

Positive association between the *PDLIM5* gene and bipolar disorder in the Chinese Han population

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Background: Bipolar disorder is a widespread and severe brain disorder that is strongly affected by genetic factors. The *PDZ* and *LIM domain 5 (PDLIM5)* gene encodes a protein as an Enigma homologue LIM domain protein, which has been widely reported as being expressed in various brain regions. The analysis of DNA microarrays in the frontal lobes of patients with bipolar disorder has indicated changes in the expression level of *PDLIM5*, and subsequent studies have suggested that *PDLIM5* might play a role in susceptibility to bipolar disorder. We sought to examine the association between *PDLIM5* and bipolar disorder. **Methods:** We recruited 502 patients with bipolar disorder and 507 controls from Anhui Province, China. We conducted a case-control study of 4 single-nucleotide polymorphisms (SNPs) of *PDLIM5* that have been reported to be significantly associated with bipolar disorder in the Japanese and Chinese population: rs10008257, rs2433320, rs2433322 and rs2438146. **Results:** We found that rs2433322 showed significantly different frequencies between patients and controls ($p = 0.002$). Three of the SNPs, rs10008257, rs2433320 and rs2438146, showed no statistical association with bipolar disorder; however, haplotypes constructed from 3 SNPs, rs2433320, rs2433322 and rs2438146, were significantly associated with bipolar disorder (global $p = 0.004$ after Bonferroni correction). **Limitations:** Our genetic association study only offered evidence for susceptibility of *PDLIM5* to bipolar disorder, but the positive SNP rs2433322 could not indicate a direct cause of this complicated brain disorder. In addition, the 4 tagged SNPs that we selected could not cover the whole region of *PDLIM5*, thus additional reproducible studies of more SNPs in large non-Asian populations are needed. **Conclusion:** Our results suggest that *PDLIM5* might play a role in susceptibility to bipolar disorder among the Chinese Han population.

Contexte : Le trouble bipolaire est un trouble mental répandu et grave dont la composante génétique est importante. Le gène 5 des domaines *PDZ* et *LIM5 (PDLIM5)* encode une protéine sous forme de protéine homologue énigmatique du domaine LIM qui, selon de nombreux rapports, est exprimée dans diverses régions du cerveau. L'analyse de microréseaux d'ADN provenant du lobe frontal de patients atteints de trouble bipolaire a révélé la présence de variations du taux d'expression du *PDLIM5* et selon des analyses subséquentes, ce dernier pourrait jouer un rôle dans la prédisposition au trouble bipolaire. Nous avons voulu étudier le lien entre le *PDLIM5* et le trouble bipolaire. **Méthodes :** Nous avons recruté 502 patients atteints de trouble bipolaire et 507 participants témoins dans la province d'Anhui en Chine. Nous avons procédé à une étude cas-témoins sur 4 polymorphismes de nucléotides simples (PNS) du *PDLIM5* qui seraient significativement associés au trouble bipolaire dans les populations japonaises et chinoises, soit les PNS rs10008257, rs2433320, rs2433322 et rs2438146. **Résultats :** Nous avons découvert que le rs2433322 se manifestait à des fréquences significativement différentes entre les patients et les témoins ($p = 0,002$). Trois des PNS, le rs10008257, le rs2433320 et le rs2438146, n'ont présenté aucun lien statistique avec le trouble bipolaire. Toutefois, les haplotypes élaborés à partir de 3 PNS, soit le rs2433320, le rs2433322 et le rs2438146, ont été significativement associés au trouble bipolaire (p global = 0,004 après correction de Bonferroni). **Limites :** Notre étude sur ce lien génétique n'a fourni des preuves qu'en regard de la prédisposition des participants porteurs du *PDLIM5* au trouble

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J Psychiatry Neurosci 2009;34(3):199-204.

Submitted Feb. 17, 2008; Revised Apr. 7, Jun. 16, 2008; Accepted Jun. 16, 2008

bipolaire, mais un PNS rs2433322 positif n'a pas pu être considéré comme une cause directe de ce trouble mental complexe. De plus, les 4 PNS marqués qui ont été sélectionnés ne couvrant pas toute la région du *PDLIM5*, il faudra procéder à d'autres études de reproductibilité avec un plus grand nombre de PNS auprès d'importantes cohortes non asiatiques. **Conclusion** : Selon nos résultats, le *PDLIM5* pourrait jouer un rôle dans la prédisposition au trouble bipolaire dans la population chinoise d'ascendance Han.

Introduction

Bipolar disorder, or manic-depressive illness, is a frequent, severe brain disorder characterized by dramatic recurrent episodes of mania and depression that affect mood, energy and ability to function. The lifetime prevalence of bipolar disorder is 1.3%–1.6%.¹ About 1% of the world's population is affected by the illness (classically defined as bipolar I disorder), and bipolar II disorder is reported to be even more prevalent.² Lithium salts are the most effective long-term preventive treatment; however, the etiology and pathophysiology of the illness remain unknown. The role of genetic factors in bipolar disorder has been consistently supported by family, twin and adoption studies. The lifetime risk of bipolar disorder in first-degree relatives of patients with the disorder is 40%–70% for monozygotic twins and 5%–10% for all other first-degree relatives.^{3,4} Genetic research suggests that bipolar disorder, like other mental illnesses, is a complicated syndrome affected by many different genes.⁵ Genome-wide linkage studies have yielded several positive results for susceptible chromosomal loci in which some candidate genes have been identified as being associated with the illness in a variety of populations.

The *PDZ and LIM domain 5 (PDLIM5)* gene localizes on chromosome 4q22.3, a region that has been linked to bipolar disorder⁶ and schizophrenia.⁷ Iwamoto and colleagues⁸ used an oligonucleotide microarray to achieve comprehensive gene expression analysis of frontal lobes obtained from the Stanley Brain Foundation and found that the expression of the *PDLIM5* gene was significantly altered. The gene was upregulated in postmortem brains and downregulated in the lymphoblastoid cell lines of patients with bipolar disorder, schizophrenia and major depression. They further confirmed the downregulation of *PDLIM5* in lymphoblastoid cells in a replication study.⁹ Kato and colleagues¹⁰ performed an expression-level analysis in a sample of post-mortem prefrontal cortices of patients with bipolar disorder and schizophrenia obtained from the Stanley Array Collection, and they validated the upregulation of *PDLIM5*. In addition, Iga and colleagues¹¹ reported in a study on the Japanese population that mRNA levels in the peripheral leukocytes were significantly lower in medication-free patients with depression than in controls. Recently, however, Numata and colleagues¹² reported contrary results in patients with schizophrenia.

The protein encoded by *PDLIM5* is a LIM domain protein; LIM domains are cysteine-rich double zinc fingers comprising 50–60 amino acids involved in protein–protein interactions. As a member of the Enigma class of proteins, a LIM domain protein possesses a 100-amino acid PDZ domain in the N terminus and 1–3 LIM domains in the C terminus, which are

involved in cytoskeleton organization, cell lineage specification, organ development and oncogenesis. The LIM–homeobox gene family, characterized by LIM domains, plays crucial roles in neurogenesis.¹³ Moreover, the Enigma homologue LIM domain protein, as a protein kinase C binding protein, is expressed in various brain regions, most notably in the hippocampus, cortex, thalamus, hypothalamus, amygdala and cerebellum.¹⁴ The *PDLIM5* gene is also a homologue of Alzheimer disease–associated neuronal thread protein (AD7c-NTP), which is overexpressed in Alzheimer disease beginning early in the course of the disease. Overexpression of AD7c-NTP would cause neuritic sprouting and cell death.¹⁵

A number of Japanese studies investigating expression shift of *PDLIM5* and the pathogenesis of mental diseases have focused on the association with genetic variants, especially in the upstream region. Kato and colleagues¹⁰ found a positive association between 3 single-nucleotide polymorphisms (SNPs), rs10008257 (A/G), rs2433320 (A/G) and rs2438146 (C/T), and bipolar disorder; however, no association was observed in case–control analysis and family-based association analysis involving patients with schizophrenia. Subsequent studies have confirmed the negative results observed in patients with schizophrenia¹² and major depression.¹¹ Meanwhile Horiuchi and colleagues¹⁶ reported that rs2433320 and rs2433322 were significantly distinct between patients with schizophrenia and controls ($p = 0.004$). Recently, Li and colleagues¹⁷ replicated the positive result for rs2433322 but obtained a negative result for rs2433320 among patients with schizophrenia in the Chinese Han population. Liu and colleagues¹⁸ reported a positive result for rs2433320 among patients with major depression in the Chinese population. We conducted a case–control study in which we examined 4 SNPs in sequence (rs10008257, rs2433320, rs2433322 and rs2438146) to provide enhanced detection power and to investigate the possible association between SNPs of *PDLIM5* and susceptibility to bipolar disorder in the Chinese Han population.

Methods

Participants

We recruited unrelated inpatients with bipolar disorder from Anhui Province, China. Bipolar disorder had been diagnosed according to DSM-IV¹⁹ criteria. Two senior psychiatrists independently reviewed the diagnoses and psychiatric records. We recruited unrelated controls without major mental illness from the same geographic region as the patients. All participants were Chinese Han. We obtained written informed consent from all participants, and the Shanghai Ethical Committee of Human Genetic Resources reviewed and approved our study.

Single-nucleotide polymorphism genotyping

To ensure that the minor allele frequencies of the 4 polymorphisms were not too low in the Chinese Han population to distinguish between patients and controls, we checked the dbSNP database (www.ncbi.nlm.nih.gov/SNP/) and the hapmap human SNP database (www.hapmap.org/). The minor allele frequencies of rs10008257, rs2433320 and rs2433322 were 0.378, 0.178 and 0.178, respectively, in the Chinese Han population (frequency data of rs2438146 was not available).

We extracted genomic DNA from the blood using the standard phenol-chloroform method. We amplified a 325bp genomic segment for the rs10008257 (A/G) polymorphism (upstream primer 5'-GCAATCAAACCTCCAGCCACT-3', downstream primer 5'-AATATGTCCCCAGCATCAGG-3'), a 445bp genomic segment including the rs2433320 (A/G) polymorphism (upstream primer 5'-TGGAAGTGGCA-GAAGCTGTA-3', downstream primer 5'-GTGTGCCTG-TAGTCCCAGGT-3'), a 267bp genomic segment for the rs2433322 (A/G) polymorphism (upstream primer 5'-CCC-CGTAGTTGTAGGGAACA-3', downstream primer 5'-GGAATGTTCAAGTCCCTGCT-3') and a 294bp genomic segment for the rs2438146 (C/T) polymorphism (upstream primer 5'-CATGCAGATTATTCTAGGCA-3', downstream primer 5'-GGCTGAGGCAGAAGAATCAC-3'). We performed the polymerase chain reaction using the GeneAmp 9700 System (Applied Biosystems) in a 15- μ L reaction containing 10 ng genomic DNA, 1.2U Taq polymerase, 0.25 μ L of each primer (10 pM), 2.5 μ L polymerase chain reaction buffer (10x; QIAGEN) and 1.5 μ L deoxyribonucleotide triphosphates (each 2 mM). The amplification process began with an initial 10-minute denaturation at 94°C, followed by replication of 35 cycles of 30 seconds at 94°C, 40 seconds at 60°C, 40 seconds at 72°C and finally an extension period at 72°C for 7 minutes for each SNP. We incubated the polymerase chain reaction products for sequencing with 0.1 U shrimp alkaline phosphatase at 37°C for 60 minutes, followed by heat inactivation at 85°C for 20 minutes. We sequenced the treated polymerase chain reaction products using an ABI Prism BigDye Terminator Cycle Sequencing

Kit, version 3.1 on an ABI Prism 3100 sequencer (Applied Biosystems).

We performed a duplicate quality-control test (48 samples for each SNP), with 100% concordance.

Statistical analysis

We conducted Hardy-Weinberg equilibrium tests, allele and genotype frequency analysis online on a robust and user-friendly software platform (<http://analysis.bio-x.cn/>)²⁰ developed by our laboratory. We estimated linkage disequilibrium measured with standardized D' , and we compared the discrepancies of allele and genotype frequencies on single loci between patients and controls using a Monte Carlo simulation strategy,²¹ a χ^2 test and odds ratios (ORs). We used Bonferroni correction for multiple tests of all SNPs and haplotypes in patients and controls. We performed post hoc power calculations with pre-established α error probability, sample size and effect size using the G*Power program.²² All reported p values are 2-tailed. We set statistical significance at $p < 0.05$.

Results

Participants

We included 502 patients with bipolar disorder (281 men and 221 women) and 507 controls (287 men and 220 women) in our study. The mean age of patients was 37.82 (standard deviation [SD] 12.69) years, and the average age at onset of disease was 26.76 (SD 10.57) years. The mean age of controls was 36.29 (SD 7.23) years.

Genotyping

Genotypic distributions of the 4 SNPs in patients and controls were in Hardy-Weinberg equilibrium ($p > 0.05$). We observed a significant difference in allele and genotype frequencies between patients and controls at rs2433322 ($p = 0.002$, OR 1.51, 95% confidence interval [CI] 1.17–1.97, $p = 0.018$ after Bonferroni correction [$\times 10$]) (Table 1). The frequency of the G allele of rs2433322 was greater among patients than

Table 1: Allele and genotype frequencies of 4 single-nucleotide polymorphisms among patients and controls

SNP; group	Allele, no. (frequency)		p_1 value	OR (95% CI)	Genotype, no. (frequency)			p_2 value
rs10008257	A	G	0.19	1.13 (0.94–1.36)	A/A	A/G	G/G	0.41
	Patient	408 (0.415)			576 (0.585)	84 (0.171)	240 (0.488)	
Control	362 (0.385)	578 (0.615)	70 (0.149)	222 (0.472)	178 (0.379)			
	rs2433320	A	G	0.44	1.10 (0.86–1.41)	A/A	A/G	G/G
Patient	166 (0.166)	836 (0.834)	10 (0.020)			146 (0.291)	345 (0.689)	
Control	137 (0.153)	761 (0.847)	11 (0.024)	115 (0.256)	323 (0.719)			
	rs2433322	A	G	0.002	1.51 (1.17–1.97)	A/A	A/G	G/G
Patient	759 (0.814)	173 (0.186)	314 (0.674)			131 (0.281)	21 (0.045)	
Control	711 (0.869)	107 (0.131)	311 (0.760)	89 (0.218)	9 (0.022)			
	rs2438146	C	T	0.23	1.18 (0.90–1.53)	C/C	C/T	T/T
Patient	844 (0.858)	140 (0.142)	362 (0.736)			120 (0.244)	10 (0.020)	
Control	829 (0.876)	117 (0.124)	366 (0.774)	97 (0.205)	10 (0.021)			

CI = confidence interval; OR = odds ratio; SNP = single-nucleotide polymorphism.

controls (18.6% v. 13.1%). Bipolar disorder can be further subdivided into bipolar disorder I and II. Allowing for distinct diagnostic features and mood patterns, we studied the relation between controls and patients with bipolar I disorder, and the positive association remained (Table 2).

The estimation of linkage disequilibrium for all pairs of SNP markers showed strong linkage disequilibrium ($D' > 0.8$ and $r^2 > 0.6$) for rs2433320, rs2433322 and rs2438146 (Table 3). We analyzed only the common haplotypes (frequency > 0.03). Haplotype analysis of 4 SNPs reported some

significant global p values (Table 4) and haplotype frequency discrepancies (Table 5). Two 2 SNP-based and two 3 SNP-based haplotypes were significantly associated with bipolar disorder even after strict Bonferroni correction ($\times 50$). The most significant SNP markers were rs2433320, rs2433322 and rs2438146 ($p = 0.004$ after Bonferroni correction [$\times 50$]) and the haplotype G-G-C (rs2433320-rs2433322-rs2438146) was observed to be strongly associated with patients ($p < 0.001$, OR 8.27, 95% CI 2.55–26.79, $p = 0.001$ after Bonferroni correction [$\times 50$]).

Table 2: Allele and genotype frequencies of 4 single-nucleotide polymorphisms among patients with bipolar disorder I and controls

SNP; group	Allele, no. (frequency)		p_1 value	OR (95% CI)	Genotype, no. (frequency)			p_2 value
rs10008257	A	G	0.18	1.14 (0.94–1.38)	A/A	A/G	G/G	0.38
	Patient	345 (0.417)			483 (0.583)	70 (0.169)	205 (0.495)	
Control	A	G	0.28	1.15 (0.89–1.49)	A/A	A/G	G/G	0.25
	Patient	144 (0.172)			694 (0.828)	8 (0.019)	128 (0.305)	
rs2433320	A	G	0.002	1.52 (1.16–1.20)	A/A	A/G	G/G	0.013
	Patient	633 (0.814)			145 (0.186)	262 (0.674)	109 (0.280)	
Control	A	G	0.24	1.18 (0.90–1.55)	A/A	A/G	G/G	0.33
	Patient	711 (0.869)			107 (0.131)	311 (0.760)	89 (0.218)	
rs2438146	C	T	0.002	1.52 (1.16–1.20)	C/C	C/T	T/T	0.013
	Patient	708 (0.857)			118 (0.143)	303 (0.734)	102 (0.247)	
Control	C	T	0.24	1.18 (0.90–1.55)	C/C	C/T	T/T	0.33
	Patient	829 (0.876)			117 (0.124)	366 (0.774)	97 (0.205)	

CI = confidence interval; OR = odds ratio; SNP = single-nucleotide polymorphism.

Table 3: Estimation of linkage disequilibrium (D' and r^2 value) among the 4 single-nucleotide polymorphisms*

$D' \setminus r^2$	rs10008257	rs2433320	rs2433322	rs2438146
rs10008257		0.014	0.018	0.005
rs2433320	0.333		0.659	0.605
rs2433322	0.379	0.823		0.637
rs2438146	0.226	0.859	0.899	

SNP = single-nucleotide polymorphism.

*For each pair of SNPs, r^2 and D' values are shown above and below the diagonal respectively; $D' > 0.8$ and $r^2 > 0.6$ are in bold.

Table 4: Global p values of estimated haplotypes of the single-nucleotide polymorphisms

SNPs, no.	Haplotype	Global p value
2	rs10008257 – rs2433320	0.58
	rs2433320 – rs2433322	0.006*
	rs2433322 – rs2438146	0.010*
3	rs10008257 – rs2433320 – rs2433322	0.045*
	rs2433320 – rs2433322 – rs2438146	0.004*
4	rs10008257 – rs2433320 – rs2433322 – rs2438146	0.21

SNP = single-nucleotide polymorphism.

*Global p value after Bonferroni correction ($\times 50$); statistical significance set at $p < 0.05$.

Table 5: Estimated haplotype frequencies and p values among patients and controls

SNPs, no.	Haplotype				Frequency, %		p value	p value*	OR (95% CI)
	rs10008257	rs2433320	rs2433322	rs2438146	Patient	Control			
2		A	A		1.7	3.1	0.05	NS	0.53 (0.28–1.01)
		G	A		79.7	83.8	0.029	NS	0.76 (0.59–0.97)
		G	G		4.0	1.1	< 0.001	0.007	3.95 (1.85–8.44)
3			A	C	80.0	86.3	0.003	NS	0.67 (0.51–0.87)
			G	C	5.8	2.1	< 0.001	0.005	2.95 (1.68–5.20)
	A	A	G		3.9	2.1	0.031	NS	1.92 (1.05–3.50)
	G	G	A		44.5	49.1	0.035	NS	0.81 (0.67–0.99)
	G	G	G		3.1	0.7	< 0.001	0.029	4.40 (1.76–11.04)
		G	A	C	78.6	83.6	0.010	NS	0.68 (0.51–0.91)
4		G	G	C	3.2	0.4	< 0.001	0.001	8.27 (2.55–26.79)
	A	A	G	T	3.7	2.2	0.05	NS	1.80 (0.99–3.29)

CI = confidence interval; NS = no significance after Bonferroni correction; OR = odds ratio; SNP = single nucleotide polymorphism.

* p values after Bonferroni correction ($\times 50$).

In power calculations using the G*Power 3 program, we found that the sample size had greater than 98% power for rs10008257, greater than 90% for rs2433320 and greater than 84% for rs2438146 to detect a relatively weak gene effect (OR 1.3) at $\alpha \leq 0.05$.

Discussion

According to DSM-IV criteria, bipolar disorder is characterized by chronic and severe recurrent episodes of mania and depression. This phenomenon is likely attributable to turbulence in nerve regulation in which some key genes play important roles. Research based on family, twin and adoption studies have established a genetic contribution to susceptibility to mental illnesses. The largest and most methodologically rigorous bipolar disorder twin study was conducted by Bertelsen and colleagues²³ using the Danish Twin Register. They found that the proband-wise concordance (the proportion of proband twins with bipolar disorder who had a twin with bipolar disorder) in monozygotic twins was 0.62, whereas the comparable figure for dizygotic twins was 0.08. Over the past decade, association studies on *DAOA*, *DTNBP1*, *COMT*, *BDNF*, *DSC1* and *PDLIM5* genes have suggested possible relations between allele and genotype frequencies and psychopathology.

The protein encoded by *PDLIM5*, known as Enigma homologue LIM domain protein, contains 1 PDZ domain and 3 LIM domains. It is known to interact with N-type calcium channels and protein kinase C.¹⁴ Protein kinase C is a common target of mood stabilizers, such as lithium and valproate, which are widely used in the long-term treatment of bipolar disorder.²⁴ The potential function of *PDLIM5* is to act as an adaptor for the PKC-ENH-N-type calcium channel complex, which is the molecular foundation of specificity and efficiency of cellular signalling in the form of a kinase-substrate complex. Therefore, *PDLIM5* may play an essential role in the process of regulation of the nervous system by interfering with the molecular cascade from protein kinase C to the calcium channel that controls intracellular calcium levels.

The identification of *PDLIM5* as a candidate gene for bipolar disorder has been linked to the expression shift in the brain, found frequently in Japanese patients.⁸⁻¹⁰ Further, genetic association analysis has suggested that polymorphisms of *PDLIM5* are associated with the risk of mental illness. Kato and colleagues¹⁰ examined a series of SNPs in the *PDLIM5* gene and reported 2 in the upstream region with significant associations with bipolar disorder in 2 independent samples. Those phenomena might be attributable to SNPs impacting the binding of trans-acting factors in the transcription process.

Our study provides further support for the association between *PDLIM5* and bipolar disorder. After genotyping 4 SNPs within the *PDLIM5* locus in a Chinese Han sample, we found significantly different allele and genotype frequencies for rs2433322 between patients and controls. Other associations were negative. In addition, to allow for distinct diagnostic features, we repeated the analysis for patients

with bipolar I disorder and also found positive associations for rs2433322. The sample of patients with bipolar II disorder was so small that we did not repeat the analysis for this subgroup. Our data suggest that the high frequency of the G allele for rs2433322 might be a risk factor for bipolar disorder.

Haplotypes constructed by contiguous SNPs will increase the statistical power for association with the disease. The estimation of linkage disequilibrium showed that rs2433320, rs2433322 and rs2438146 had strong linkage disequilibrium ($D' > 0.8$ and $r^2 > 0.6$). Further analysis of 2-, 3- and 4 SNP-based haplotypes showed some significant global associations with bipolar disorder even after strict Bonferroni correction, which was necessary but overly conservative for multiple testing (Table 4). The most significant haplotype was G-G-C (rs2433320-rs2433322-rs2438146; $p = 0.001$ after Bonferroni correction). When we removed the rs2433322 locus and examined the haplotype rs2433320-rs2438146, we observed no positive result, which suggests that the results of haplotype analysis were mainly impacted by rs2433322.

Although the Chinese and Japanese populations are genetically close, heterogeneity is still a factor, especially for a complicated mental disease. Our study suggested that polymorphisms in the upstream region of *PDLIM5* might not play a major role in susceptibility to bipolar disorder; however, we found that the G allele of rs2433322 was a risk allele and *PDLIM5* might be related to bipolar disorder. It is possible that the G allele of rs2433322 is involved in impaired *PDLIM5* function, or that the SNP is in high linkage disequilibrium with some unknown functional variants. Although the G allele of rs2433322 has higher frequency among patients than controls, we cannot conclude that *PDLIM5* is a disease gene. Unlike a single-gene disease, bipolar disorder should be considered to be associated with contributions from a series of susceptibility alleles and genes. The molecular variation may play a role in a complex multicomponent network and contribute in an additive way to the final disease phenotype. In addition, association studies are mainly concerned with the minor effect of genes or genotypes, so samples have to be large enough and have sufficient statistical power to deliver satisfactory results. Previous studies with positive results have been based on several relatively small samples of no more than 300 participants, so further studies should have larger and more varied samples. Our investigation on *PDLIM5* constitutes the largest sample to date relating to bipolar disorder in the Chinese population.

Limitations

Our study had some limitations. One is that the positive SNP, rs433322, is not likely to have a direct effect on bipolar disorder. In addition, the 4 tagged SNPs that we selected could not cover the whole region of the *PDLIM5* gene, thus additional replication studies using more SNPs in large non-Asian populations are needed.

In conclusion, our case-control study provided consistent evidence that *PDLIM5* might play a potential role in the susceptibility to bipolar disorder in the Chinese Han population.

We hope it may act as a reference point for further replication studies on *PDLIM5* and bipolar disorder in other ethnic groups and for the comprehensive meta-analyses that are required for validation.

Acknowledgements: This work was supported by grants from the Chinese Academy of Sciences (KSCX2-YW-R-01), the National Natural Science Foundation of China, the national S973 and 863 Programs, and the Shanghai Municipal Commission for Science and Technology. Shanghai Leading Academic Discipline Project (B205). We appreciate the contribution of all of the members participating in this study, as well as of the psychiatrists who helped us in the diagnosis.

Competing interests: None declared.

Contributors: Drs. Zhao, Liu, Zhou, Zhang, Chen, Feng, Yu and He designed the study. Drs. P. Wang, Li, Xu and Feng acquired the data, which Drs. Zhao, Liu, Zhou, Zhang, Chen and T. Wang analyzed. Dr. Zhao wrote the article, which all other authors reviewed. All authors approved the final version for publication.

References

- Muller-Oerlinghausen B, Berghofer A, Bauer M. Bipolar disorder. *Lancet* 2002;359:241-7.
- Belmaker RH. Bipolar disorder. *N Engl J Med* 2004;351:476-86.
- Craddock N, Jones I. Genetics of bipolar disorder. *J Med Genet* 1999;36:585-94.
- Potash JB, DePaulo JR Jr. Searching high and low: a review of the genetics of bipolar disorder. *Bipolar Disord* 2000;2:8-26.
- Hyman SE. Introduction to the complex genetics of mental disorders. *Biol Psychiatry* 1999;45:518-21.
- Dettera-Wadleigh SD, Badner JA, Yoshikawa T, et al. Initial genome scan of the NIMH genetics initiative bipolar pedigrees: chromosomes 4, 7, 9, 18, 19, 20, and 21q. *Am J Med Genet* 1997;74:254-62.
- Mowry BJ, Ewen KR, Nancarrow DJ, et al. Second stage of a genome scan of schizophrenia: study of five positive regions in an expanded sample. *Am J Med Genet* 2000;96:864-9.
- Iwamoto K, Kakiuchi C, Bundo M, et al. Molecular characterization of bipolar disorder by comparing gene expression profiles of postmortem brains of major mental disorders. *Mol Psychiatry* 2004;9:406-16.
- Iwamoto K, Bundo M, Washizuka S, et al. Expression of HSPF1 and LIM in the lymphoblastoid cells derived from patients with bipolar disorder and schizophrenia. *J Hum Genet* 2004;49:227-31.
- Kato T, Iwayama Y, Kakiuchi C, et al. Gene expression and association analyses of LIM (PDLIM5) in bipolar disorder and schizophrenia. *Mol Psychiatry* 2005;10:1045-55.
- Iga J, Ueno S, Yamauchi K, et al. Gene expression and association analysis of LIM (PDLIM5) in major depression. *Neurosci Lett* 2006;400:203-7.
- Numata S, Ueno S, Iga J, et al. Gene expression in the peripheral leukocytes and association analysis of PDLIM5 gene in schizophrenia. *Neurosci Lett* 2007;415:28-33.
- Shawlot W, Behringer RR. Requirement for Lim1 in head-organizer function. *Nature* 1995;374:425-30.
- Maeno-Hikichi Y, Chang S, Matsumura K, et al. A PKC epsilon-ENH-channel complex specifically modulates N-type Ca²⁺ channels. *Nat Neurosci* 2003;6:468-75.
- Wu M, Li Y, Ji C, et al. Cloning and identification of a novel human gene PDLIM5, a homolog of AD-associated neuronal thread protein (AD7c-NTP). *DNA Seq* 2004;15:144-7.
- Horiuchi Y, Arai M, Niizato K, et al. A polymorphism in the PDLIM5 gene associated with gene expression and schizophrenia. *Biol Psychiatry* 2006;59:434-9.
- Li C, Tao R, Qin W, et al. Positive association between PDLIM5 and schizophrenia in the Chinese Han population. *Int J Neuropsychopharmacol* 2008;11:27-34.
- Liu Z, Liu W, Xiao Z, et al. A major single nucleotide polymorphism of the PDLIM5 gene associated with recurrent major depressive disorder. *J Psychiatry Neurosci* 2008;33:43-6.
- American Psychiatric Association. *Diagnostic and statistical manual of mental disorders*. 4th ed. Washington (DC): The Association; 1994.
- Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res* 2005;15:97-8.
- Sham PC, Curtis D. Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. *Ann Hum Genet* 1995;59:97-105.
- Faul F, Erdfelder E, Lang AG, et al. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 2007;39:175-91.
- Bertelsen A, Harvald B, Hauge M. A Danish twin study of manic-depressive disorders. *Br J Psychiatry* 1977;130:330-51.
- Manji HK, Lenox RH. Ziskind-Somerfeld Research Award. Protein kinase C signaling in the brain: molecular transduction of mood stabilization in the treatment of manic-depressive illness. *Biol Psychiatry* 1999;46:1328-51.