Polymorphisms and Haplotypes in the *YWHAE* Gene Increase Susceptibility to Bipolar Disorder in Chinese Han Population

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ABSTRACT

Background: Schizophrenia and bipolar disorder are 2 major psychiatric illnesses sharing some specific genetic risk factors. Increasing evidence suggests the 2 illnesses might be more closely related than previously considered.

Objective: To test this hypothesis, we investigated the allele and genotype frequencies of 11 single nucleotide polymorphisms (SNPs) and the haplotypes in these SNPs of the *YWHAE* gene.

Method: 1,982 patients were interviewed by 2 independent, experienced psychiatrists. Bipolar disorder diagnoses were made in strict accordance with *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (*DSM-IV*) criteria using the Structured Clinical Interview for *DSM-IV* Axis I Disorders. In 2011, we conducted this genetic association analysis between 11 SNPs in *YWHAE* and bipolar disorder, involving a male group and a female group.

Results: In the analysis of allele and genotype frequencies, the SNP rs1873827 increased susceptibility to bipolar disorder in the male group. The haplotype analysis of CAC in rs3752826, rs2131431, and rs1873827 in the male group ($\chi^2 = 25.744$, $P = 3.97E-07$, OR = 0.478 [95% CI, 0.358–0.639]) and of ACT and CAC in rs3752826, rs2131431, and rs1873827 in the female group (for ACT, $\chi^2 = 30.365$, $P = 3.67E-08$, OR = 0.040 [95% CI, 0.007–0.218]; for CAC, $\chi^2 = 16.874$, $P = 4.04E-05$, OR = 0.597 [95% CI, 0.466–0.765]) showed they are protective factors for bipolar disorder. However, the haplotype analysis of CAT in the male group ($\chi^2 = 19.874$, $P = 8.39E-06$, OR = 2.314 [95% CI, 1.587–3.374]) and of AAC and CAT in the female group (for AAC, $\chi^2 = 38.561$, $P = 5.47E-10$, OR = 7.104 [95% CI, 3.471–14.540]; for CAT, $\chi^2 = 25.497$, $P = 4.52E-07$, OR = 2.076 [95% CI, 1.556–2.770]) showed they are risk factors for bipolar disorder.

Conclusions: Considering the size of our sample, the results suggest that *YWHAE* does play a major role in bipolar disorder in the Han Chinese population.

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Bipolar disorder (Online Mendelian Inheritance in Man [OMIM] 125480) is a major psychiatric illness that causes extreme shifts in mood, energy, and functioning. It is a genetically complex neuropsychiatric disorder like other psychiatric diseases such as schizophrenia and major depressive disorder. Psychiatrists have debated whether schizophrenia and bipolar disorder are 2 distinct mental disorders with different etiopathogenesis, course, and prognosis or whether they are more connected mental disorders, possibly representing different ends of the common pathophysiologic processes.[1,2] And increasing evidence suggests that schizophrenia and bipolar disorder might be more closely related than previously considered in that they share certain specific genetic and environmental risk factors.[3–6] Bipolar disorder is a manic-depressive illness that is estimated to have a lifetime prevalence of 0.8%–2.6%.[7] Data from family, twin, and adoption studies provide strong evidence that bipolar disorder is predominantly a polygenic genetic disorder with high heritability.[8–12]

Recently, Ikeda et al[13] identified the *YWHAE* gene as a possible susceptibility gene for schizophrenia in the Japanese population. However, in an earlier study,[14] we found no association of the *YWHAE* gene with schizophrenia, major depressive disorder, or bipolar disorder in the Han Chinese Population. *YWHAE* encodes 14-3-3epsilon, which is an interacting partner of DISC1. *DISC1* has been identified as a potential susceptibility gene for major psychiatric disorders, including schizophrenia, depressive disorder, and bipolar disorder. In a large Scottish pedigree, *DISC1* was found to be disrupted by a translocation that cosegregated with major psychiatric illness, including schizophrenia, bipolar disorder, recurrent depression, and childhood-onset behavioral disorder.[15] *DISC1* has also been associated with bipolar disorder in several studies. Many researchers have recently conducted studies of its function, and DISC1 has been found to be widely distributed throughout neurons, nuclei, mitochondria, and neurites. It interacts with many proteins and is related to many functions, such as neurite extension, neuronal migration, dendrite plasticity, and neurotransmitter signaling. Several DISC1 interactors such as *PDE4B* have also been defined as independent genetic susceptibility factors for psychiatric illness. DISC1 is a hub protein in a multidimensional risk pathway for major psychiatric illness, and studies of this pathway are opening up opportunities for a better understanding of causality and possible intervention mechanisms.[16]

An interactor of DISC1, 14-3-3epsilon is encoded by *YWHAE* and is a subtype of the 14-3-3 protein family. This family consists of 7 subtypes (β, γ, ε, σ, η, θ/τ, and ζ) encoded by separate genes. They are involved in many processes, including cell cycle regulation, metabolism control, apoptosis, and control of gene transcription.[17] *YWHAH*, which encodes 14-3-3ζ, is a positional and functional candidate gene for both schizophrenia and bipolar disorder.[18,19] As an interacting partner of DISC1, *YWHAE* may also have a role to play in these psychiatric disorders. In 2005, Yanagi et al[20] showed that *YWHAE* is associated with suicide in

the Japanese population. In 2008, Ikeda et al[13] reported that *YWHAE* is a possible susceptibility gene for schizophrenia in the Japanese population. They first identified this gene by screening 25 tag single nucleotide polymorphisms (SNPs) that covered 5 DISC1-interacting molecules (*NUDEL, LIS1, YWHAE, GRB2, KIF5A*). Their study also showed that rs28365859, rs34041110, rs7224258, rs3752826, rs11655548, rs2131431, and rs1873827 were significantly associated with schizophrenia in both allele and genotype analyses. Other studies[21,22] have shown that patients with deletion of 17p13.3 involving *YWHAE* were affected by Miller-Dieker syndrome, along with cognitive impairment, shared craniofacial features, and structural abnormalities of the brain. In our earlier study[14] investigating the possible relationship between the *YWHAE* gene and major psychiatric disorders, we genotyped 11 SNPs of *YWHAE* in 1,140 schizophrenia patients, 1,140 major depressive disorder patients, 1,140 bipolar disorder patients, and 1,140 normal controls of Chinese Han origin but found no association between the *YWHAE* gene and these major mental disorders in the Han Chinese population. However, to date, no other studies of the relationship between *YWHAE* and bipolar disorder have been reported.

To investigate whether the *YWHAE* gene plays a significant role in men or women with bipolar disorder, we focused on the *YWHAE* gene in bipolar disorder in Han Chinese samples involving 993 bipolar disorder patients (533 men and 460 women) and 989 normal controls (296 men and 693 women). We genotyped 11 SNPs in *YWHAE*, consisting of the 7 positive SNPs in the study by Ikeda et al[13] (rs34041110, rs7224258, rs3752826, rs11655548, rs2131431, rs1873827, and rs28365859) and 4 additional tag SNPs (rs12452627, rs1532976, rs8064578, and rs7225165) selected from the HapMap database (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap28_B36/; version: release #21/phase II, July 2006; population: Han Chinese in Beijing, minor allele frequencies more than 0.05) to provide a good coverage of the gene.

- Our study suggests that some susceptibility may be common to both schizophrenia and bipolar disorder and that a putative, sex-dependent relationship exists between the *YWHAE* gene and bipolar disorder.
- Evidence from this study will be useful in influencing psychiatric research to move from reliance on a diagnostic and classification system that is based only on clinical description to a scheme that better reflects the underlying biology of the psychiatric entities encountered in our clinics.

Clinical Points

METHOD

Samples

In total, 993 unrelated bipolar patients (men, n = 533; women, n = 460; mean ± SD age = 36.8 ± 5.8 years), and 989 unrelated controls (men, n = 296; women, n = 693; mean ± SD age = 57.2 ± 3.7 years) were recruited. All the subjects were from Shanghai and were of Han origin. Among the 993 bipolar patients, 851 (85.6%) were diagnosed with bipolar I disorder. Two independent psychiatrists made a final diagnosis on the basis of interview data and hospital case notes. Diagnoses were made in strict accordance with *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (*DSM-IV*) criteria based on the Structured Clinical Interview for *DSM-IV* Axis I Disorders. All participating subjects signed an informed consent statement. The complete details of the entire study design and procedures involved were approved by the ethics committee of Bio-X Institutes, Shanghai Jiao Tong University. Controls were randomly selected from the Shanghai general population. DNA was extracted from peripheral blood samples of the subjects by using the phenol-chloroform method. The study was conducted in 2011.

Genotyping

Of the 11 SNPs, rs3752826, rs2131431, rs1873827, rs12452627, rs8064578, rs7224258, and rs11655548 are in the intron, and rs34041110, rs1532976, rs28365859, and rs7225165 are in the intergenic region. All the SNPs were genotyped on the ABI 7900 DNA detection platform (Applied Biosystems, Foster City, California) by using TaqMan technology. All probes and primers were designed by the Assay-by-DesignTM or Assayon-DemandTM service of Applied Biosystems. The standard polymerase chain reactions (PCRs) of 4.435 μL were carried out using TaqMan Universal PCR Master Mix reagent kits (Applied Biosystems, Foster City, California) under the guidelines provided. To ensure the accuracy of genotyping results, 4 negative controls (no DNA) and 4 duplicated samples were included in each of the 384-well plates for the quality control. Five percent of the samples were repeated, and the results were 100% concordant. All genotypes were called blind to their case status in the genotyping process.

Statistical Analysis

The χ^2 test for goodness of fit was used to check for Hardy-Weinberg equilibrium in genotype distributions in patients and controls. The differences in the genotype and allele distributions between patients and controls were examined by using the χ^2 test for independence. All the analyses were carried out online on a robust and user-friendly software platform.[23,24] All tests were 2-tailed, and statistical significance was assumed at $P < .05$.

RESULTS

In the 1,982 samples, genotype distributions were in Hardy-Weinberg equilibrium for all the SNPs. The allele and genotype frequencies of the 11 SNPs of the male patient sample group and the healthy controls are listed in Table 1. The allele and genotype frequencies of the 11 SNPs of the female patient sample group and the healthy controls are listed in Table 2. The linkage disequilibrium among the

Table 1. Allele and Genotype Frequency of the 11 Loci in Bipolar Disorder: Male Patient Sample Group and Healthy Controls

SNP	Position	Allele (frequency)[a]		OR (95% CI)	*P* Value[b]	*P*-Permutation (10,000 times)	Genotype (frequency)[a]			HWE *P*	*P* Value[b]	*P*-Permutation (10,000 times)
rs34041110	1193642	A	G				A/A	A/G	G/G			
Case		603 (0.567)	461 (0.433)	1.138702 (0.929021–1.395708)	.210831	0.9467	165 (0.310)	273 (0.513)	94 (0.177)		.399205	0.9981
Control		309 (0.535)	269 (0.465)				77 (0.266)	155 (0.536)	57 (0.197)	0.185801		
rs3752826	1211814	A	C				A/A	A/C	C/C			
Case		565 (0.554)	455 (0.446)	1.101958 (0.897112–1.353578)	.354761	0.9966	157 (0.308)	251 (0.492)	102 (0.200)		.592852	1.0000
Control		302 (0.530)	268 (0.470)				78 (0.274)	146 (0.512)	61 (0.214)	0.633808		
rs2131431	1241645	A	C				A/A	A/C	C/C			
Case		826 (0.781)	232 (0.219)	1.086341 (0.854180–1.381603)	.499553	1.0000	326 (0.616)	174 (0.329)	29 (0.055)		.448938	0.9998
Control		449 (0.766)	137 (0.234)				170 (0.580)	109 (0.372)	14 (0.048)	0.511201		
rs1873827	1247690	C	T				C/C	C/T	T/T			
Case		367 (0.364)	641 (0.636)	0.768045 (0.623133–0.946658)	**.013298**	0.1666	69 (0.137)	229 (0.454)	206 (0.409)		**.025579**	0.2902
Control		246 (0.427)	330 (0.573)				48 (0.167)	150 (0.521)	90 (0.312)	0.275152		
rs12452627	1249222	A	G				A/A	A/G	G/G			
Case		121 (0.116)	921 (0.884)	1.018187 (0.737871–1.404994)	.912622	1.0000	2 (0.004)	117 (0.225)	402 (0.772)		.780072	1.0000
Control		64 (0.114)	496 (0.886)				2 (0.007)	60 (0.214)	218 (0.779)	0.327888		
rs1532976	Unknown	A	G				A/A	A/G	G/G			
Case		581 (0.554)	467 (0.446)	1.192631 (0.971861–1.463551)	.091527	0.6891	161 (0.307)	259 (0.494)	104 (0.198)		.236511	0.9684
Control		290 (0.511)	278 (0.489)				73 (0.257)	144 (0.507)	67 (0.236)	0.806461		
rs28365859	1250528	C	G				C/C	C/G	G/G			
Case		720 (0.706)	300 (0.294)	1.026895 (0.822083–1.282733)	.815104	1.0000	254 (0.498)	212 (0.416)	44 (0.086)		.629878	1.0000
Control		409 (0.700)	175 (0.300)				139 (0.476)	131 (0.449)	22 (0.075)	0.23927		
rs8064578	1201625	A	G				A/A	A/G	G/G			
Case		590 (0.558)	468 (0.442)	1.015891 (0.826476–1.248716)	.880942	1.0000	164 (0.310)	262 (0.495)	103 (0.195)		.894151	1.0000
Control		309(0.554)	249 (0.446)				83 (0.297)	143 (0.513)	53 (0.190)	0.535697		
rs7225165	Unknown	A	G				A/A	A/G	G/G			
Case		89 (0.085)	963 (0.915)	0.797889 (0.565433–1.125910)	.198032	0.9361	0 (0.000)	89 (0.169)	437 (0.831)		.105468	0.7383
Control		60 (0.104)	518 (0.896)				2 (0.007)	56 (0.194)	231 (0.799)	0.481133		
rs7224258	1202252	C	G				C/C	C/G	G/G			
Case		250 (0.239)	798 (0.761)	0.966324 (0.762686–1.224334)	.776627	1.0000	37 (0.071)	176 (0.336)	311 (0.594)		.165842	0.8966
Control		142 (0.245)	438 (0.755)				14 (0.048)	114 (0.393)	162 (0.559)	0.282627		
rs11655548	1230748	C	T				C/C	C/T	T/T			
Case		350 (0.339)	682 (0.661)	0.977774 (0.786405–1.215713)	.8397	1.0000	72 (0.140)	206 (0.399)	238 (0.461)		.269156	0.9820
Control		190 (0.344)	362 (0.656)				32 (0.116)	126 (0.457)	118 (0.428)	0.852061		

[a]Values represent absolute number and proportion of total of the indicated allele or genotype.
[b]Values in bold indicate statistical significance.
Abbreviations: HWE = Hardy-Weinberg equilibrium, SNP = single nucleotide polymorphism.

Table 2. Allele and Genotype Frequency of the 11 Loci in Bipolar Disorder: Female Patient Sample Group and Healthy Controls

SNP	Position	Allele (frequency)[a]		OR (95% CI)	*P* Value	*P*-Permutation (10,000 times)	Genotype (frequency)[a]			HWE *P*	*P* Value	*P*-Permutation (10,000 times)
rs34041110	1193642	A	G				A/A	A/G	G/G			
Case		515 (0.562)	401 (0.438)	1.008720 (0.851760–1.194604)	.919837	1.0000	150 (0.328)	215 (0.469)	93 (0.203)		.995074	1.0000
Control		755 (0.560)	593 (0.440)				219 (0.325)	317 (0.470)	138 (0.205)	0.23684		
rs3752826	1211814	A	C				A/A	A/C	C/C			
Case		495 (0.554)	399 (0.446)	1.054769 (0.889652–1.250532)	.5393	1.0000	147 (0.329)	201 (0.450)	99 (0.221)		.620232	1.0000
Control		721 (0.540)	613 (0.460)				202 (0.303)	317 (0.475)	148 (0.222)	0.264488		
rs2131431	1241645	A	C				A/A	A/C	C/C			
Case		704 (0.770)	210 (0.230)	1.044396 (0.856581–1.273390)	.667592	1.0000	273 (0.597)	158 (0.346)	26 (0.057)		.868628	1.0000
Control		1,040 (0.762)	324 (0.238)				397 (0.582)	246 (0.361)	39 (0.057)	0.912592		
rs1873827	1247690	C	T				C/C	C/T	T/T			
Case		346 (0.391)	538 (0.609)	0.992828 (0.834859–1.180688)	.935097	1.0000	71 (0.161)	204 (0.462)	167 (0.378)		.86358	1.0000
Control		537 (0.393)	829 (0.607)				116 (0.170)	305 (0.447)	262 (0.384)	0.093809		
rs12452627	1249222	A	G				A/A	A/G	G/G			
Case		106 (0.117)	800 (0.883)	1.265161 (0.961605–1.664544)	.092358	0.6943	6 (0.013)	94 (0.208)	353 (0.779)		.232703	0.9588
Control		124 (0.095)	1,184 (0.905)				5 (0.008)	114 (0.174)	535 (0.818)	0.689223		
rs1532976	Unknown	A	G				A/A	A/G	G/G			
Case		502 (0.555)	402 (0.445)	1.039487 (0.877172–1.231837)	.654833	1.0000	145 (0.321)	212 (0.469)	95 (0.210)		.868984	1.0000
Control		728 (0.546)	606 (0.454)				204 (0.306)	320 (0.480)	143 (0.214)	0.402913		
rs28365859	1250528	C	G				C/C	C/G	G/G			
Case		630 (0.705)	264 (0.295)	1.020583 (0.848517–1.227542)	.82876	1.0000	228 (0.510)	174 (0.389)	45 (0.101)		.252038	0.9683
Control		954 (0.700)	408 (0.300)				329 (0.483)	296 (0.435)	56 (0.082)	0.350676		
rs8064578	1201625	A	G				A/A	A/G	G/G			
Case		497 (0.552)	403 (0.448)	1.050807 (0.885904–1.246404)	.569355	1.0000	141 (0.313)	215 (0.478)	94 (0.209)		.84364	1.0000
Control		703 (0.540)	599 (0.460)				197 (0.303)	309 (0.475)	145 (0.223)	0.255168		
rs7225165	Unknown	A	G				A/A	A/G	G/G			
Case		85 (0.095)	813 (0.905)	1.161164 (0.864592–1.559466)	.32033	0.9924	0 (0.000)	85 (0.189)	364 (0.811)		.048452	0.4705
Control		113 (0.083)	1,255 (0.917)				5 (0.007)	103 (0.151)	576 (0.842)	0.866562		
rs7224258	1202252	C	G				C/C	C/G	G/G			
Case		207 (0.228)	699 (0.772)	1.008809 (0.825228–1.233229)	.931781	1.0000	24 (0.053)	159 (0.351)	270 (0.596)		.984001	1.0000
Control		305 (0.227)	1,039 (0.773)				34 (0.051)	237 (0.353)	401 (0.597)	0.893711		
rs11655548	1230748	C	T				C/C	C/T	T/T			
Case		306 (0.345)	582 (0.655)	1.010744 (0.844941–1.209082)	.906921	1.0000	51 (0.115)	204 (0.459)	189 (0.426)		.520546	0.9998
Control		451 (0.342)	867 (0.658)				85 (0.129)	281 (0.426)	293 (0.445)	0.174985		

[a]Values represent absolute number and proportion of total of the indicated allele or genotype.
Abbreviations: HWE = Hardy-Weinberg equilibrium, SNP = single nucleotide polymorphism.

Figure 1. Linkage Disequilibrium Among the 11 Single Nucleotide Polymorphisms: Male Group Patients and Controls

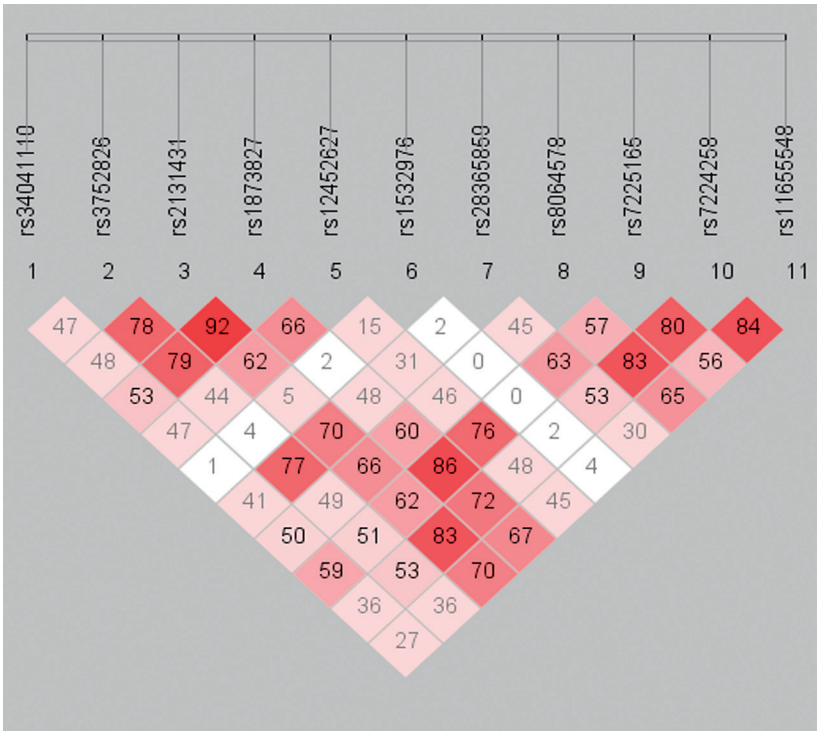
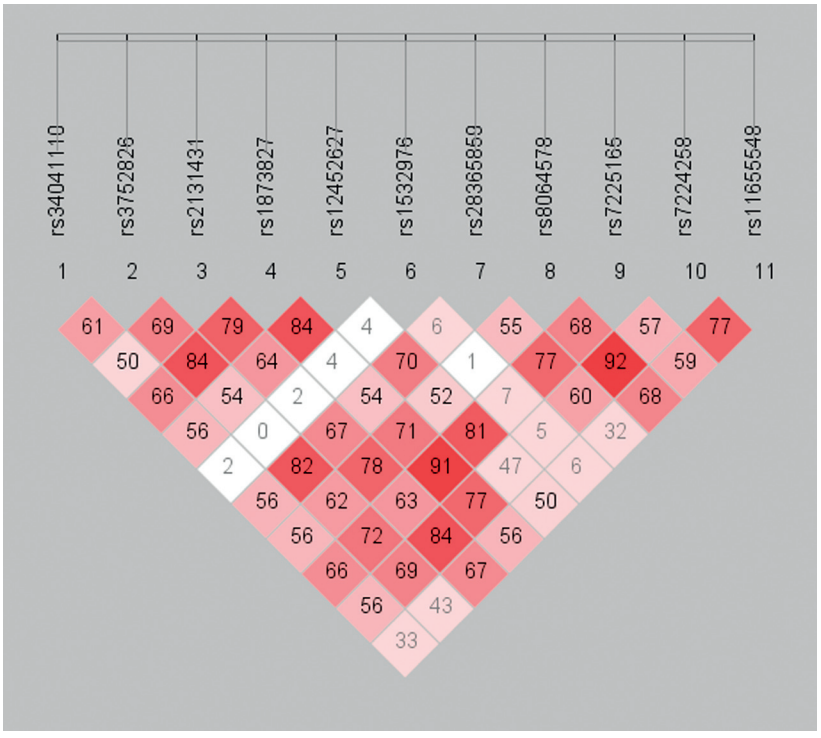


Figure 2. Linkage Disequilibrium Among the 11 Single Nucleotide Polymorphisms: Female Group Patients and Controls



11 SNPs is shown in Figures 1 and 2. The selection criteria for haplotypes used in the haplotype analyses were the adjacent SNPs with pairwise $D' > 0.70$. In the analysis, haplotypes with frequencies above 0.03 were tested. According to the selection criteria, 3 SNPs (rs3752826, rs2131431, and rs1873827) with strong pairwise $D' > 0.70$ were in 1 block, both in the male and female groups. Total results for haplotypes in the male and female groups are listed in Table 3.

In the male group, only the allele and genotype *P* values for rs1873827 are associated with bipolar disorder (allele, $P = 0.013298$; genotype, $P = 0.025579$; OR = 0.768045 [95% CI, 0.623133–0.946658]) (Table 1). In Table 2, all the allele and genotype *P* values for the 11 SNPs are greater than .05.

In Table 3, the haplotype analysis of CAC in rs3752826, rs2131431, and rs1873827 in the male group ($\chi^2 = 25.744$, $P = 3.97E-07$, OR = 0.478 [95% CI, 0.358–0.639]) and of ACT and CAC in rs3752826, rs2131431, and rs1873827 in the female group (for ACT, $\chi^2 = 30.365$, $P = 3.67E-08$, OR = 0.040 [95% CI, 0.007–0.218]; for CAC, $\chi^2 = 16.874$, $P = 4.04E-05$, OR = 0.597 [95% CI, 0.466–0.765]) showed they are protective factors for bipolar disorder. The haplotype analysis of CAT in the male group ($\chi^2 = 19.874$, $P = 8.39E-06$, OR = 2.314 [95% CI, 1.587–3.374]) and of AAC and CAT in the female group (for AAC, $\chi^2 = 38.561$, $P = 5.47E-10$, OR = 7.104 [95% CI, 3.471–14.540]; for CAT, $\chi^2 = 25.497$, $P = 4.52E-07$, OR = 2.076 [95% CI, 1.556–2.770]) showed they are risk factors for bipolar disorder.

DISCUSSION

Although no significant associations were shown between the 11 SNPs of *YWHAE* gene and bipolar disorder when we investigated male and female subjects collectively in our prior study,[14] significant associations may arise when looking at these SNPs in the male and female samples separately. Thus, the main purpose of the current study was to investigate whether there is a gender-limited association between the *YWHAE* gene and bipolar disorder. We tested 11 SNPs of *YWHAE* gene between the bipolar disorder cases and controls. By the angle of both allele and genotype frequencies analysis, our results indicate that, in the male group, rs1873827

Table 3. Haplotype Analysis With SNPs rs3752826, rs2131431, and rs1873827 in Male and Female Groups

Group	Haplotype	Case Frequency (%)	Control Frequency (%)	χ^2	*P*	*P*-Permutation (10,000 times)	OR (95% CI)
rs3752826, rs2131431, rs1873827	AAC	34.41 (0.036)	9.60 (0.017)	4.286	.038452	0.0468	2.112 (1.025–4.354)
Male[a]	AAT	475.55 (0.492)	271.79 (0.482)	0.041	.83983	0.7340	1.022 (0.828–1.262)
	CAC	105.94 (0.110)	114.02 (0.202)	25.744	3.97E-07	0.0000	0.478 (0.358–0.639)
	CAT	138.10 (0.143)	37.60 (0.067)	19.874	8.39E-06	0.0000	2.314 (1.587–3.374)
	CCC	190.96 (0.198)	112.95 (0.200)	0.049	.824277	0.7277	0.971 (0.748–1.260)
Female[b]	AAC	40.58 (0.047)	9.36 (0.007)	38.561	5.47E-10	0.0000	7.104 (3.471–14.540)
	AAT	404.59 (0.472)	649.23 (0.493)	0.246	.620186	0.4652	0.957 (0.804–1.139)
	ACT	1.37 (0.002)	51.91 (0.039)	30.365	3.67E-08	0.0000	0.040 (0.007–0.218)
	CAC	102.99 (0.120)	249.94 (0.190)	16.874	4.04E-05	0.0002	0.597 (0.466–0.765)
	CAT	114.83 (0.134)	93.47 (0.071)	25.497	4.52E-07	0.0000	2.076 (1.556–2.770)
	CCC	167.98 (0.196)	252.20 (0.191)	0.245	.620902	0.8401	1.057 (0.850–1.314)

[a]Male group global result: $\chi^2_4 = 43.697289$ (frequency < 0.03 in both control and case has been dropped), Pearson $P = 7.42E\text{-}009$, permutation $P = 0.0002$.
[b]Female group global result: $\chi^2_5 = 104.692055$ (frequency < 0.03 in both control and case has been dropped), Pearson $P = 5.41E\text{-}021$, permutation $P = 0.0000$.

showed statistically significant differences between the 533 cases and 296 controls. Also in the male group, the CAC haplotype and CAT haplotype of rs3752826, rs2131431, and rs1873827 were positively associated with bipolar disorder. For the CAC haplotype, the OR value and the upper limit of 95% CI were less than 1, which indicates that CAC was a protective factor for bipolar disorder. For haplotype CAT, the OR value and the lower limit of 95% CI were greater than 1, which indicates that CAT was a risk factor for bipolar disorder. When we applied the same metric to the female group, the AAC haplotype and the CAT haplotype of rs3752826, rs2131431, and rs1873827 were positively associated with bipolar disorder, and they were both risk factors of the disease. On the other hand, the ACT and CAC were 2 protective factors for bipolar disorder. Therefore, the conclusion above shows that the combination of rs3752826, rs2131431, and rs1873827 may therefore play an important role in the development of bipolar disorder in both male and female groups. Compared with our prior study,[14] the present one showed that, when analyzed separately by genders, the rs1873827 polymorphism, which was not significantly associated with bipolar disorder when analyzed in all cases and controls, was associated with the male group. The reason for this finding is that rs1873827 plays an important role in the male group with bipolar disorder, but not in the female group with bipolar disorder. Therefore, in our prior study, which analyzed subjects that were not divided into gender groups, the role of rs1873827 was masked. Also, since we did not subdivide bipolar disorder patients in our prior study, the associations shown with the haplotypes in the present study were not detected in the prior study.

In summary, by analyzing from the allele angle and the genotype angle, this study provides further evidence for the hypothesis that some susceptibility may be common to both schizophrenia and bipolar disorder, and its findings imply a putative, sex-dependent relationship between the *YWHAE* gene and bipolar disorder. The relatively weak association obtained in our study may result from the weak effect of the gene. However, analyzing the gene from the angle of haplotype, it is true that several important haplotypes may significantly affect bipolar disorder, in both the protective direction and the risk direction and in both the male group and the female group.

Certainly, there is an important limitation of our previous study and present study. The samples we used were all from Shanghai area. The results would be more convincing if we could use samples from all over the country or even from all over the world. Although caution is needed in drawing conclusions from the current data, our study and previous findings should encourage further investigation into *YWHAE* and its potential role in the etiology of neurologic and psychiatric diseases.

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