction between them with PLINK. Independence of the two SNPs was tested using the—logistic and—condition function. We tested the effect of rs10761482 while adding the allelic dosage for rs10994336 as a covariate, and vice versa. Interaction between rs10761482 and rs10994336 was tested using the interaction function, which is identical to the interaction term in a standard logistic regression analysis.

Meta Analysis

Studies included in the meta analysis were identified using MEDLINE database using the following keywords “ANK3” and “Schizophrenia” in combination with “polymorphism,” “genetics,” or “association.” All the data analyzed was previously published. To conduct case–control and TDT meta-analyses of independent samples, the extended method of DerSimonian and Laird statistics on random effects model was applied when there was heterogeneity of the genetic effects, while fixed-effect estimates described by Kazeem and Farrall were applied when there was no heterogeneity [DerSimonian and Laird, 1986; Kazeem and Farrall, 2005]. The Cochran's chi-squared-based Q statistical test was performed to assess possible heterogeneity between the individual studies, and heterogeneity was considered significant when $P < 0.1$ for the Q statistic. All P -values were two-tailed with a significance level of 0.05. All statistical meta analyses were performed using the Case–control And TDT Meta-Analysis Package (catmap) in the R-project program (<http://www.r-project.org>) [Nicodemus, 2008].

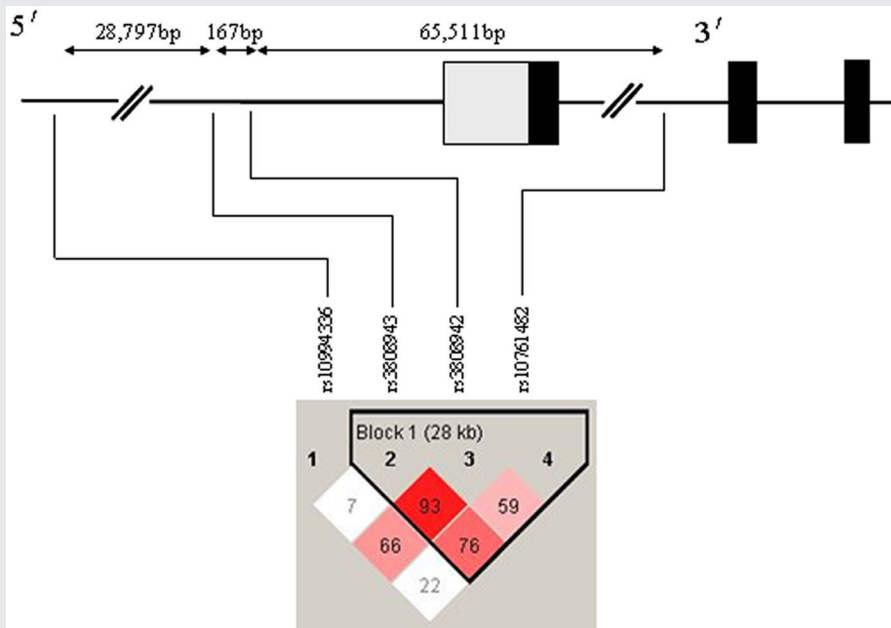


FIG. 1. Linkage disequilibrium measures (D') across the ANK3 gene. From left to right, the SNPs are aligned from 5' to 3'. Black boxes represent exons and gray box represents 5-untranslated region. LD patterns and haplotype blocks were defined by the "solid spine of LD" using the Haploview software. A standard color scheme is used to display LD pattern, with dark red for very strong LD, white for no LD, and shades of red for intermediate LD. Increasing intensity of red indicates increasing degrees of LD.

RESULTS

Case–Control Study

None of the genotype distributions in the patients and control population deviated from Hardy–Weinberg equilibrium. Table I shows the allele and genotype frequencies for rs10761482, rs10994336, rs3808942, and rs3808943 among the 516 patients and 400 controls. The SNP rs10761482 showed significant association with schizophrenia in our sample ($P=0.0057$; $P=0.011$, after FDR correction). The frequency of C allele of rs10761482 was significantly higher in patients than that in controls (OR = 1.45, 95% CI: 1.15–1.82, $P=0.007$; $P=0.014$, after FDR correction). The SNP rs10994336 was also found to be significantly associated with schizophrenia ($P=0.0002$; $P=0.0008$, after FDR correction). The T allele

of rs10994336 was more frequent in patients than that in controls and remained reliably frequent even after correction for multiple testing was applied (OR = 1.40, 95% CI: 1.14–1.72, $P=0.0001$; $P=0.0004$, after FDR correction). The SNPs rs3808942 and rs3808943 did not differ significantly in both genotype and allele distributions.

To perform haplotype-based association analyses, we examined LD structure within the genotype data of case–control and parents subjects and identified one haplotype-block. Block 1 was 28 kb long and consisted of three SNPs (rs10761482, rs3808942, and rs3808943) as shown in Figure 1. Further haplotype analysis revealed a significant association between the T–T–T haplotype and schizophrenia ($P=0.002$) and a trend of association between the C–C–C haplotype and schizophrenia ($P=0.067$). The global P -value was 0.038 (Table II).

TABLE II. Differences in the rs10761482–rs3808942–rs3808943 Haplotype Between Patients With Schizophrenia and Controls

Haplotype	Frequency		Individual P -value	OR (95% CI)	Global P -value
	Patients	Controls			
rs10761482–rs3808942–rs3808943					
C–C–C	0.26	0.22	0.067	1.00	0.038
C–T–C	0.51	0.50	0.839	0.86 [0.68–1.09]	
C–T–T	0.05	0.04	0.105	1.24 [0.74–2.09]	
T–T–T	0.17	0.22	0.002	0.63 [0.47–0.85]	

OR, odds ratio; CI, confidence interval.

TABLE III. Transmission Disequilibrium Test for SNPs in the ANK3 Gene and the rs10761482–rs3808942–rs3808943 Haplotype

SNP ID	Risk Allele	T	NT	P-value	FDR adjusted	Haplotype rs10761482–rs3808942–rs3808943				OR (95% CI)	Global P-value
						OR (95% CI)	N	NT	Individual P-value		
rs10761482	C	0.79	0.68	0.023	0.046	1.78 (1.07–2.97)	0.07	0.01	0.013	1	0.015
rs10994336	T	0.29	0.19	0.021	0.084	1.89 (1.09–3.30)	0.53	0.47	1.354	0.42 (0.08–2.16)	
rs3808942	C	0.22	0.19	0.605	0.807	1.14 (0.69–1.89)	0.18	0.20	0.743	0.30 (0.05–1.74)	
rs3808943	C	0.75	0.73	0.696	0.696	1.11 (0.66–1.85)	0.07	0.06	0.794	0.38 (0.05–2.69)	

OR, odds ratio; FDR, false discovery rate; CI, confidence interval.

Family-Based Association

Genotype distributions of the SNPs conformed to Hardy–Weinberg equilibrium in the parents and no Mendelian inheritance error was found. Allele and haplotype frequencies analysis are shown in Table III. Both the C allele of rs10761482 and the T allele of rs10994336 were found over-transmitted from heterozygous parents to affected offspring ($P = 0.023$ and $P = 0.021$, respectively). However, only the significant association of rs10761482 with schizophrenia survived the multiple test ($P = 0.046$, after FDR correction). The lack of association in family-based analysis might be due to the modest sample size of family trios.

There were four different haplotypes (frequencies ≥ 0.01) in block 1: CCC, CTC, CTT, and TTT. Haplotype analysis showed haplotype C–C–C was significantly associated with schizophrenia ($P = 0.013$). The global P -value was 0.015 (Table III).

Dissecting the Relationship Between rs10761482 and rs10994336

There was no detectable LD between rs10761482 and rs10994336. The r^2 value was 0.009 and D' value was 0.281 in the case–control samples. Each SNP was a significant and independent predictor of affection status ($t = 2.285$, $P = 0.02$ for rs10761482, conditioned on rs10994336; $t = 3.217$, $P = 0.001$ for rs10994336, conditioned on rs10761482). No evidence of interaction was detected between rs10761482 and rs10994336 ($P = 0.91$).

Meta-Analysis

For rs10761482, we combined and analyzed the data from two previous and current studies, including a total of 1,637 cases, 1,187 controls, and an additional 81 trios. The statistical summary of the meta analysis for rs10761482 was shown in Table IV and Figure 2. Heterogeneity was found to be present among the four studies ($\chi^2 = 10.79$, $P_{\text{heterogeneity}} = 0.01$), thus, the random-effects model was employed. Pooled OR and 95% CI values were calculated for the rs10761482 variant “C” allele versus wild-type “T” allele. Significant association was found between the “C” allele and schizophrenia risk (pooled OR = 1.29, 95% CI = 1.03–1.62, $P = 0.029$). For rs10994336, the meta analysis covering five studies including a total of 3,119 cases, 15,204 controls, and an additional 81 trios. Figure 2 shows the combined results of case–control and TDT studies of rs10994336 “T” allele in association with schizophrenia. No evidence of heterogeneity was found among studies ($\chi^2 = 5.98$, $P_{\text{heterogeneity}} = 0.20$). Using the fixed-effects model, “T” allele was found to be significantly associated with schizophrenia (pooled OR = 1.19, 95% CI = 1.06–1.33, $P = 0.002$).

DISCUSSION

Confirmation of findings from a GWAS, in a well-characterized population, is an important and necessary step towards mapping susceptibility loci for schizophrenia. In the present study, we firstly investigated the association of ANK3 polymorphisms and schizophrenia in 516 cases and 400 healthy control subjects of the Han Chinese population. We detected four SNPs of ANK3 gene in our

TABLE IV. Meta Analysis of SNPs rs10761482 and rs10994336 in the ANK3 Gene and Schizophrenia

SNP ID	Refs.	Allele counts				TDT		Random-effect		Fixed-effect		$P_{\text{heterogeneity}}$					
		Minor/ major allele	SCZ	CON	OR	P -value ^c	T	NT	OR	P -value	OR		P -value				
														SCZ	CON	OR	P -value
rs10761482	Gella et al. [2011]	T/C	450	1,390	238	722	1.02 ^a	0.079									
		T/C	974	4,754	7,326	20,848	1.72 ^a	5.115									
	Present study [2012]	T/C	181	839	190	608	1.45 ^a	2.155	34	128	52	110	1.78 ^a	0.024	0.029	0.013	
		T/C	110	1,730	62	898	1.09 ^a	0.079					1.29	0.029	(1.03–1.62)		
rs10994336	Gella et al. [2011]	T/C	150	1,958	394	5,396	1.05 ^b	0.194									
		T/C	70	1,188	1,142	2,716	1.08 ^b	0.252									
	Present study [2012]	T/C	342	688	203	573	1.40 ^b	4.000	47	113	30	130	1.90 ^b	0.021	1.19	0.002	0.201
		T/C													(1.06–1.33)		

SCZ, patients with schizophrenia; CON, healthy controls; OR, odds ratio; CI, confidence interval; $P_{\text{heterogeneity}}$, P -value for heterogeneity.

^aTo ease comparisons, the OR (95% CI) is calculated with respect to C-allele and T-allele, respectively.

^bTo ease comparisons, the OR (95% CI) is calculated with respect to C-allele and T-allele, respectively.

^c P -values are calculated with $-\log_{10} P$ -values.

samples. Significant differences were found in allele and genotype frequencies between patients and controls at rs10761482 and rs10994336. Given that false positive association may arise from case-control study, because of the confounding influence of population stratification [Shi et al., 2004; Fang et al., 2008], and that schizophrenia patients with early-age onset tend to have a greater genetic loading [Kumra and Charles Schulz, 2008], family-based study using EOS subjects was conducted as an effective approach to follow up the findings from the case-control study. In our family samples, the C allele of rs10761482 was preferentially transmitted, rather than non-transmitted, from heterozygous parents to probands, which supported the results from our case-control study. In addition, the meta analysis covering the published and current studies also showed that the C allele rs10761482 and T allele of rs10994336 were significantly associated with schizophrenia.

The original reports of Athanasiu et al. [2010] found rs10761482 [C] in ANK3 to be associated with schizophrenia in Norwegian samples ($P = 7.68 \times 10^{-6}$, OR = 1.72). In the Han Chinese population, we detected the same C allele was associated with schizophrenia in the case-control study ($P = 0.028$, OR = 1.45) and in the family study ($P = 0.046$, OR = 1.78). The meta analysis also demonstrated significant pooled ORs for rs10761482 [C] in the combined samples from the previous and current studies ($P = 0.029$, OR = 1.29). For rs10994336 [T], we found a significant association with schizophrenia in case-control study ($P = 0.028$, OR = 1.45) and a trend of association with schizophrenia in the family study ($P = 0.084$, OR = 1.89). The previous study showed that rs10994336 [T] was not associated with schizophrenia ($P = 0.560$, OR = 1.08) [Tesli et al., 2011]. Thus, the distribution of allelic frequencies across groups and the directions of effect size for rs10761482 and rs10994336 in the previous reported and current studies was the same. However, the effect size varied among the studies, both the OR for rs10761482 C allele in our family study and the OR for rs10994336 T allele in our studies were greater than that in the reported association studies.

There may be several explanations for the discrepancy of effect size between the previous and current results. The first possibility relates to the different ethnicities of the subjects. Inconsistent effect size in association studies between different ethnic populations are partly due to the differences in the allele frequency of each population. According to the data bank of the dbSNP Short Genetic Variations (<http://www.ncbi.nlm.nih.gov/snp>), allele frequencies of rs10761482 and of rs10994336 are different between European and Chinese population (Supplementary Table I). Therefore, the differences in allele frequencies may, at least partly, explain the inconsistent effect sizes. The second possibility relates to the heterogeneity of schizophrenia. The previous and current studies did not record the clinical background of patients, such as diagnostic subtypes, family history, and other biological parameters. Difference in biological mechanisms may also contribute to these discrepancies. In addition, the allele ORs in our early onset schizophrenia family study (OR = 1.78 for rs10761482 and OR = 1.89 for rs10994336) are greater than that in our case-control study (OR = 1.45 for rs10761482 and OR = 1.40 for rs10994336). Genetic epidemiology data suggested that schizophrenic patients with early onset age (e.g., less than 18 years old) tended to have a greater genetic predisposition than their adult counterparts [Pulver et al.,

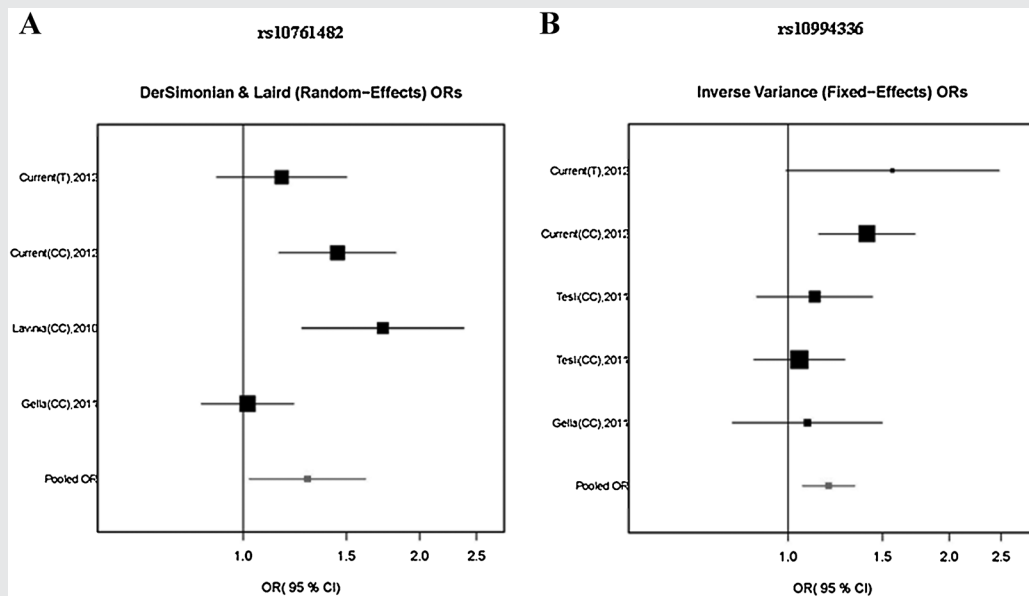


FIG. 2. Summary estimates (OR and 95% CI) for risk of schizophrenia associated with rs10761482 and rs10994336 variants. **A:** Schizophrenia risk for rs10761482 T > C variant: DerSimonian & Laird (random-effects) pooled OR = 1.29, 95% CI: 1.03–1.62, $P = 0.029$, $P_{\text{heterogeneity}} = 0.01$. **B:** Schizophrenia risk for rs10994336 T > C variant: Inverse variance (fixed-effects) pooled OR = 1.19, 95% CI: 1.06–1.33, $P = 0.002$, $P_{\text{heterogeneity}} = 0.20$. Current, current study; T, trio study; CC, case-control study; OR, odds ratio; CI, confidence interval.

1990; Sham et al., 1994] Therefore, our results may imply that greater genetic loading may relate to the greater effect size.

Interestingly, our data also showed that each of the two SNPs, rs10761482 and rs10994336 in *ANK3* gene, are an independent risk factor for schizophrenia. The two markers are located over 94 kb apart and there was little detectable LD between them. Furthermore, each marker was a significant, independent predictor in a logistic regression analysis that included the both markers. We could not detect any significant effect about marker \times marker interaction. Our findings may be due to true allelic heterogeneity, where more than one variant in the same locus confer risk to disease. Allelic heterogeneity is usually the rule for Mendelian disorders, while our data supported it was still true for a complicated disease such as schizophrenia.

In addition, in the study finished by Ferreira et al. [2008], the T allele at rs10994336 in *ANK3* was initially reported to confer increased risk for BD. Thus, our study has demonstrated an overlap in risk at the level of individual allele between schizophrenia and BD. This data did not explain the reason why, among all those carrying risk allele(s), some develop BD, others develop schizophrenia, and still others remained obviously well. This phenomenon may reflect a lot of risk alleles, few of which have been detected to date, environmental influences, and/or perhaps epigenetic factors.

Genetic variations in *ANK3* may interact with the formation and maintenance of myelinated axons and may lead to dysfunctional neuronal connectivity. These variations may be responsible for an individual patient's vulnerability for the development of schizophrenia. Nodes of Ranvier, major myelin sheath-free axon segments where ion (sodium and potassium) exchange can take place in

central nervous system [Zhou et al., 1998], provide action potentials from the axon initial segment down the length of the axon to synaptic terminals by invoking the ion channels distribution, and changes in *ANK3* activity may cause alterations in ion channel-triggered signal transduction and subsequent neuronal activity. Indeed, in an *ANK3* knockout mouse model, the generation and persistence of action potentials were dramatically inhibited [Zhou et al., 1998]. In addition, a recent study reported the novel role of *ANK3* gene in the maintenance of neuronal polarity represented by the establishment of axonal and somatodendritic domains [Hedstrom et al., 2008; Sobotzik et al., 2009]. The ankyrin-G-depleted axons would develop protrusions and the dysfunction of glutamate transmitters systems as has been described in schizophrenia brain [Bullock et al., 2008]. These potential connections indicate that further studies may examine the functions of *ANK3* and its interacting partners in the molecular, development, and pathophysiological processes in schizophrenia.

There were two limitations in the present study that should be noted. First, the most suspicious patients did not want to participate in the study. This is probably a real problem for many studies of schizophrenia. Second, our sample size was small compared with initial genome-wide association study samples. Total relatively, the statistical power to detect the moderate effect size for complex human diseases such as schizophrenia was not strong. Further studies with enlarged sample size are required to verify our findings.

In summary, using the two individual samples for our association analysis, we reported that two markers with relatively low LD to each other in the *ANK3* gene were independently associated with schizophrenia. In addition, our data also provided further evidence

for the existence of shared susceptibility loci between schizophrenia and BD. Future studies would focus on determining the mechanistic links between the genetic variation at the two loci and schizophrenia.

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