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ORIGINAL ARTICLE

Genome-wide association study of bipolar I disorder in the Han Chinese population

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We report the first genome-wide association study in 1000 bipolar I patients and 1000 controls, with a replication of the top hits in another 409 cases and 1000 controls in the Han Chinese population. Four regions with most strongly associated single-nucleotide polymorphisms (SNPs) were detected, of which three were not found in previous GWA studies in the Caucasian populations. Among them, SNPs close to specificity protein 8 (*SP8*) and *ST8* α-*N*-acetyl- neuraminide α-2,8-sialyltransferase (*ST8SIA2*) are associated with Bipolar I, with *P*-values of 4.87×10^{-7} (rs2709736) and 6.05×10^{-6} (rs8040009), respectively. We have also identified SNPs in potassium channel tetramerization domain containing 12 gene (*KCTD12*) (rs2073831, $P = 9.74 \times 10^{-6}$) and in *CACNB2* (Calcium channel, voltage-dependent, β-2 subunit) gene (rs11013860, $P = 5.15 \times 10^{-5}$), One SNP nearby the rs1938526 SNP of *ANK3* gene and another SNP nearby the SNP rs11720452 in chromosome 3 reported in previous GWA studies also showed suggestive association in this study ($P = 6.55 \times 10^{-5}$ and $P = 1.48 \times 10^{-5}$, respectively). This may suggest that there are common and population-specific susceptibility genes for bipolar I disorder.
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Introduction

Studies have shown that there is a strong genetic component associated with bipolar disorder with a heritability estimated to be as high as 79%.[1] Even though numerous linkage and candidate gene studies have been performed, genes consistently found to be associated with bipolar disorder still remain elusive. Recently, several genome-wide association studies

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have been performed to identify disease susceptibility loci for bipolar disorder.[2–5]

The Wellcome Trust Case Control Consortium Study, consisted of 2000 patients with bipolar I, bipolar II and schizoaffective disorders, identified a region (16p12) associated with these disorders.[2] This study also identified *KCNC2* (Shaw-related voltage-gate potassium channel) to be associated with bipolar disorder in the expanded reference group analysis. The involvement of ion channels in the pathogenesis of bipolar disorder was further supported by a GWA meta-analysis, which implicated *CACNA1C* (α-1C subunit of the L-type voltage-gated calcium channel) in bipolar disorder.[4] Calcium channel blockers such as verapamil and nimodipine have been used to treat bipolar patients[6,7] and elevated intracellular calcium has been found in lymphocytes from patients suffering from bipolar disorder.[8] These suggest that imbalance of cellular calcium level might be one of the causes for bipolar disorder. However, the exact roles of calcium and calcium channel in the pathogenesis of bipolar disorder remain to be elucidated.

Most of the earlier studies have been carried out under the assumption that bipolar disorder and schizophrenia are separate diseases with separate etiologies (known as the Kraepelian dichotomy). However, a considerable proportion of patients with bipolar disorder also manifest psychotic features during manic and/or depressive episodes. Thus, an affective spectrum hypothesis with syndromes ranging from unipolar depression to bipolar disorder, and further to schizoaffective disorder with both affective and psychotic features has been proposed.[9] In recent years, increasing genetic evidence from family,[10,11] twin[12,13] and linkage studies[14,15] have shown a degree of overlap between the two diseases. These studies might suggest that while certain genes are shared by both disorders, each of them has its own specific genes.[15]

Nearly all of the recent GWA studies of bipolar disorder have been performed in the Caucasian population, and not all of them separate bipolar I from bipolar II and schizoaffective disorder. In this paper, we present results of the first GWA study focusing on bipolar I disorder in a Han-Chinese population.

Materials and methods

Study subjects and phenotype definition

A Taiwan Bipolar Consortium was established for this study by ATAC with members from the Institute of Biomedical Sciences, Academia Sinica and 25 psychiatric departments of general hospitals and psychiatric institutions in Taiwan. A total of 1409 unrelated bipolar I patients were consecutively recruited from inpatient units of these psychiatric departments and institutions, including 665 (47.2%) males and 744 females (52.8%). All of them were diagnosed according to DSM-IV criteria[16] for bipolar I disorder with at least one inpatient treatment for manic episode, and nearly all of them (96.3%) had recurrent episodes of mania. Patients with other psychotic and affective disorders were excluded.

Clinical phenotype assessment was performed by trained psychiatric nurses and psychiatrists using a cross-culturally validated and reliable Chinese version of the Schedules for Clinical Assessment in Neuropsychiatry (SCAN),[17] supplemented by available medical records. In a further study, the interrater reliability for all SCAN symptom/course items among 23 psychiatric patients were found to be acceptable, with overall percentage agreement ranging from 61 to 100% and the generalized kappa coefficient ranging from 0.47 to 0.88.[18] The agreement on the diagnosis of bipolar disorder was excellent, being 100% both in this study and in the earlier crosscultural study of the SCAN between US/UK and Taiwan senior psychiatrists.[17] Diagnosis was generated from the computer diagnostic algorism for the SCAN (I-Shell).[19]

Only the Han-Chinese population, which accounted for 98% of the population in Taiwan, was considered for the recruitment of patients in this study. The control group ($N = 2000$, with nearly equal proportions of men and women) was randomly selected from the Han-Chinese Cell and Genome Bank in Taiwan reported previously.[20] In brief, more than 3300 healthy controls were recruited via a stratified, 3-staged probability clustering sampling scheme through the registry of all the 329 non-aboriginal townships or city districts in Taiwan. The study was approved by the institutional review board of all the participating hospitals and Academia Sinica, Taiwan and written informed consent were obtained from all of the participants.

Study design and power calculation

The study began with a GWA analysis of the first 1000 bipolar 1 disorder cases recruited and 1000 controls. After cross-platform validation, the most significant single-nucleotide polymorphisms (SNPs) associated with bipolar 1 disorder and nearby tag SNPs in the same LD blocks were genotyped in additional 409 cases and 1000 controls. Joint association analysis was then performed with all of the 1409 bipolar I cases and 2000 controls, this could achieve a power of 0.85 to detect a disease allele with frequency of 0.15 and odds ratio of 1.5, assuming a disease prevalence of 0.02, at a significant level of 0.05 (Supplementary Table 1).

Genotyping and quality control

GWA was conducted among the first 1000 cases and 1000 controls using the Illumina HumanHap550-Duo BeadChip (Illumina, San Diego, CA, USA) and genotyping was performed by deCODE genetics (Reykjavík, Iceland). Genotype calling was determined by Beadstudio (Illumina) using default parameters.

Quality control of genotype data was performed by examining several summary statistics. First, the ratio of loci with heterozygous calls on the X chromosome

was calculated to double check the subject gender. Total successful call rate and the minor allele frequency of cases and controls were also calculated for each SNP. SNPs were excluded for further analysis if one of the following conditions applied: (1) only one allele appeared in both cases and controls; (2) the total call rate was less than 95%; (3) the total minor allele frequency was less than 5% and the total call rate was less than 99%. In addition, SNPs significantly departed from Hardy–Weinberg equilibrium proportions were also excluded (P-value $< 10^{-7}$).

Population stratification
Detection of possible population stratification that might influence association analysis was carried out using principle component analysis, structure analysis and genomic control, with genotype data from 100 000 SNPs located at equally spacing across the human genome.

GWA analysis
GWA analysis was carried out by comparing allele/genotype frequencies between cases and controls using five single-point methods: genotype, allele-type and Cochran-Armitage trend test along with tests considering dominant and recessive inheritance models. Empirical P-values were also obtained with 10^7 simulations. SNPs with P-values less than $\alpha = 10^{-7}$, a cut-off for the multiple-comparison adjusted by Bonferroni correction were considered to be significantly associated with the traits. SNPs with P-values between 10^{-7} and 10^{-5} were considered to have suggestively significant association. Quantile–quantile (Q–Q) plots were then used to examine P-value distributions (Supplementary Figure 1).

Two-point analysis was performed using logistic regression model, regressing the affected status on two SNPs and their interaction. SNPs with P-values less than 10^{-3} in the single point association analysis were chosen to facilitate the analysis. SNPs were coded as 0, 1 and 2 for the number of minor alleles and treated as continuous variables.

In addition, multi-point/haplotype analysis was performed using the Haplotype Score Test[21] implemented in haplo.stat, a suite of S-PLUS/R routines for the analysis of indirectly measured haplotypes, with 3- and 5-point sliding windows across the genome. Regions showing global P-values less than 10^{-5} in permutation test of 10^5 simulations were further tested with independent 10^7 simulations.

Validation and fine mapping
Top SNPs (P-value $< 10^{-5}$) from the GWA analysis in 1000 cases and 1000 controls were further validated using MALDI-TOF mass spectrometry (SEQUENOM MassARRAY, Sequenom, San Diego, CA, USA). Re-sequencing of the SNPs was performed if the two platforms showed inconsistency. Fine mapping was performed at the SNPs showing consistency in cross-platform validation, using tag SNPs based on the HapMap Asian group data in the same LD block. SNPs retained after cross-platform validation were then genotyped in additional 409 cases and 1000 controls for replication.

Results

Data quality
Call rates were greater than 97% for all subjects genotyped in this study. The average call rate was 99.67% (s.d. = 0.37%). Gender determined from the genotyping results for all the individuals were consistent with recorded data. A total of 516 919 SNPs (92.3%) passed the quality control filter with an average call rate of 99.90% and were advanced to GWA analysis (Supplementary Table 2).

Assessment of population stratification
The results of principle component analysis showed no significant evidence of population stratification between bipolar I patients and controls ($P = 0.06$, and Fst statistics between populations < 0.001) (Supplementary Figure 2). Structure analysis also showed similar results. Furthermore, a genomic control coefficient $\lambda = 1.05$, estimated posterior to the regular GWA study, also indicated no substantial population stratification. Hence the use of genomic control did not largely change the results from this GWA study.

Association analysis
Analysis was first performed in 1000 cases and 1000 controls. Fifteen out of the 516 919 SNPs were associated with bipolar 1 disorder ($P < 10^{-5}$). However, only five SNPs were retained after cross-validation between the Illumina and Sequenom platforms, in which they reached an overall 99.9% consistency (Supplementary Table 3). Re-sequencing of the 10 non-validated SNPs in 94 cases and 94 controls revealed that the inconsistent calls resulted from polymorphism sites near these SNP, which caused the failure of PCRs in Illumina genotyping. They were excluded from further analysis (Supplementary Figure 3).

The five SNPs retained after cross-validation were then genotyped in additional 409 cases and 1000 controls. Their nearby tag SNPs were also genotyped in the entire cohort. A joint analysis with 1409 cases and 2000 controls found seven significant SNPs in four chromosome regions, listed in Table 1 and also shown in Figure 1. Findings of the joint analysis with the best statistical model for SNP are described below.

The rs2709736 SNP located on chromosome 7 showed the strongest statistical evidence for association in this study ($P = 4.87 \times 10^{-7}$). Its nearby SNP, rs2709722, also showed suggestive significant association ($P = 4.65 \times 10^{-6}$) in joint analysis. These two SNPs are not located on any known genes but the nearest gene, *SP8*, is approximately 35 kb away (Figure 1b). The rs8040009 SNP ($P = 6.05 \times 10^{-6}$), is located in the 3′UTR of chromosome 15 open reading frame 32 (C15orf32), whose function is still not known. This SNP, with the nearby tag SNP

Table 1 Regions of the genome showing the strongest evidence: a genome-wide association study of bipolar I disorder in Han Chinese

Chromosome	*SNP*[a]	*Position*	*Nearby gene or transcript*	*Genotype*	*Case (%)*	*Control (%)*	*OR (95% CI), P*[b]	*Genotype*	*Case (%)*	*Control (%)*	*OR (95% CI), P*[c]
7	**rs2709736**	20828827	SP8	AA	14.4	16.5	1.00 (reference)	AA + AG	60.2	68.5	1.00 (reference)
				AG	45.7	52.1	1.25 (1.13–1.38)	GG	39.8	31.5	1.44 (1.25–1.66)
				GG	39.8	31.5	1.56 (1.28–1.91)				$P = 4.87 \times 10^{-7}$
							$P = 1.36 \times 10^{-5}$				
7	rs2709722	20834333	SP8	TT	14.4	16.3	1.00 (reference)	TT + CT	59.9	67.6	1.00 (reference)
				CT	45.5	51.3	1.23 (1.11–1.36)	CC	40.1	32.4	1.39 (1.21–1.61)
				CC	40.1	32.4	1.51 (1.23–1.84)				$P = 4.65 \times 10^{-6}$
							$P = 6.59 \times 10^{-5}$				
15	**rs8040009**	90845343	ST8SIA2/ C15orf32	TT	73.1	79.7	1.00 (reference)	T	85.6	89.2	1.00 (reference)
				CT	24.9	19.1	1.40 (1.21–1.62)	C	14.4	10.8	1.40 (1.21–1.61)
				CC	2.0	1.2	1.96 (1.46–2.62)				$P = 6.05 \times 10^{-6}$
							$P = 6.38 \times 10^{-6}$				
15	rs7174854	90844980	ST8SIA2/ C15orf32	AA	73.4	80.1	1.00 (reference)	A	85.7	89.5	1.00 (reference)
				AT	24.6	18.8	1.41 (1.22–1.64)	T	14.3	10.5	1.41 (1.22–1.64)
				TT	2.0	1.2	2.00 (1.49–2.68)				$P = 3.50 \times 10^{-6}$
							$P = 3.79 \times 10^{-6}$				
13	**rs2073831**	76396700	BTF3L1/ KCTD12	CC	32.1	41.4	1.00 (reference)	C	56.6	63.5	1.00 (reference)
				CT	49.1	44.2	1.32 (1.17–1.5)	T	43.4	36.5	1.33 (1.17–1.51)
				TT	18.9	14.4	1.75 (1.36–2.26)				$P = 9.74 \times 10^{-6}$
							$P = 1.35 \times 10^{-5}$				
13	rs1323038	76395176	BTF3L1/ KCTD12	AA	3.4	6.1	1.00 (reference)				
				AT	33.8	37.3	1.30 (1.15–1.46)				
				TT	62.8	56.6	1.68 (1.33–2.13)				
							$P = 1.56 \times 10^{-5}$				
10	**rs11013860**	18694033	CACNB2	CC	28.5	35.1	1.00 (reference)	CC	28.5	35.1	1.00 (reference)
				AC	52.9	48.0	1.19 (1.08–1.31)	AA + AC	71.5	64.9	1.36 (1.17–1.58)
				AA	18.6	16.9	1.42 (1.16–1.73)				$P = 5.15 \times 10^{-5}$
							$P = 5.54 \times 10^{-4}$				
9	**rs576026**	18723462	ADAMTSL1	AA	25.5	30.5	1.00 (reference)	AA	25.5	30.5	1.00 (reference)
				AG	53.2	48.7	1.12 (1.01–1.23)	AG + GG	74.5	69.5	1.28 (1.1–1.49)
				GG	21.3	20.9	1.25 (1.03–1.52)				$P = 1.51 \times 10^{-3}$
							$P = 0.02476$[d]				

[a]The bold SNPs indicate the five validated SNPs from the initial GWA analysis.
[b]*P*-values were obtained using logistic regression with SNPs codes as 0, 1 and 2 copies of reference alleles. Results of all SNPs, except for rs1323038, were based on genotype data of 1409 cases and 2000 controls. The rs2073831 SNP could not be genotyped by Sequenom Mass Array and the *P*-value was based on 1000 cases and 1000 controls. However, the genotyping calls for this SNP was validated by direct sequencing. There is high consistency (100%) between Illumina genotyping calls and re-sequencing results in 200 cases and 200 controls.
[c]Minimal *P*-values of the five association tests: genotype, allele-type, trend, dominant and recessive.
[d]Result calculated from Illumina550 K with 1000 cases and 1000 controls, which gave $P = 7.42 \times 10^{-6}$ in a dominant model.

rs7174854 ($P = 3.50 \times 10^{-6}$) are located approximately 30 kb downstream of a more functionally relevant gene, ST8 α-*N*-acetyl-neuraminide α-2,8-sialyltransferase 2 (*ST8SIA2*). Both *SP8* and *ST8SIA2* involve the development of the brain.

The third SNP rs2073831 with *P*-value 9.74×10^{-6} located on chromosome 13. Its nearby tag SNP, rs1323038, also showed association with $P = 1.56 \times 10^{-5}$. These two SNPs lie between basic transcription factor 3, like 1 (*BTF3L1*, approximately 3.4 kb upstream) and potassium channel tetramerization domain containing 12 (*KCTD12*, approximately 38 kb upstream).

The fourth SNP rs11013860 with *P*-value 5.15×10^{-5} located on chromosome 10 in the intronic region of Calcium channel, voltage-dependent, β-2 subunit (*CACNB2*). *CACNB2*, together with the previously identified *CACNA1C*[4] are parts of the calcium channel complex. Both *KCTD12* and *CACNB2* genes encode for ion channels.

The last SNP rs576026 showed association ($P = 7.42 \times 10^{-6}$) in the initial analysis of 1000 cases and 1000 controls in a dominant model. However, the significance of this association was considerably reduced in the joint analysis ($P = 1.51 \times 10^{-3}$).

All the top SNPs detected in this study all showed modest effect with odds ratio (OR) between 1.2 and 1.4. The highest OR was observed with SNPs on chromosomes 7 and 15 (OR = 1.4) (Table 1).

When Multipoint/haplotype analysis was conducted to detect whether any specific haplotypes were associated with bipolar I disorder, none could be found (data not shown). A total of 5700 SNPs were chosen and resulted in 16 242 150 pairs for two-point analysis. The corresponding cutoff considering Bonferroni correction was thus determined at 3.08×10^{-9}. None of the pairs showed significant interaction (P-values $< 10^{-7}$).

Comparison of our results with previous GWA studies

The large GWA collaborative study identified *ANK3, CACNA1C*, 15q14 and SNPS located in additional

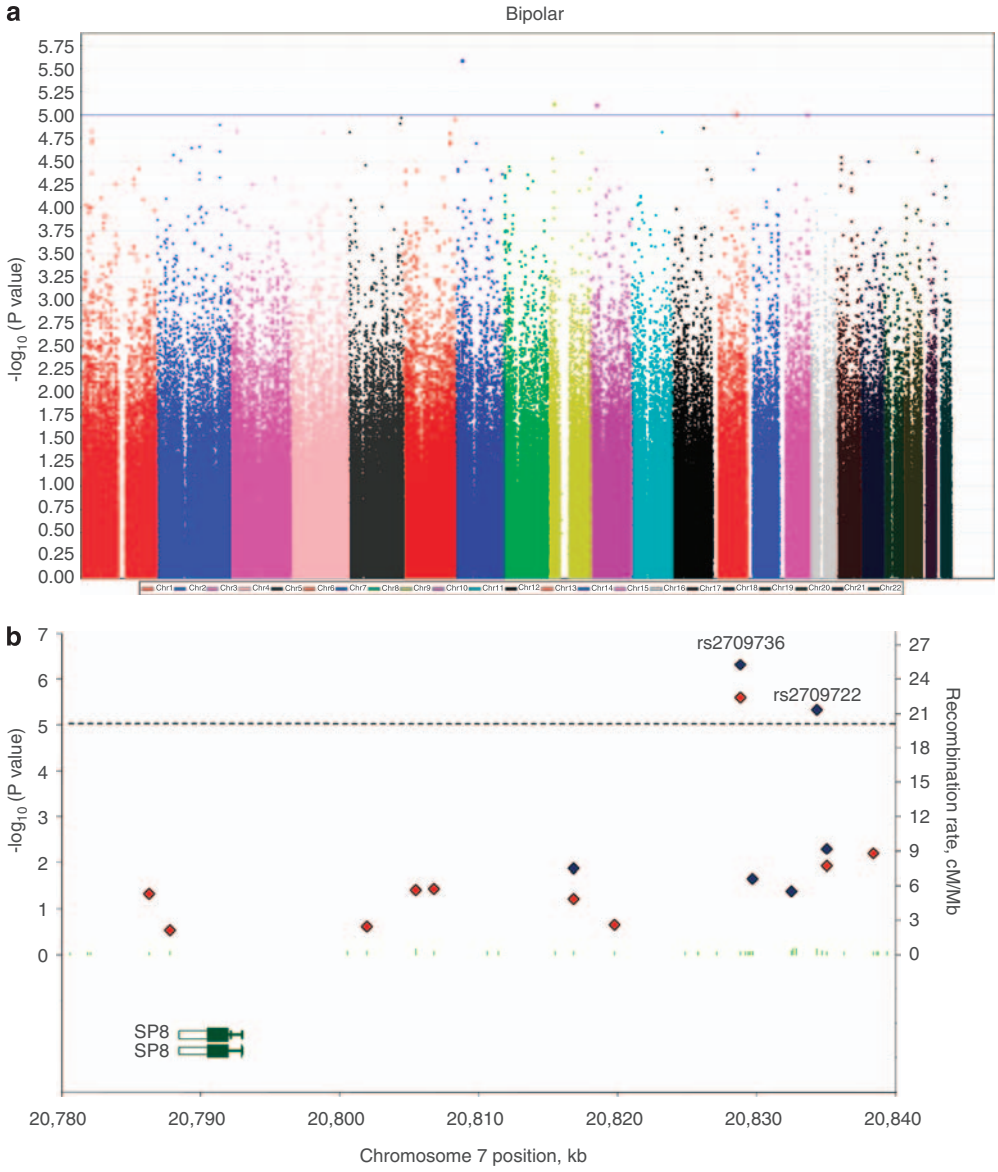

Figure 1 Results from the bipolar I disorder GWA analysis in Han Chinese. (**a**) Results of genome-wide association analysis ($-\log_{10}P$) are shown in chromosomal order for 516 919 SNPs that were tested for association in the initial sample of 1000 bipolar cases and 1000 controls. The horizontal line indicates a P-value of 10^{-5}. (**b–e**) Refined association plots surrounding each significant locus $-\log_{10}$ P-values from the Illumine 550K Duo SNPs in 1000 cases and 1000 controls are shown as red diamonds and the blue diamonds represent the additional tag SNPs genotyped. The recombination rate (right y axis) based on the Chinese HapMap population is plotted as vertical light green bars. The dashed horizontal line indicates significant association at $P = 10^{-5}$. Genes located in these regions are shown as dark horizontal green lines with the exons represented by vertical bars.

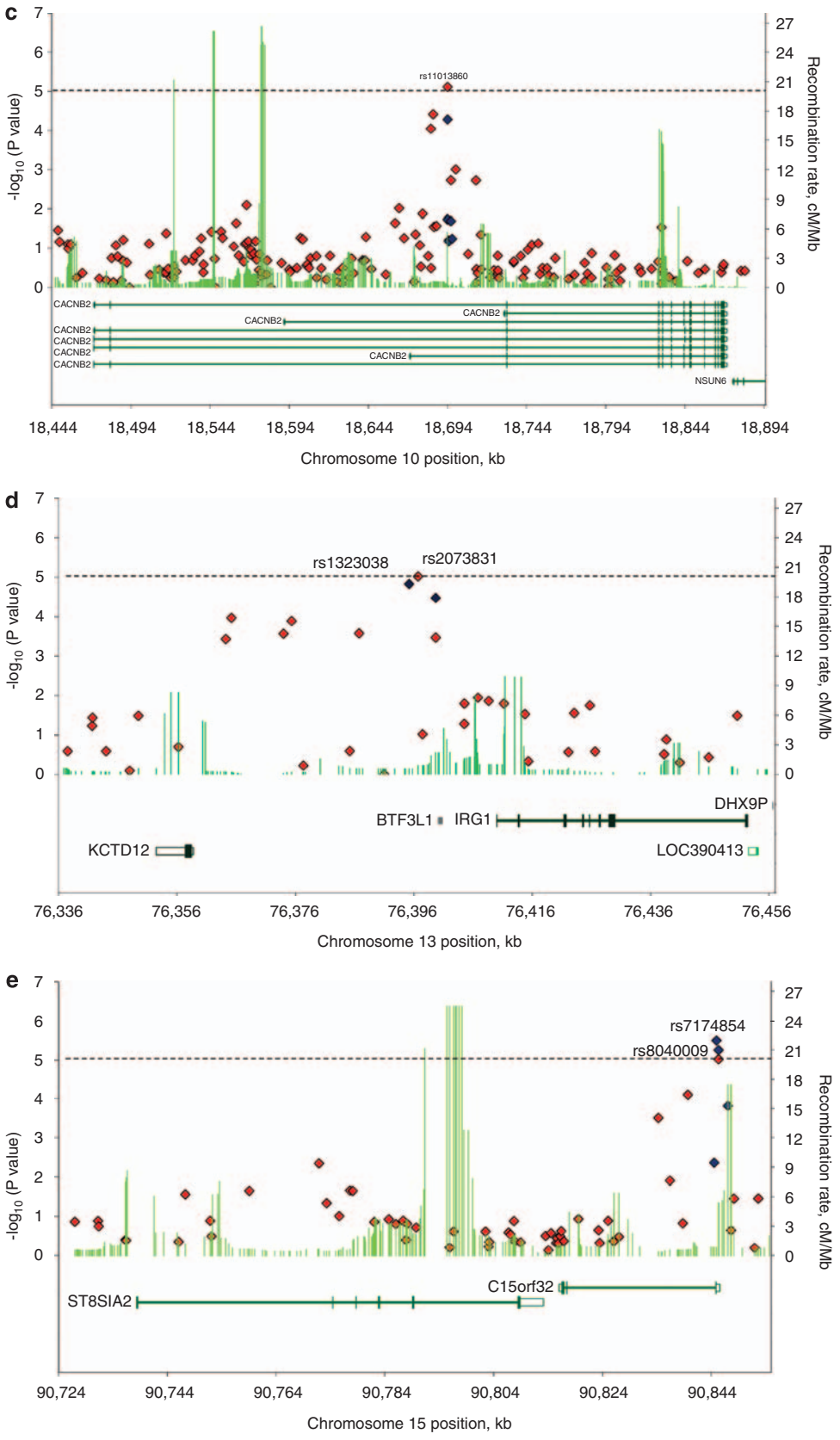

Figure 1 Continued.

18 regions with *P*-values less than 10^{-5} in Caucasian populations.[4] We examined these regions (within +/− 200 kb) in our 1000 cases and 1000 controls to identify the loci with the smallest *P*-values of association, without correction for multiple testing.

One SNP nearby the rs1938526 SNP of ANK3 gene and another SNP nearby the SNP rs11720452 in chromosome 3 reported in previous GWA studies also showed suggestive association in this study ($P = 6.55 \times 10^{-5}$ and $P = 1.48 \times 10^{-5}$, respectively). Other regions harboring three SNPs (rs3821396, rs17082664 and rs7226677) showed nominal associations (Supplementary Figure 4 and Supplementary Table 4).

We also examined the seven regions surrounding the most strongly associated SNPs ($P < 10^{-6}$) identified in a combined sample of European Americans and African Americans.[22] Only one region harboring rs7078071 showed nominal association ($P = 5.22 \times 10^{-4}$) in our Han-Chinese sample (Supplementary Table 5).

Discussion

This study reported results of the first large-scale GWA study of bipolar I disorder conducted in a non-Caucasian population, the Han Chinese. Our sample size is comparable with individual GWA studies of bipolar disorder in the Caucasian population,[2,3,5] though relatively smaller than the Euro-American collaborative GWA association analysis.[4] We have employed a cross-culturally valid and reliable standardized psychiatric interview in phenotype assessment and have produced both novel and replicative findings. Our study population consisted entirely of homogenous bipolar I patients as previous studies indicated that reliability is higher for bipolar I than for any other affective spectrum disorders.[23] Moreover, there is controversy regarding whether bipolar I and II share the same neuropsychological basis,[24,25] and genetic homogeneity of affective spectrum disorders are not supported by familial aggregation studies.[26]

Among the four suggestive significant chromosome regions identified in this study, the strongest signals were observed at two SNPs, rs2709736 and rs8040009 located on chromosome 7 and chromosome 15, respectively. These two SNPs were located near two functionally related genes.

The gene *SP8* located nearest to the rs2709736 SNP has been shown to be essential during the early specification of the cellular diversity within the frontal and motor areas of neocortex.[27] It is a crucial developmental event as the neocortical areas form the basis for sensory perception, control of movements and mediate our behavior.[28]

The gene *ST8SIA2* (previously known as *SIAT8B*) located close to rs8040009 on a hypothetical protein C15orf32, whose role in the pathogenesis of bipolar I disorder is not clear. However, *ST8SIA2* has been found to associate with both schizophrenia and bipolar disorder in one recent study using genome-wide microsatellite scan,[29] implying that *ST8SIA2* may lie in a common pathway for the development of bipolar and schizophrenia. It is a polysialytransferase, which modulates the function of neural cell adhesion molecule 1 (*NCAM1*) by catalyzing the transfer of transferpolysialic acid[30] to *NCAM*1. *NCAM1* has an important role during the development of the central nervous system and also has been shown to associate with bipolar disorder in a previous study.[31] Additional evidence to support the genetic overlap between these two diseases comes from a study, which showed that several *NCAM1* SNPs contribute differential risk for both bipolar disorder and schizophrenia by alternative splicing.[32]

The identification of these two genes might suggest that embryonic brain development/neurogenesis has an important role in the pathogenesis of bipolar I disorder. A neurodevelopmental model has been proposed for schizophrenia with the notion that early insults in the brain could impair neurocognitive development.[33]

The remaining two suggestive regions identified in this study contain genes involving the ion channel transport. The rs2073831 SNP locates between *BTF3L1* and *KCTD12*, however, the role of *BTF3L1*, a hypothetical basic transcription factor 3, like 1, has no obvious connection to the pathogenesis of bipolar I disorder. Thus, it is likely that *KCTD12*, which is highly expressed in fetal brain[34] and involved in potassium ion transport, might associate with bipolar I via ion channelopathy. The Wellcome Trust Case Control Consortium Study identified *KCNC2*, also a potassium channel, to be associated with bipolar disorder.[3] A recent study revealed that genetic variations in Na^+, K^+-ATPase (a major plasma membrane transporter for sodium and potassium) are associated with bipolar disorder, suggesting a role of potassium ion in the etiology of this disease.[35]

The other ion channel gene identified in this study is *CACNB2*, the β-subunit of calcium channel complex. A previous meta-analysis revealed that *CACNA1C*, the α-1 subunit, was strongly associated with bipolar disorder. The calcium channel complex consists of α-1, α-2/delta, β and γ-subunits. The α-1 subunit forms the pore through which ions pass into the cell, whereas the β-subunit modulates the ion flow of the channel and G protein inhibition and controls the α-1 subunit membrane targeting. Thus, polymorphism in *CACNB2*, identified in this study, could lead to ion imbalance in the brain. The altered calcium levels in cells derived from bipolar patients and the neuroprotective effects of calcium-acting mood stabilizers suggest that calcium might affect the vulnerability or impaired resilience of neurons in bipolar disorder.[36]

Among the regions implicated in previous GWA studies, two (*ANK3* and rs11720452) were found to have suggestive association in our replication study (Supplementary Figure 4 and Supplementary Table 4). This may suggest that there are common and population-specific susceptibility genes for bipolar I

disorder. As reported in the recent GWA study,[22] differential associations were found in European-American and African-American populations.

The regions identified in this study await future replication in other populations. All the GWA studies conducted so far have now strongly suggested ion-channelopathy, specifically calcium channels, is involved in the pathogenesis of bipolar I disorder, with the identification of *CACNB2* in our study and *CACNA1C* from a previous study.[4]

The major limitation of our study is that the sample size in this study only allowed us to detect a disease allele with frequency of 0.15 and odds ratio of 1.5 with a power of 0.85 (assuming a disease prevalence of 0.02, at a significant level of 10^{-7} after correcting for multiple comparisons). Hence, disease alleles with smaller effects might not be identified. Although majority of the genome was covered by the SNPs used in this study, we speculate that some disease alleles might not be tagged by these SNPs. Future GWA studies using denser SNPs in a larger sample might be warranted.

In summary, we have reported the first GWA study in the Han Chinese population for bipolar I disorder. Our data have indicated that neurodevelopmental defect and ion-channelopathy are two of the main pathological mechanisms for the development of bipolar I disorder. Further studies are required to confirm the association of the genes discovered in this study across populations; to identify the causative variants for these genes; and to elucidate the mechanisms of them.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

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References

1 Smoller JW, Finn CT. Family, twin, and adoption studies of bipolar disorder. *Am J Med Genet C Semin Med Genet* 2003; **123C**: 48–58.
2 The Welcome Trust Case Control Consortium. Genome-wide association study of 14 000 cases of seven common diseases and 3000 shared controls. *Nature* 2007; **447**: 661–678.
3 Baum AE, Akula N, Cabanero M, Cardona I, Corona W, Klemens B *et al.* A genome-wide association study implicates diacylglycerol kinase eta (DGKH) and several other genes in the etiology of bipolar disorder. *Mol Psychiatry* 2008; **13**: 197–207.
4 Ferreira MA, O'Donovan MC, Meng YA, Jones IR, Ruderfer DM, Jones L *et al.* Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. *Nat Genet* 2008; **40**: 1056–1058.
5 Sklar P, Smoller JW, Fan J, Ferreira MA, Perlis RH, Chambert K *et al.* Whole-genome association study of bipolar disorder. *Mol Psychiatry* 2008; **13**: 558–569.
6 Brotman AW, Farhadi AM, Gelenberg AJ. Verapamil treatment of acute mania. *J Clin Psychiatry* 1986; **47**: 136–138.
7 Goodnick PJ. The use of nimodipine in the treatment of mood disorders. *Bipolar Disord* 2000; **2**: 165–173.
8 Hough C, Lu SJ, Davis CL, Chuang DM, Post RM. Elevated basal and thapsigargin-stimulated intracellular calcium of platelets and lymphocytes from bipolar affective disorder patients measured by a fluorometric microassay. *Biol Psychiatry* 1999; **46**: 247–255.
9 Moller HJ. Bipolar disorder and schizophrenia: distinct illnesses or a continuum? *J Clin Psychiatry* 2003; **64**(Suppl 6): 23–27; discussion 28.
10 Tsuang MT, Winokur G, Crowe RR. Morbidity risks of schizophrenia and affective disorders among first degree relatives of patients with schizophrenia, mania, depression and surgical conditions. *Br J Psychiatry* 1980; **137**: 497–504.
11 Valles V, Van Os J, Guillamat R, Gutierrez B, Campillo M, Gento P *et al.* Increased morbid risk for schizophrenia in families of in-patients with bipolar illness. *Schizophr Res* 2000; **42**: 83–90.
12 Cardno AG, Rijsdijk FV, Sham PC, Murray RM, McGuffin P. A twin study of genetic relationships between psychotic symptoms. *Am J Psychiatry* 2002; **159**: 539–545.
13 McGuffin P, Reveley A, Holland A. Identical triplets: non-identical psychosis? *Br J Psychiatry* 1982; **140**: 1–6.
14 Badner JA, Gershon ES. Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol Psychiatry* 2002; **7**: 405–411.
15 Berrettini W. Evidence for shared susceptibility in bipolar disorder and schizophrenia. *Am J Med Genet C Semin Med Genet* 2003; **123C**: 59–64.
16 American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 4th edn. American Psychiatric Association: Washington, DC, 1994.
17 Cheng ATA, Tien AY, Chang CJ, Brugha TS, Cooper JE, Lee CS *et al.* Cross-cultural implementation of a Chinese version of the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) in Taiwan. *Br J Psychiatry* 2001; **178**: 567–572.
18 Lee CS, Liu CY, Chang CJ, Chang JC, Cheng TA, Yu WY *et al.* Inter-rater Reliability of the Chinese Version of the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) in Taiwan. *Taiwan J Psychiatry* 2002; **16**: 35–45.
19 World Health Organization. *SCAN I-Shell. Computer assisted personal interviewing application for the Schedules for Clinical Assessment in Neuropsychiatry version 2.1 and diagnostic algorithms for WHO ICD-10 Chapter V DCR and for American Psychiatric Association Diagnostic and Statistical Manual.* Version 1.0.4.6. World Health Organization: Geneva, 2005.
20 Pan WH, Fann CSJ, Wu JY, Hung YT, Ho MS, Tai TH *et al.* Han Chinese cell and genome bank in Taiwan: purpose, design and ethical considerations. *Hum Hered* 2006; **61**: 27–30.
21 Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet* 2002; **70**: 425–434.
22 Smith EN, Bloss CS, Badner JA, Barrett T, Belmonte PL, Berrettini W *et al.* Genome-wide association study of bipolar disorder in European American and African American individuals. *Mol Psychiatry* 2009; **14**: 755–763.
23 Regier DA, Kaelber CT, Roper MT, Rae DS, Sartorius N. The ICD-10 clinical field trial for mental and behavioral disorders: results in Canada and the United States. *Am J Psychiatry* 1994; **151**: 1340–1350.

24 Harkavy-Friedman JM, Keilp JG, Grunebaum MF, Sher L, Printz D, Burke AK; *et al.* Are BPI and BPII suicide attempters distinct neuropsychologically? *J Affect Disord* 2006; **94**: 255–259.
25 Fossion P, Staner L, Dramaix M, Kempenaers C, Kerkhofs M, Hubain P *et al.* Does sleep EEG data distinguish between UP, BPI or BPII major depressions? An age and gender controlled study. *J Affect Disord* 1998; **49**: 181–187.
26 Maier W, Lichtermann D, Minges J, Hallmayer J, Heun R, Benkert O *et al.* Continuity and discontinuity of affective disorders and schizophrenia. *Arch Gen Psychiatry* 1993; **50**: 871–883.
27 Zembrzycki A, Griesel G, Stoykova A, Mansouri A. Genetic interplay between the transcription factors Sp8 and Emx2 in the patterning of the forebrain. *Neural Dev* 2007; **2**: 8.
28 O'Leary DD, Sahara S. Genetic regulation of arealization of the neocortex. *Curr Opin Neurobiol* 2008; **18**: 90–100.
29 Vazza G, Bertolin C, Scudellaro E, Vettori A, Boaretto F, Rampinelli S *et al.* Genome-wide scan supports the existence of a susceptibility locus for schizophrenia and bipolar disorder on chromosome 15q26. *Mol Psychiatry* 2007; **12**: 87–93.
30 Angata K, Suzuki M, McAuliffe J, Ding Y, Hindsgaul O, Fukuda M. Differential biosynthesis of polysialic acid on neural cell adhesion molecule (NCAM) and oligosaccharide acceptors by three distinct alpha 2,8-sialyltransferases, ST8Sia IV (PST), ST8Sia II (STX), and ST8Sia III. *J Biol Chem* 2000; **275**: 18594–18601.
31 Arai M, Itokawa M, Yamada K, Toyota T, Arai M, Haga S *et al.* Association of neural cell adhesion molecule 1 gene polymorphisms with bipolar affective disorder in Japanese individuals. *Biol Psychiatry* 2004; **55**: 804–810.
32 Atz ME, Rollins B, Vawter MP. NCAM1 association study of bipolar disorder and schizophrenia: polymorphisms and alternatively spliced isoforms lead to similarities and differences. *Psychiatr Genet* 2007; **17**: 55–67.
33 Murray RM, Sham P, Van Os J, Zanelli J, Cannon M, McDonald C. A developmental model for similarities and dissimilarities between schizophrenia and bipolar disorder. *Schizophr Res* 2004; **71**: 405–416.
34 Resendes BL, Kuo SF, Robertson NG, Giersch AB, Honrubia D, Ohara O *et al.* Isolation from cochlea of a novel human intronless gene with predominant fetal expression. *J Assoc Res Otolaryngol* 2004; **5**: 185–202.
35 Goldstein I, Lerer E, Laiba E, Mallet J, Mujaheed M, Laurent C *et al.* Association between sodium- and potassium-activated adenosine triphosphatase alpha isoforms and bipolar disorders. *Biol Psychiatry* 2009; **65**: 985–991.
36 Kato T. Molecular neurobiology of bipolar disorder: a disease of 'mood-stabilizing neurons'? *Trends Neurosci* 2008; **31**: 495–503.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)