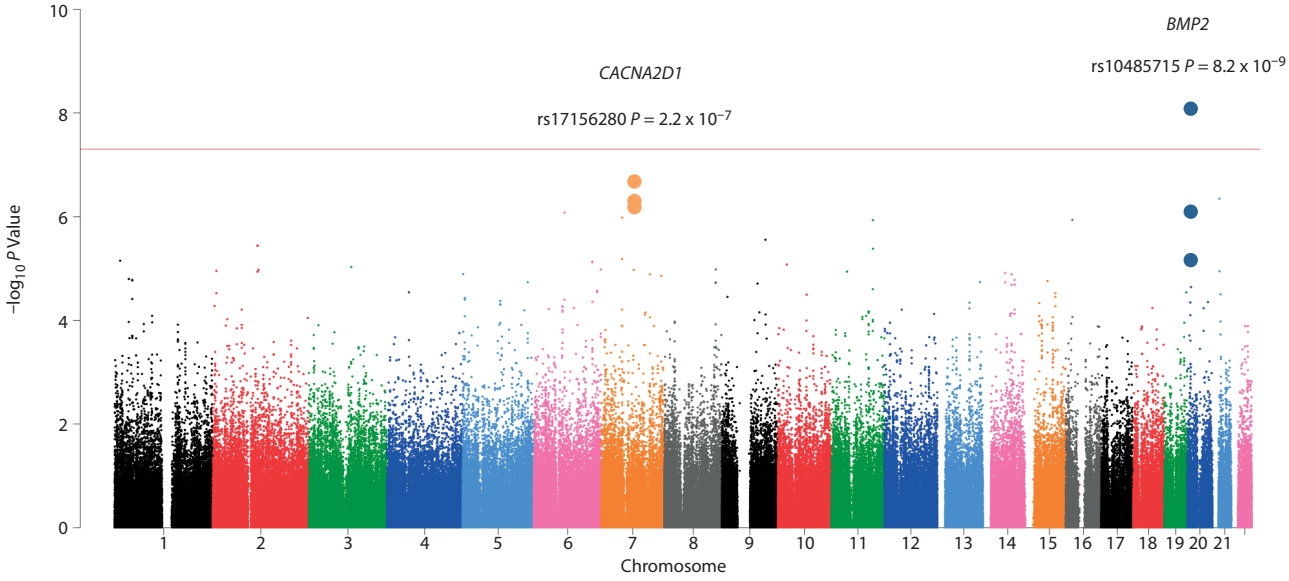
Genome-Wide Environment Interaction Between Depressive State and Stressful Life Events

To the Editor: Although recent advances in genetic studies have identified numerous susceptibility genes for psychiatric disorders, the definitive gene(s) for major depressive disorder (MDD) has not been detected. One possible strategy to detect "MDD susceptibility genes" is to examine gene-environment (G × E) interaction. In this study, we conducted a genome-wide environment interaction study (GWEIS), with longitudinal follow-up, on the depressive state and stressful life events (SLEs) of hospital staff.

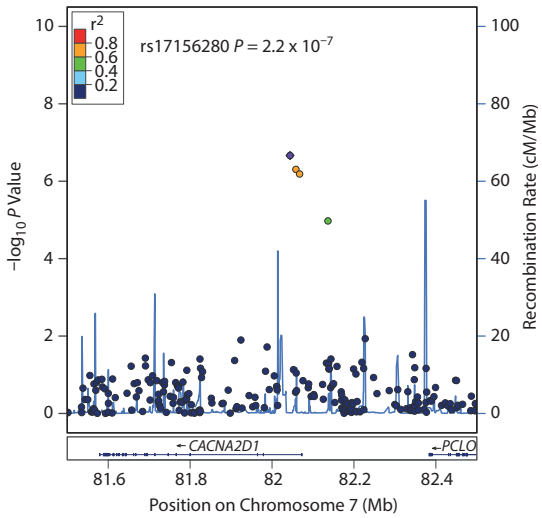
Method. A subset of the subjects was previously analyzed.[1] Individuals were evaluated for depressive symptoms and private SLEs using the Beck Depression Inventory-I (BDI)[2] and the List of Threatening Experiences (LTE) questionnaire[3] (12 life events within 6 months), respectively. We approached 1,559 subjects, and a total of 1,112 (100 men and 1,012 women; mean ± SD age was 28.5 ± 8.1 years) agreed to participate. Samples were collected in 3 phases as part of the Depression Protection Program in Fujita (Supplementary Text and Supplementary eFigure 1). Phase 1 began in April 2012 (828 subjects); phase 2, April 2013 (91 subjects); and phase 3, April 2014 (193 subjects). We evaluated the subjects every 3

Figure 1. Summary of the Association Results

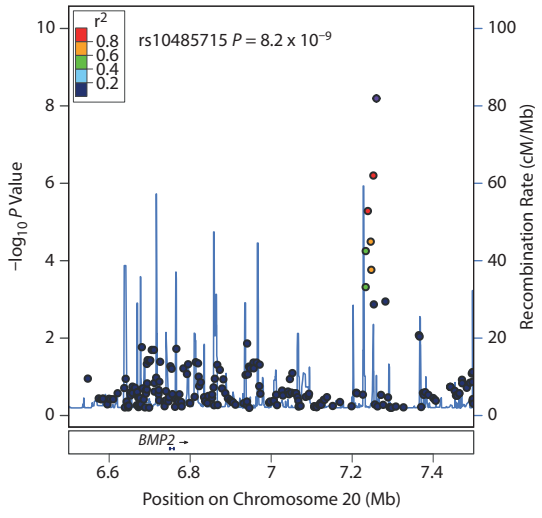
A. Manhattan Plot[a]



B. Regional Association Plot for SNPs in Chromosome 7[b]



C. Regional Association Plot for SNPs in Chromosome 20[b]



[a]Red line indicates the threshold of genome-wide significance (5×10^{8}).
[b]Genome build and linkage disequilibrium population is based on hg19 and Asian population of 1000 Genomes Project.[5]
Abbreviations: *BMP2* = bone morphogenetic protein 2; *CACNA2D1* = calcium channel, voltage-dependent, alpha 2/delta subunit 1; *PCLO* = piccolo presynaptic cytomatrix protein.

months (April, July, October, and January) for the first 2 years, then every 6 months thereafter. Data collection for this study ended in January 2015. Subject participation was voluntary and 31.5% (3,076 of 9,780) of the surveys were not returned.

We performed genome-wide single-nucleotide polymorphism (SNP) genotyping using the HumanOmniExpressExome (Illumina Inc). We followed this with stringent quality control protocol, including principal component analysis for population stratification (1,088 subjects; 527,599 SNPs with minor allele frequency > 1%; Supplementary Text and Supplementary eFigure 2).

Subjects who scored ≥ 19 at least once on the BDI were classified as depressive subjects (n = 308), and those who always scored < 19 on the BDI were classified as nondepressive subjects (n = 780) (Supplementary eTable1).

To assess genome-wide G × E interaction, we used a recently developed robust joint test.[4] This analysis can combine the effect of SNP (main effect: additive model) and SNP-SLE interaction with greater power, but it can reduce genome-wide inflation. To analyze the binary phenotype of a depressive state ("depressive"/"nondepressive") as major outcome in this model, we used the SNP and SNP-SLE interaction (presence of SLE when the BDI score was the worst) terms, with adjustment of sex and age as covariates. Single-nucleotide polymorphisms with minor allele frequency of ≥ 10% (418,225 SNPs) were analyzed according to a previous study.[4]

Results. The quantile-quantile plot is shown in Supplementary eFigure 3. The lambda value based on −2ln (*P*) of χ^2 distribution was 1.027, indicating minimal genome-wide inflation.

Figure 1 shows the Manhattan plot of the GWEIS. A significant joint effect (rs10485715, $P = 8.2 \times 10^{-9}$, Figure 1 and Supplementary eTable 2) was obtained downstream of the bone morphogenetic protein 2 (*BMP2*). Although there was no direct relationship between *BMP2* and mood disorder susceptibility, *BMP2* was widely expressed in neurons and exerted neurotrophic effects.[6]

The second region that showed suggestive association (rs17156280, $P = 2.2 \times 10^{-7}$, Figure 1 and Supplementary eTable 2) was located at *CACNA2D1* (calcium channel, voltage-dependent, alpha 2/delta subunit 1). Interestingly, *CACNA2D1* was reported as one of the candidate genes for bipolar disorder.[7]

Stress plays a substantial role in the etiology of MDD. However, MDD can be difficult to study because there is a large amount of variability in SLEs. A major advantage of this study was the longitudinal data collection of depressive states and private SLEs in a homogeneous population that was subjected to similarly significant stressors in the workplace.

We detected a joint effect of SNP and G × E interaction in *BMP2* and *CACNA2D1* for depressive state, although there were some critical limitations in this study: we did not evaluate MDD or lifetime psychiatric diagnoses, subjects with unreturned surveys were assigned to "depressive" or "nondepressive" state based on their worst BDI scores, and the sample size was small. On the basis of our findings, it is stressed that first, the effect of "risk" SNP (as main effect) or SNP-SLE interaction on depressive state was not extremely large (Supplementary eTable 3 and Supplementary eFigure 4); and second, we detected no significant joint effect on SNP and SNP-SLE interaction in the known candidate genes (Supplementary eFigure 5), such as the serotonin transporter (*SLC6A4;* solute carrier family 6 [neurotransmitter transporter], member 4), serotonin-2A receptor (*HTR2A;* 5-hydroxytryptamine [serotonin] receptor 2A, G protein-coupled), and brain-derived neurotrophic factor (*BDNF*). Replication is essential to verify our results. However, this type of "controlled" sample, with detailed information on phenotype and relevant environmental exposure, is crucial to detect the risks for depressive state and, presumably, for MDD that is moderately heritable.

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Supplementary material: See supplementary text and graphics in the accompanying pages.

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Supplementary material follows this letter.

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Supplementary Material

Article Title: Genome-Wide Environment Interaction Between Depressive State and Stressful Life Events

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List of Supplementary Material for the article

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Genome-wide environment interaction between depressive state and stressful life events

Ikeda et al.

Supplementary Text

Ethics statement

The ethics committee of Fujita Health University approved this study. After providing a complete description of the study to the subjects, written informed consent was obtained. All participants were nurses working at Fujita Health University Hospital. We reiterate that in order to avoid discrimination (the current work was not intended to promote all kind of genetic discriminations, e.g employment discrimination, promotion discrimination or genetic discrimination in health insurance), we did not share the subjects' personal data, such as mental state and genetic variants, with any of the administrative units of our hospital (except the health care unit, if the "depressive" subject needed immediate medical care, as mentioned below).

Depression Protection Program in Fujita

1) Registration (eFigure 1)

This program started in April 2012. All subjects working at that time were approached to join this program (Phase I). New subjects joined Phase II in April 2013, Phase III in April 2014, and Phase IV in April 2015.

On registration, subjects were asked to reply to several questionnaires, including (1) the Beck Depressive Inventory I (BDI, a 20-item questionnaire); (2) a questionnaire regarding stressful life events (SLEs) according to the List of Threatening Experiences (LTE) questionnaire (12 life events, within 6 months, that were found to have long-term negative effects on most people); (3) personality traits according to the Neuroticism–Extraversion–Openness Five-Factor Inventory (NEO–FFI; 60 items that assessed five personality traits, including neuroticism, extraversion, openness to experience, agreeableness, and conscientiousness); (4) the SF-8 (8 items that assess quality of life); (5) the Brief Stress check list (written in Japanese, http://www.tmu-ph.ac/topics/stress_table.php); and 6) other general questions. For this

current GWEIS analysis, we did not include data from NEO-FFI, SF-8, and Brief Stress checklist because several scores from these questionnaires were correlated with BDI score (data not shown).

2) Protection program for MDD

We evaluated the subjects' responses to several questionnaires, including BDI, LTE, SF-8, and Brief Stress checklist. Subjects were evaluated every 3 months (April, July, October, and January) after registration for the first 2 years and every 6 months thereafter (eFigure1). The responses to this approach were voluntarily obtained. One-third of the information was missing (summarized on January 2015) because the subjects did not respond to the questionnaires.

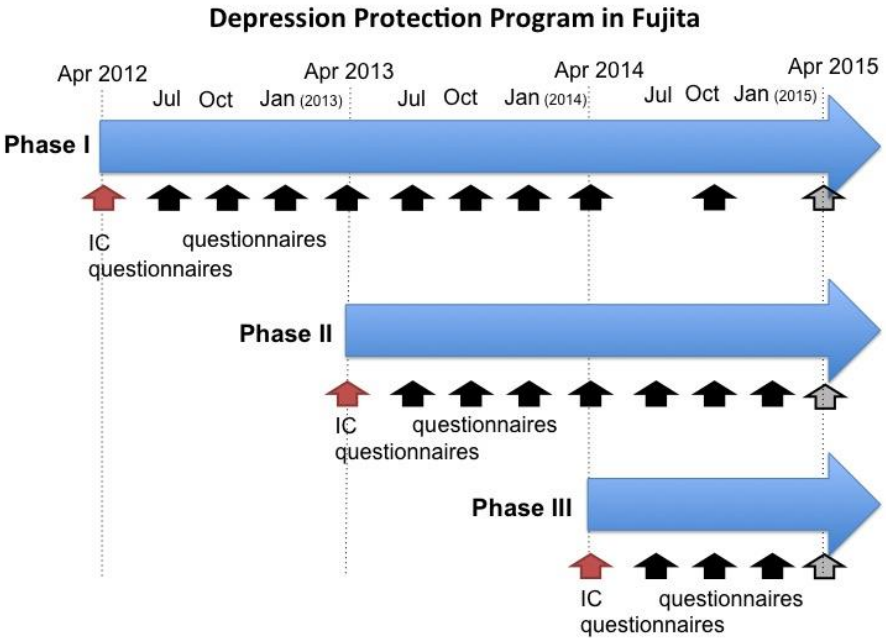
If a subject had a moderate to high BDI score (≥19) or rapid worsening the score, a research staff informed him/her that he/she was at risk for developing MDD or other psychiatric disorders. The subject then had the option to meet with psychologists for counseling. If the subject happened to be part of the management, the psychologists advised them to consult with a psychiatrist in the university hospital or in another hospital. The decision to seek help was made by the subject, unless the psychologists judged the subject to be in need of immediate help in which case the psychologists inform the health administration unit. Subjects agreed to this protocol prior to participating in the study.

SNP genotyping and quality controls (QCs)

We performed genome-wide single nucleotide polymorphism (SNP) genotyping: we genotyped Phase I samples using the HumanOmniExpressExome v1.0 (Illumina Inc.) and II/III samples using the HumanOmniExpressExome v1.2 (Illumina Inc.). We followed this with a stringent quality control procedure including principal component analysis: 1) Extracting overlapping SNPs between v1.0 and v1.2 chips, 2) gender consistency by investigating the SNPs on chromosome X, 3) removing the subjects with a low call rate (<0.99), and 4) removing the subjects with two or fewer degrees of relatedness using an identity-by-state analysis. After this filtering, 1,103 subjects and 527,599 SNPs with a minor allele frequency of >1% were included for

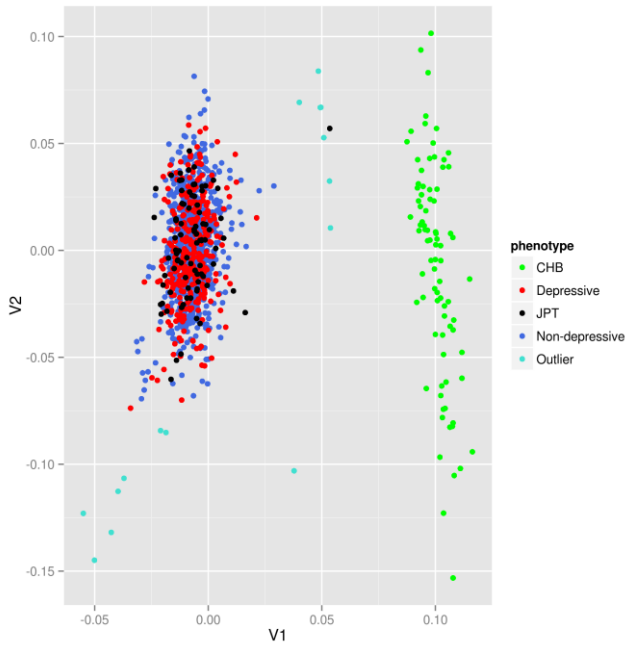
further analysis. We used principal component analysis to investigate population structures. We confirmed that all subjects belonged to the East Asian population cluster by comparing our sample with Japanese and Chinese samples from HapMap. We then further classified the population clusters and 15 subjects were excluded (total 1,088 subjects and 527,599 SNPs: eFigure 2).

eFigure 1: Overview of the Depression Protection Program

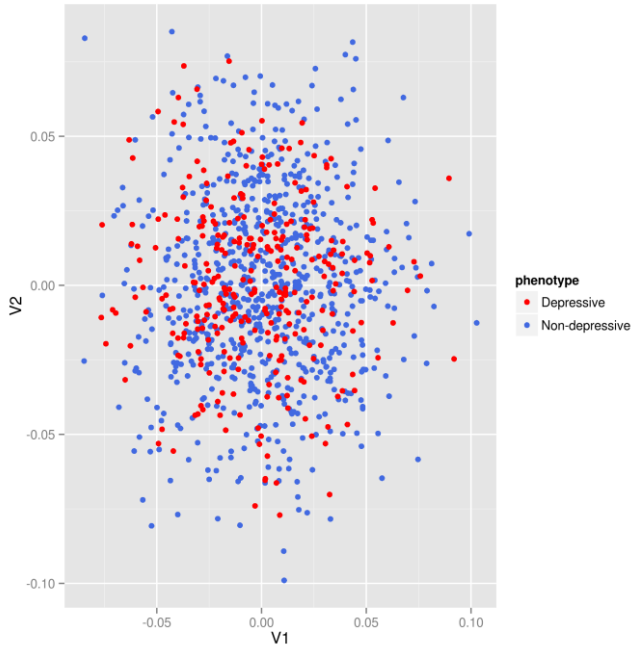


eFigure 2: Population stratification (PCA analysis: first and second Eigen vectors) (A) HapMap samples (JPT-Japanese and CHB-Chinese) and our samples (depressive and non-depressive): (B) Our samples only (depressive and non-depressive) after removing outliers based on PCA vectors from (A).

(A)

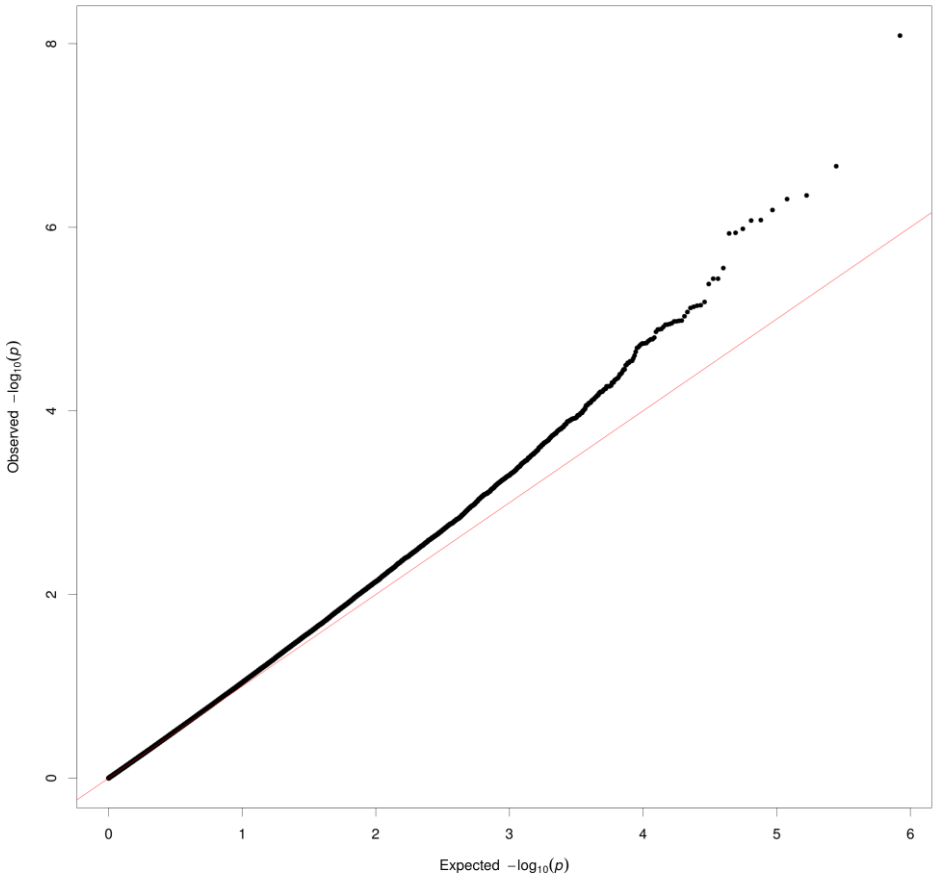


(B)



eFigure 3: Quantile-quantile plot

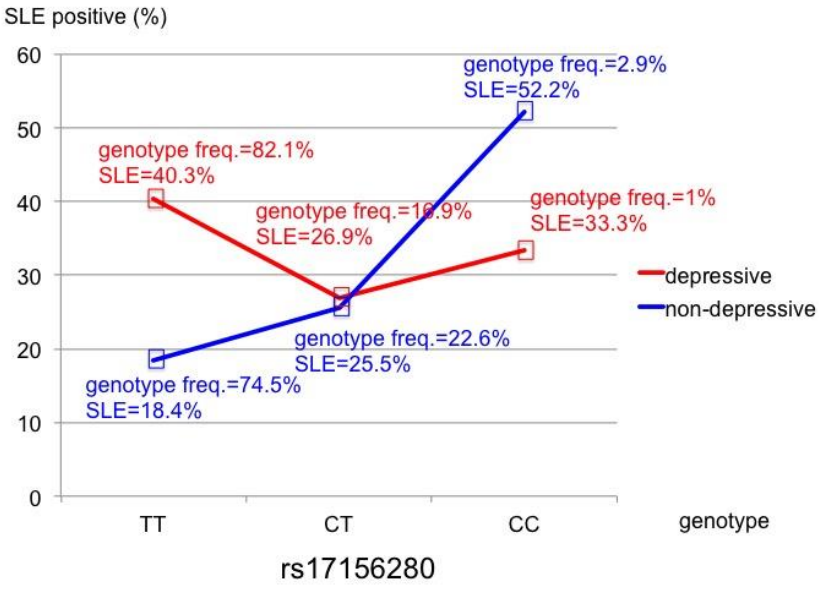
The lambda value based on -2ln(P) of chi-square distribution was 1.027.



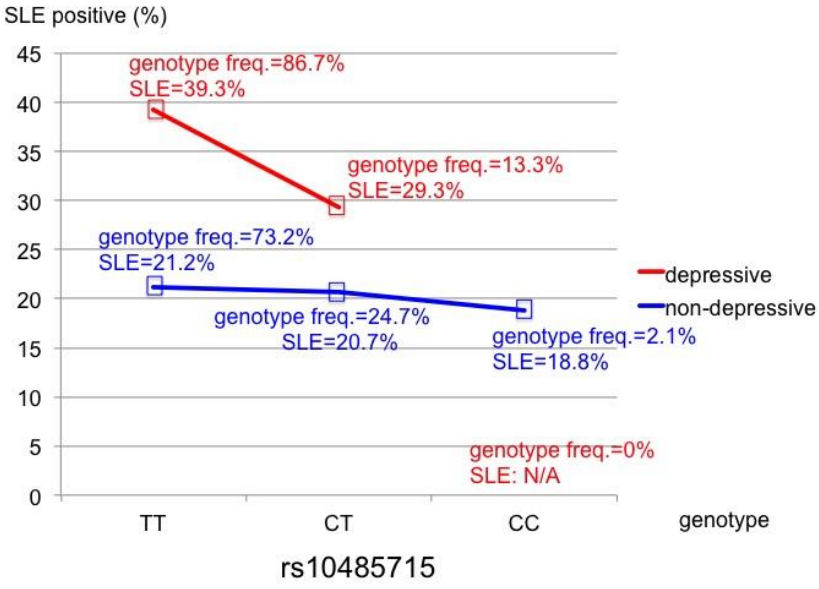
eFigure 4: Association results of *CACNA2D1* and *BMP2*

The Y-axis indicates the percentage of the subjects with positive SLEs (i.e., SLE >=1) at the worst BDI score. freq., frequency; SLE, stressful life event

(A) *CACNA2D1* (rs17156280): P-value (Joint test) = 2.2×10^{-7}



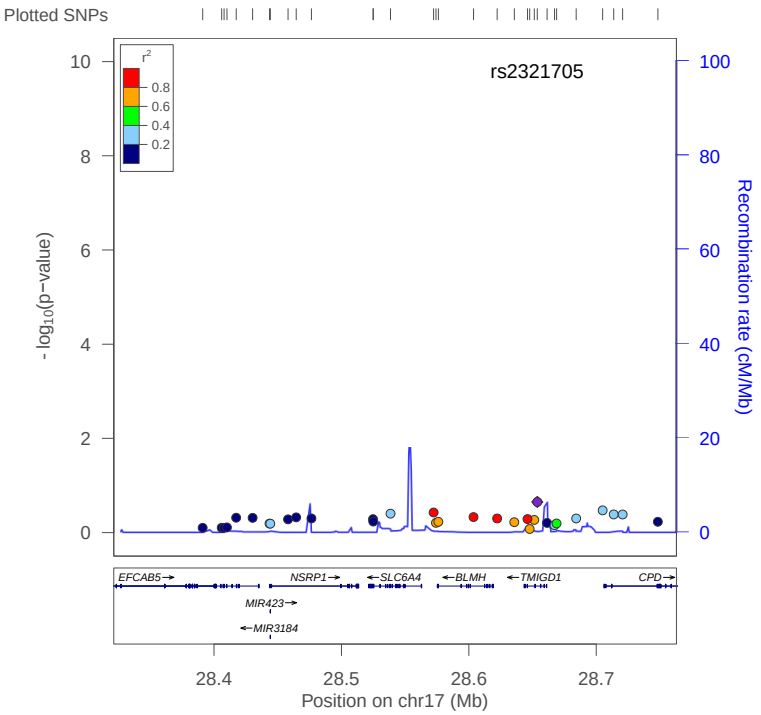
(B) *BMP2* (rs10485715): P-value (Joint test) = 8.2×10^{-9}



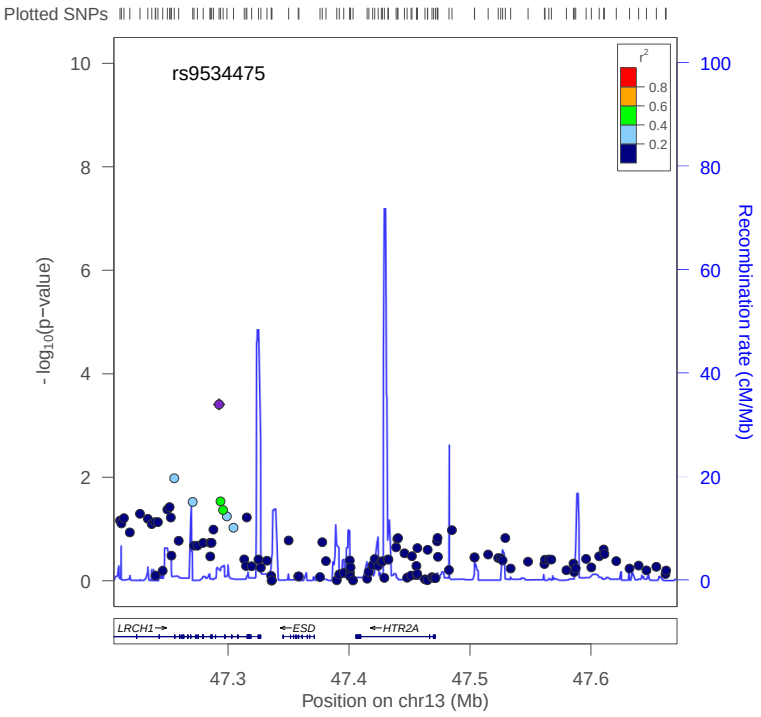
eFigure 5: Regional association plots for SNPs in the implicated loci

Genome build and linkage disequilibrium population is based on hg19 and Asian population of 1000 Genome Project (2012 Nov).

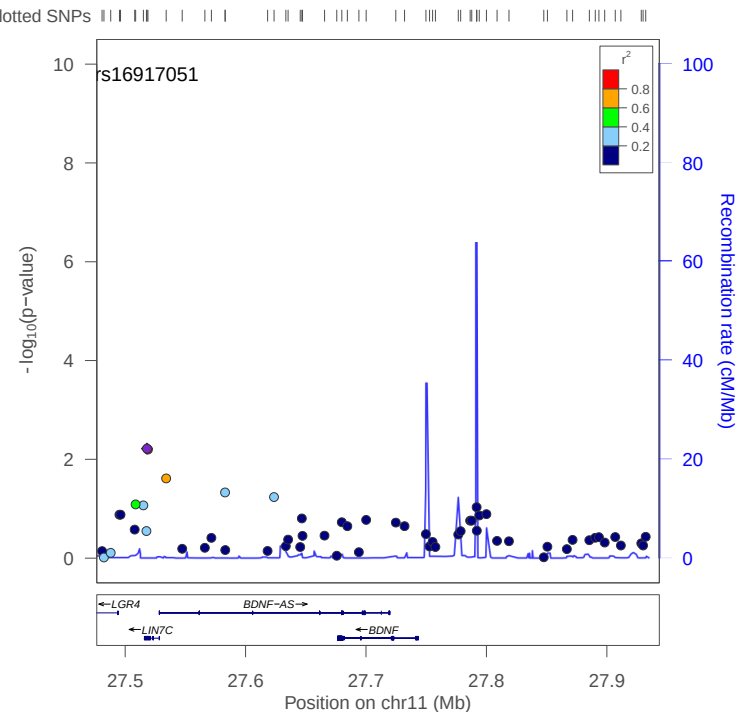
(A) Serotonin transporter gene (*SLC6A4*)



(B) serotonin 2A receptor gene (*HTR2A*)



(C) brain-derived neurotrophic factor (*BDNF*)



eTable 1. Demographic data of our samples (after quality control)

BDI: Beck Depression Inventory-I, SLE: Stressful life event

	"Depressive" group	"Non-depressive" group
Number	308	780
female/male	277/31	715/65
Age (mean +/- SD)	28.6 +/- 7.9	28.5 +/- 8.1
BDI worst score (mean +/- SD)	25.5 +/- 6.0	8.9 +/- 5.0
Subjects with positive SLEs (i.e. SLE >=1) at the worst BDI score	37.9% (=117/308)	21.0% (=164/780)

eTable 2. Top hit association in the GWEIS (robust joint test)
Chr: Chromosome, BP: base position based on hg19, A1: minor allele (based on whole sample), Freq: frequency of A1, A2: major allele
Bold number represents significant association with genome-wide significance based on the robust joint test (5×10^{-8}).

This analysis was based on the robust join test[4] on the combined effect of SNP (additive) and SLE (0 or >=1), with depressive status ("depressive"/"non-depressive") as dependent variable and with adjustments made for age and sex

Chr	SNP	BP	closest gene	A1	Freq. (Depressive)	Freq. (non-depressive)	A2	$P_{joint\ test}$
7	rs17156280	82043990	*CACNA2D1*	C	0.0942	0.142	T	2.17E-07
	rs17156308	82058270		A	0.0974	0.146	C	4.94E-07
	rs3801664	82067309		G	0.109	0.155	A	6.50E-07
	rs2158636	82136552		T	0.112	0.153	C	1.06E-05
20	rs6085948	7233350	*BMP2*	G	0.114	0.178	A	0.000695
	rs6077166	7233568		G	0.104	0.174	A	7.96E-05
	rs6117724	7238710		G	0.0763	0.146	A	7.16E-06
	rs6117728	7245805		C	0.089	0.155	T	4.52E-05
	rs6054856	7247239		G	0.102	0.168	A	0.000246
	rs7275039	7252352		G	0.0700	0.140	T	8.46E-07
	rs6107955	7253477		G	0.438	0.514	A	0.00197
	rs10485715	7259925		C	0.0666	0.144	T	**8.19E-09**

eTable 3. Logistic regression analysis of top hit association detected in robust joint test (rs17156280 and rs10485715)
Chr: Chromosome, BP: base position based on hg19, A1: minor allele (based on whole sample), A2: major allele, NMISS: number of non-missing genotypes, OR: odds ratio (for A1, i.e. A2 is reference), ADD: SNP (additive model), SLE: stressful life event.

This was a logistic regression analysis with depressive status ("depressive"/"non-depressive") as dependent variable and SNP (additive), SLE (0 or >=1), age, and sex as independent variables

Chr	SNP	BP	A1	A2	TEST	NMISS	OR	P
7	rs17156280	82043990	C	T	ADD	1088	0.6096	0.001312
					SLE	1088	2.384	4.59E-09
					age	1088	1	0.9763
					sex	1088	1.21	0.415
20	rs10485715	7259925	C	T	ADD	1088	0.4265	2.54E-06
					SLE	1088	2.257	4.28E-08
					age	1088	1.002	0.8581
					sex	1088	1.234	0.3712