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***PDLIM5* and susceptibility to bipolar disorder: a family-based association study and meta-analysis**

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Abstract

Objectives—The postsynaptic density-95/discs large/zone occludens-1 (PDZ) domain and LIM (Lin-11, Isl-1, and Mec-3) domain 5 (*PDLIM5*) gene has been analyzed as a candidate gene for both schizophrenia (SZ) and bipolar disorder (BP) in Japanese samples. We performed a family-based association study to test the hypothesis that variants in *PDLIM5* increase susceptibility to BP in European Americans and a meta-analysis to clarify whether there is a single marker consistently contributing to risk for BP.

Methods—Five single nucleotide polymorphisms (SNPs) in the *PDLIM5* gene were genotyped in 290 European American BP families. Programs Sibling-Transmission/Disequilibrium Test (sib_tdt) and PDTPhase were used for allelic and haplotypic association, respectively. We carried out a meta-analysis combining our family-based data and case-control data from two Japanese sample sets and from two genome-wide association (GWA) studies.

Results—Our association analysis showed no single SNP associated with BP. A rare haplotype consisted of rs10008257 and rs2433320 had nominal association ($p = 0.045$), which failed to survive correction for multiple tests. The meta-analysis identified a significant allelic association at rs2433320 in all combined samples (excluding overlapped samples in GWA: overall OR = 0.897, 95% CI: 0.838–0.961, adjusted $p = 0.012$) and in all Caucasian samples (excluding overlapped samples in GWA: overall OR = 0.905, 95% CI: 0.843–0.971, adjusted $p = 0.032$), but not in the Japanese samples.

Conclusion—*PDLIM5* may play a minor effect on susceptibility to bipolar disorder in Caucasians. The findings in Japanese need further confirmation in larger independent samples.

Keywords

PDLIM5; bipolar disorder; association study; haplotype; single nucleotide polymorphism

Introduction

The *PDLIM5* gene encodes an enigma-homologue (ENH) protein containing a postsynaptic density-95/discs large/zone occludens-1 (PDZ) domain and three LIM (Lin-11, Isl-1, and Mec-3) domains (Ueki *et al.*, 1999). There are two splicing isoforms of *PDLIM5* gene. The long gene spans 216.3 Kb with one non-coding and 12 coding exons while the short gene spans 136.3 Kb with one non-coding and 9 coding exons. Several lines of evidence suggest *PDLIM5* as a candidate gene for both schizophrenia (SZ) and bipolar disorder (BP). First, *PDLIM5* is significantly upregulated in postmortem brains of patients with SZ or BP from the Stanley Medical Research Institute's (SMRI) brain collections (Iwamoto *et al.*, 2004b; Kato

et al., 2005) and abnormally expressed in the peripheral leukocytes derived from patients with each disorder (Iwamoto *et al.*, 2004a; Numata *et al.*, 2007). Second, *PDLIM5* is an adomer protein interacting with N-type calcium channel (Maeno-Hikichi *et al.*, 2003), a signaling pathway implicated in the pathophysiology of BP (Warsh *et al.*, 2004). Third, *PDLIM5* is mapped to 4q22, a potential linkage region of BP (Detera-Wadleigh *et al.*, 1997) and SZ (Mowry *et al.*, 2000).

Recently, *PDLIM5* was reported to be associated with SZ in Japanese (Horiuchi *et al.*, 2006). However, other two Japanese groups failed to confirm this association (Kato *et al.*, 2005; Numata *et al.*, 2007). Kato *et al.* (Kato *et al.*, 2005) reported that two adjacent SNPs, rs10008257 and rs2433320, were associated with BP in two independent Japanese sample sets, respectively. In addition, the haplotype containing these two SNPs was also associated with disease in the combined sample (Kato *et al.*, 2005).

To investigate whether *PDLIM5* also increase susceptibility to BP in European Americans, we performed a family-based association. To see whether there is a consistent allelic association across different ethnic samples, we carried out a meta-analysis combining our family-based data and case-control data from two Japanese sample sets and from two latest genome-wide association (GWA) studies (Baum *et al.*, 2008; Wellcome Trust Case Control Consortium 2007).

Materials and methods

Subjects

A total of 1103 samples from 290 families were recruited in the association study. The participants included 831 samples from the National Institute of Mental Health (NIMH) Genetics Initiative for Bipolar Disorder Waves 1-4 series (37 trios and 180 quads), 45 samples from the Clinical Neurogenetics (CNG) series (7 trios and 6 quads), and 227 samples from the Chicago-Hopkins-Intramural Project (CHIP) series (13 trios and 47 quads). Most of the CNG and NIMH wave 1-2 samples were included in a previous linkage study and showed linkage to 4q22 (Detera-Wadleigh *et al.*, 1997). All patients met DSM-III-R or DSM-IV criteria for bipolar or schizoaffective bipolar type. All samples are Caucasian descendants identified by self-reported ethnicity information and further confirmed by STRUCTURE (Pritchard *et al.*, 2000) analysis using 254 unlinked SNPs (data not shown).

SNP selection and genotyping

A series of bioinformatics tools developed in our laboratory (<http://bioinfo.bsd.uchicago.edu/index.html>) were used to select tag SNPs (tSNPs) and described elsewhere (Shi *et al.*, 2007). Briefly, we first downloaded the 30 CEPH trios' HapMap genotype data within the 256.3-kb region contains the *PDLIM5* gene and 20 kb upstream and downstream (Rel21a/phaseII Jan07, on NCBI B35 assembly, dbSNP b125). SNPs with minor allele frequency (MAF) greater than 0.1 in individuals of European descent from the HapMap Project were examined for linkage disequilibrium (LD). Then, the ldSelect algorithm was used to select tSNPs to represent SNPs in LD with each other (Carlson *et al.*, 2004). This approach clusters the SNPs into different LD bins according to the pairwise correlation coefficient r^2 values. A criterion of $r^2 \geq 0.85$ was chosen to define the bins. Our analysis generated 19 tSNPs and 13 singleton SNPs (only one SNP in each bin). However, in the present replication study, we selected only rs10008257 and rs2433320 (significant association with BP (Kato *et al.*, 2005) or SZ (Horiuchi *et al.*, 2006)), one tSNPs (tagging rs11732668, which showed nominal association with SZ (Horiuchi *et al.*, 2006)), and two other tSNP for a better physical coverage of the gene region.

Genotyping was carried out using TaqMan 5' exonuclease assays (Applied Biosystems Inc., Foster City, CA, USA). We used PedCheck1.1 (O'Connell and Weeks 1998) to detect any Mendelian inconsistency or genotyping error, and Merlin (Abecasis *et al.*, 2002) to detect unlikely genotypes. All genotype errors were manually resolved by checking the raw genotype data, either assigning a genotype as missing (if unable to be resolved) or correcting the genotype prior to statistical analysis. The average genotyping success rate was > 97.3%.

Statistical analyses

Association—Tests for Hardy-Weinberg equilibrium (HWE) of genotype distribution in founders (unrelated parents in this study) were performed with the software PEDSTATS (Wigginton and Abecasis 2005). Pairwise D' and r^2 linkage disequilibrium (LD) between any two markers was analyzed using the program Haploview (version 4.0) (Barrett *et al.*, 2005). Haplotype blocks were identified using the solid spine of LD method in Haploview (Barrett *et al.*, 2005). Allelic association of individual SNPs was tested using the Sibling-Transmission/Disequilibrium Test (sib_tdt) program in the program package ASPEX 2.5 (<http://aspex.sourceforge.net/>). Haplotypic association tests were carried out using PDTPHASE in the program package UNPHASED (Dudbridge 2003). PDTPHASE analyzes whole pedigrees for association, correcting for the effects of linkage. It gives results for the individual haplotypes and a global probability value for a particular combination of SNPs. Given an association with schizophrenia shown in a previous study (Horiuchi *et al.*, 2006), we also analyzed our data based on the presence of psychosis in affected offspring.

Meta-analysis—We performed a meta-analysis of SNPs rs10008257 and rs2433320, combining our family-based association data and case-control data from the Japanese sample sets (Kato *et al.*, 2005) and two latest GWA studies (Baum *et al.*, 2008; Wellcome Trust Case Control Consortium 2007). The NIMH samples in Baum *et al.*'s GWA study (Baum *et al.*, 2008) overlap substantially with ours, thus data from these two samples sets was separately combined with other data.

Counts of alleles in case and control groups in population-based studies were summarized in two-by-two tables, and numbers of transmitted alleles or haplotypes from heterozygous parents to affected offspring in family-based studies were summarized in two-by-one tables. Odds ratios (ORs) and their 95% confidence intervals (CIs) and standard errors (SEs) were calculated based on the allele or haplotype data (Kazeem and Farrall 2005). Cochran's chi-square-based Q-statistic test was applied to assess between-study heterogeneity. Q is distributed approximately as a chi-square statistic with $k-1$ degrees of freedom (k = number of studies). Overall ORs and their 95% CIs were estimated under the Mantel-Haenszel's fixed-effects model (MANTEL and HAENSZEL 1959) if there is no evidence of between-study heterogeneity, otherwise using DerSimonian-Laird's random-effects model (DerSimonian and Laird 1986). Significance of overall OR was examined using a Z-test. For a sensitivity analysis, each individual study was removed in turn from the analysis, and meta-analysis was re-performed. This approach can determine whether a finding in the meta-analysis is due to contribution of a single study. Evidence for publication bias was assessed using the Egger's regression asymmetry statistics with a funnel plot of natural logarithm of OR (LnOR) against inverse SE in each study (Egger *et al.*, 1997). The meta-analyses were carried out using the program Meta-analysis with Interactive eXplanations (MIX, version 1.54) (Bax *et al.*, 2006).

To detect potential effects of different samples of ancestry (European versus Asian), overall ORs and SEs were obtained for each group, then heterogeneity of overall ORs between different ancestries was assessed using a chi-square test with 1 degree of freedom, similar to the method introduced by Kazeem and Farrall (Kazeem and Farrall 2005).

A Fisher chi-square method (Fisher 1954) was used to pool global p -values of haplotype associations across 3 sample sets. This method is based on the additivity of independent chi-squares, and on the fact that when a null hypothesis is true, the statistics is distributed as chi-square with 2 degree of freedom.

A significance level was set at $P = 0.1$ in the Q-test and Egger's test. Two SNPs and 4 haplotypes were meta-analyzed. The precise number of multiple tests is difficult to calculate since the results seem to be correlated. We used a conservative Bonferroni correction for 6 tests ($p = 0.0083$) to test the significance of overall ORs.

Results and discussion

No deviation from HWE was found in the founders (data not shown). The genomic positions and LD plot of 5 SNPs tested are shown in Figure 1. The results of association analyses are summarized in Table 1. The SNP rs10008257 reveals a borderline association with BP ($p = 0.068$). A rare haplotype consisted of rs10008257 and rs2433320 shows nominal association ($p = 0.045$), which is not significant after correction for multiple tests (Bonferroni corrected $p = 0.225$). Borderline association signals are also seen at the common haplotype GG in individual haplotype association analysis and in a global analysis (Table 1). This rs10008257-rs2433320 haplotype block shows a global p -value of 0.001 in the original Japanese "MDMD" samples set (Kato *et al.*, 2005), and gives nearly significant global p -values in the replication "MPS" samples of Japanese ($p = 0.099$) (Kato *et al.*, 2005) as well as in our study in European American ($p = 0.071$). The Fisher p -value that combined global p -values of the rs10008257-rs2433320 haplotype analyses from 3 samples sets is 0.0006. However, the associated haplotype is not the same across all three studies. This makes the significance of this finding difficult to interpret. There is no evidence of association in the subsample with psychotic symptoms (data not shown).

The allele A at rs2433320 has a disease-protective role in our meta-analysis under the fixed-effects model (Table 2). This association survives a conservative Bonferroni correction for 6 tests for 4 haplotypes and 2 SNPs in our meta-analysis. When the NIMH samples in the GWA study is excluded, the overall ORs are 0.905 (corrected $p = 0.032$) in Caucasian samples and 0.897 (corrected $p = 0.012$) in all samples (Caucasians plus Japanese). When our data, which includes partial NIMH samples, is excluded, the overall ORs are 0.903 (corrected $p = 0.018$) in Caucasian samples and 0.897 (corrected $p = 0.007$) in all samples. No heterogeneity between samples from two different ancestries and no publication bias was found by Egger's regression analyses (data not shown). No allelic association was found in the combined Japanese sample (overall OR = 0.779, 95% CI, 0.580-1.046, unadjusted $p = 0.097$), may due to significant between-study heterogeneity ($p_Q = 0.087$) and underpowered sample size. Therefore, the relationship of rs2433320 with bipolar disorder in Japanese needs further clarification in larger independent Japanese samples.

We did not detect significant haplotypic disease-association under either the random-effects model, or the fixed-effects model (Table 2).

Several limitations exist in the present study. First, 5 SNPs have poor coverage for the gene region (about 50 Kb/SNP). As shown above, at least 19 tSNPs and 13 singletons at the *PDLIM5* gene region should be analyzed to test the Common Disease/Common Variants (e.g. MAF > 0.1) (CV/CD) hypothesis in BP. In addition, two common SNPs with potential functional effects on the gene (the synonymous mutation Gln170Gln/rs11097431 and the missense mutation Ala272Thr /rs7690296) should be considered too, although they are respectively tagged by rs2452594 ($r^2 = 0.847$, LOD = 18.8) and rs1543013 ($r^2 = 1$, LOD = 32.8) in the Caucasian samples in the HapMap project, which have been tested in our study.

Secondly, rare variants may also account for susceptibility to common disease (the Common Disease/Multiple Rare Variants (CD/MRV) hypothesis) (Pritchard 2001; Pritchard and Cox 2002), which has received experimental support (Cohen *et al.*, 2005; Cohen *et al.*, 2004; Cohen *et al.*, 2006; Fearnhead *et al.*, 2004; Johnson *et al.*, 2007; Meyer *et al.*, 2005; Romeo *et al.*, 2007; Zhu *et al.*, 2005). Resequencing the *PDLIM5* gene region may help identify rare variants that confer susceptibility to BP. Finally, our sample has limited capacity to detect alleles with weak effects (e.g., OR < 1.3). With 233 quads and 57 trios, at the significance level of $p < 0.01$ (to correct for 5 SNPs), and under a multiplicative model, power calculation using the PBAT software (<http://www.biostat.harvard.edu/~clange/default.htm>) showed our sample set with 80% power to detect association with an allele frequency of 0.1 and 0.5, odds ratio of 1.6 and 1.4, respectively.

To conclude, the present replication study shows a potential association of the *PDLIM5* gene with bipolar disorder in European Americans. Our meta-analysis identifies a significant allelic association at rs2433320 in the Caucasians with ancestry of Europe. Previous findings in Japanese need to be confirmed in larger independent samples. Variants in the *PDLIM5* gene may influence risk for bipolar disorder with weak effects.

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Reference List

- Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet* 2002;30:97–101. [PubMed: 11731797]
- Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263–265. [PubMed: 15297300]
- 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. [PubMed: 17486107]
- Bax L, Yu LM, Ikeda N, Tsuruta H, Moons KG. Development and validation of MIX: comprehensive free software for meta-analysis of causal research data. *BMC Med Res Methodol* 2006;6:50. [PubMed: 17038197]
- Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. *Am J Hum Genet* 2004;74:106–120. [PubMed: 14681826]
- Cohen J, Pertsemlidis A, Kotowski IK, Graham R, Garcia CK, Hobbs HH. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. *Nat Genet* 2005;37:161–165. [PubMed: 15654334]
- Cohen JC, Kiss RS, Pertsemlidis A, Marcel YL, McPherson R, Hobbs HH. Multiple rare alleles contribute to low plasma levels of HDL cholesterol. *Science* 2004;305:869–872. [PubMed: 15297675]

- Cohen JC, Pertsemlidis A, Fahmi S, Esmail S, Vega GL, Grundy SM, et al. Multiple rare variants in NPC1L1 associated with reduced sterol absorption and plasma low-density lipoprotein levels. *Proc Natl Acad Sci U S A* 2006;103:1810–1815. [PubMed: 16449388]
- DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986;7:177–188. [PubMed: 3802833]
- Detera-Wadleigh SD, Badner JA, Yoshikawa T, Sanders AR, Goldin LR, Turner G, 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;74:254–262. [PubMed: 9184307]
- Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. *Genet Epidemiol* 2003;25:115–121. [PubMed: 12916020]
- Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;315:629–634. [PubMed: 9310563]
- Fearnhead NS, Wilding JL, Winney B, Tonks S, Bartlett S, Bicknell DC, et al. Multiple rare variants in different genes account for multifactorial inherited susceptibility to colorectal adenomas. *Proc Natl Acad Sci U S A* 2004;101:15992–15997. [PubMed: 15520370]
- Fisher, R. Statistical methods for research workers. 12th ed. Oliver & Boyd; Edinburgh, United Kingdom: 1954.
- Horiuchi Y, Arai M, Niizato K, Iritani S, Noguchi E, Ohtsuki T, et al. A polymorphism in the PDLIM5 gene associated with gene expression and schizophrenia. *Biol Psychiatry* 2006;59:434–439. [PubMed: 16213469]
- Iwamoto K, Bundo M, Washizuka S, Kakiuchi C, Kato T. Expression of HSPF1 and LIM in the lymphoblastoid cells derived from patients with bipolar disorder and schizophrenia. *J Hum Genet* 2004a;49:227–231. [PubMed: 15362566]
- Iwamoto K, Kakiuchi C, Bundo M, Ikeda K, Kato T. Molecular characterization of bipolar disorder by comparing gene expression profiles of postmortem brains of major mental disorders. *Mol Psychiatry* 2004b;9:406–416. [PubMed: 14743183]
- Johnson N, Fletcher O, Palles C, Rudd M, Webb E, Sellick G, et al. Counting potentially functional variants in BRCA1, BRCA2 and ATM predicts breast cancer susceptibility. *Hum Mol Genet*. 2007
- Kato T, Iwayama Y, Kakiuchi C, Iwamoto K, Yamada K, Minabe Y, et al. Gene expression and association analyses of LIM (PDLIM5) in bipolar disorder and schizophrenia. *Mol Psychiatry* 2005;10:1045–1055. [PubMed: 16044170]
- Kazeem GR, Farrall M. Integrating case-control and TDT studies. *Ann Hum Genet* 2005;69:329–335. [PubMed: 15845037]
- Maeno-Hikichi Y, Chang S, Matsumura K, Lai M, Lin H, Nakagawa N, et al. A PKC epsilon-ENH-channel complex specifically modulates N-type Ca²⁺ channels. *Nat Neurosci* 2003;6:468–475. [PubMed: 12665800]
- MANTEL N, HAENSZEL W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 1959;22:719–748. [PubMed: 13655060]
- Meyer J, Johannssen K, Freitag CM, Schraut K, Teuber I, Hahner A, et al. Rare variants of the gene encoding the potassium chloride co-transporter 3 are associated with bipolar disorder. *Int J Neuropsychopharmacol* 2005;8:495–504. [PubMed: 16098236]
- Mowry BJ, Ewen KR, Nancarrow DJ, Lennon DP, Nertney DA, Jones HL, et al. Second stage of a genome scan of schizophrenia: study of five positive regions in an expanded sample. *Am J Med Genet* 2000;96:864–869. [PubMed: 11121199]
- Numata S, Ueno S, Iga J, Yamauchi K, Hongwei S, Hashimoto R, et al. Gene expression in the peripheral leukocytes and association analysis of PDLIM5 gene in schizophrenia. *Neurosci Lett* 2007;415:28–33. [PubMed: 17287082]
- O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 1998;63:259–266. [PubMed: 9634505]
- Pritchard JK. Are rare variants responsible for susceptibility to complex diseases? *Am J Hum Genet* 2001;69:124–137. [PubMed: 11404818]
- Pritchard JK, Cox NJ. The allelic architecture of human disease genes: common disease-common variant...or not? *Hum Mol Genet* 2002;11:2417–2423. [PubMed: 12351577]

- Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics* 2000;155:945–959. [PubMed: 10835412]
- Romeo S, Pennacchio LA, Fu Y, Boerwinkle E, Tybjaerg-Hansen A, Hobbs HH, et al. Population-based resequencing of ANGPTL4 uncovers variations that reduce triglycerides and increase HDL. *Nat Genet* 2007;39:513–516. [PubMed: 17322881]
- Shi J, Hattori E, Zou H, Badner JA, Christian SL, Gershon ES, et al. No evidence for association between 19 cholinergic genes and bipolar disorder. *Am J Med Genet B Neuropsychiatr Genet* 2007;144B: 715–723. [PubMed: 17373692]
- Ueki N, Seki N, Yano K, Masuho Y, Saito T, Muramatsu M. Isolation, tissue expression, and chromosomal assignment of a human LIM protein gene, showing homology to rat enigma homologue (ENH). *J Hum Genet* 1999;44:256–260. [PubMed: 10429367]
- Warsh J, Andreopoulos S, Li P. Role of intracellular calcium signaling in the pathophysiology and pharmacotherapy of bipolar disorder: current status. *Clin Neurosci Res* 2004;4:201–204.
- Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661–678. [PubMed: 17554300]
- Wigginton JE, Abecasis GR. PEDSTATS: descriptive statistics, graphics and quality assessment for gene mapping data. *Bioinformatics* 2005;21:3445–3447. [PubMed: 15947021]
- Zhu X, Fejerman L, Luke A, Adeyemo A, Cooper RS. Haplotypes produced from rare variants in the promoter and coding regions of angiotensinogen contribute to variation in angiotensinogen levels. *Hum Mol Genet* 2005;14:639–643. [PubMed: 15649942]

Figure 1. Linkage disequilibrium (LD) plot of 5 single nucleotide polymorphisms in the *PDLIM5* gene region on chromosomal region 4q22

LD structure is based on genotypes for CNG, CHIP and waves 1-4 samples in this study. LD color is schemed by standard D'/LOD ; blocks are determined as solid spine of $D' > 0.8$.

Table 1 Allelic and haplotypic association of the *PDLIM5* gene with bipolar disorder

SNP	Chromosome position ^a	Alleles (1/2)	Allele 1 frequency ^b	Proportion of allele 1 transmitted	TDT <i>p</i> -value
rs10008257	95713506 95727983 95775472 95846260 95919860	G/A	0.47	0.52	0.068
rs2433320		G/A	0.58	0.55	0.13
rs774992		C/T	0.75	0.46	0.39
rs2452594		A/G	0.22	0.52	0.75
rs1543013		A/C	0.42	0.51	0.26
Haplotype			Frequency ^b		<i>p</i> -value
Block 1: rs10008257-rs2433320					
GA			0.03		0.045
GG			0.48		0.069
AG			0.12		0.16
AA			0.37		0.77
Block 2: rs2452594-rs1543013					
GA			0.43		0.47
AC			0.21		0.70
GC			0.36		0.70

^aBased on human genome sequence (NCBI Build 35 assembly).

^b Allele 1 and haplotype frequencies are calculated based the parents' genotypes. The global *p*-values for haplotype analysis of blocks 1 and 2 are 0.071 and 0.089, respectively. *p* < 0.05 is shown in bold type.

Table 2
Meta-analysis of association studies between *PDLIM5* and bipolar disorder

Study	Number of		Ethnicity	rs1008257 (A) ^a		rs2433320 (A) ^{a,b}		rs2433320 (A) ^{a,c}	
	Cases	Controls		OR	95% CI	OR	95% CI	OR	95% CI
Kato et al (MDMD)	128	130	Japanese	1.761	1.231-2.521	1.116	0.672-1.854	1.116	0.672-1.854
Kato et al (MPS)	240	240	Japanese	1.047	0.807-1.359	0.648	0.451-0.930	0.648	0.451-0.930
This study	290	290 families	European American	0.920	0.722-1.171	0.827	0.642-1.065	—	—
WTCCC	1863	2929	European UK	—	—	0.925	0.851-1.005	0.925	0.851-1.005
Baum et al (German)	679	543	European German	—	—	0.860	0.731-1.013	0.860	0.731-1.013
Baum et al (NIMH)	412	563	European American	—	—	—	—	0.850	0.709-1.019
European Caucasian	—	—	—	—	—	0.905	0.843-0.971	0.903	0.843-0.966
Significance	—	—	—	—	—	Z = 2.786	$p_Z = 0.005$	Z = 2.945	$p_Z = 0.003$
Heterogeneity	—	—	—	—	—	Q = 1.124	$p_Q = 0.570$	Q = 1.077	$p_Q = 0.584$
Pooled	—	—	—	1.165	0.826-1.643	0.897	0.838-0.961	0.896	0.838-0.958
Significance	—	—	—	Z ^d = 0.871	$p_Z = 0.384$	Z = 3.096	$p_Z = 0.002$	Z = 3.243	$p_Z = 0.001$
Heterogeneity	—	—	—	Q ^e = 8.876	$p_Q = 0.012$	Q = 4.894	$p_Q = 0.289$	Q = 4.914	$p_Q = 0.296$

Study	Number of		Ethnicity	GG		GA		AG		AA	
	Cases	Controls		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Kato et al (MDMD)	128	130	Japanese	0.492	0.346-0.699	1.719	0.929-3.181	2.092	1.444-3.032	0.392	0.150-1.027
Kato et al (MPS)	240	240	Japanese	1.164	0.902-1.501	0.623	0.418-0.930	1.088	0.835-1.418	0.740	0.346-1.582
This study	290	290 families	European American	1.227	0.944-1.594	0.620	0.317-1.232	0.933	0.651-1.338	0.908	0.700-1.179
Pooled	—	—	—	0.827	0.523-1.308	0.860	0.457-1.640	1.295	0.762-2.202	0.845	0.666-1.073
Significance	—	—	—	Z = 0.812	$p_Z = 0.417$	Z = 0.458	$p_Z = 0.647$	Z = 0.955	$p_Z = 0.340$	Z = 1.379	$p_Z = 0.168$
Heterogeneity	—	—	—	Q = 15.329	$p_Q = 0.0005$	Q = 7.947	$p_Q = 0.019$	Q = 10.01	$p_Q = 0.007$	Q = 2.864	$p_Q = 0.239$

Abbreviations: OR, odds ratio; CI, confidence interval.

^aThe associated alleles reported by Kato T et al.

^bMeta-analyses are performed for all studies by excluding Baum et al's genome-wide association data from NIMH sample set and our data, respectively.

^cMeta-analyses are performed for all studies by excluding Baum et al's genome-wide association data from NIMH sample set and our data, respectively.

^dZ test was used to determine the significance of the overall odds ratio; unadjusted $p_Z < 0.05$ are shown in bold type. Results under the fix-effects model are reported here if there is no evidence of between-study heterogeneity, otherwise under random-effects model.

^eCochran's Q test was used to assess the heterogeneity between samples; $p_Q < 0.1$ indicate significant between-study heterogeneity and are shown in bold type.

^fHaplotypes are consisted of rs1008257 (G/A) and rs2433320 (G/A).