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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.

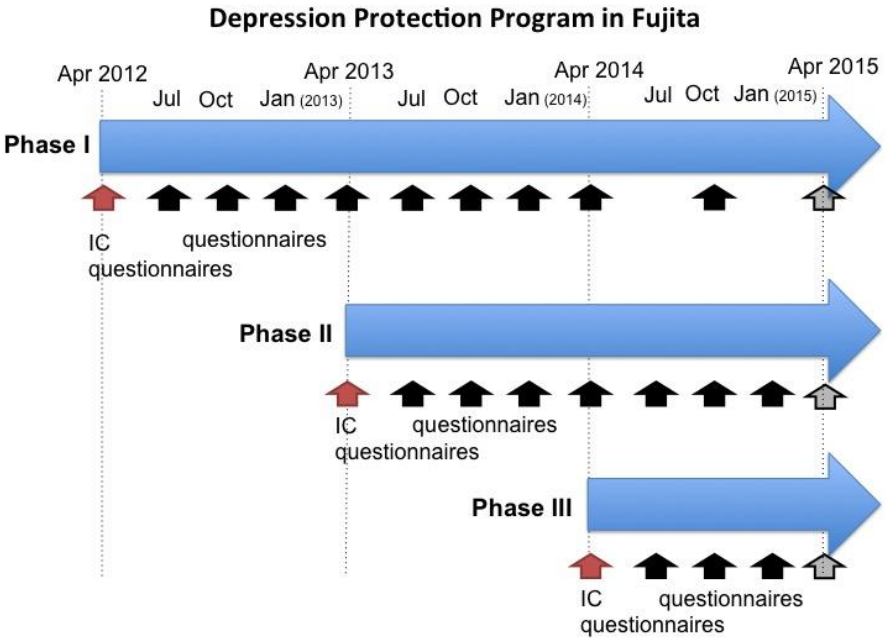
If a subject had a moderate to high BDI score (≥19) or rapid worsening the score, a research staff informed him/her that he/she was at risk for developing MDD or other psychiatric disorders. The subject then had the option to meet with psychologists for counseling. If the subject happened to be part 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

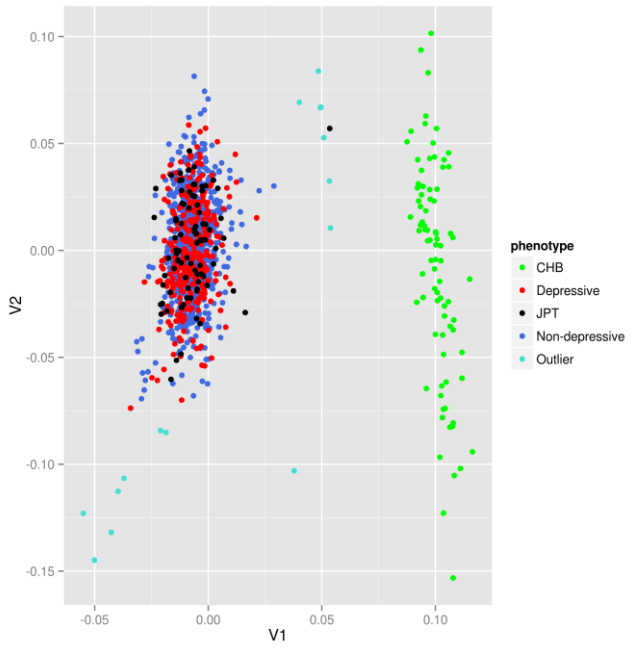
further analysis. We used principal component analysis to investigate population structures. We confirmed that all subjects belonged to the East Asian population cluster by comparing our sample with Japanese and Chinese samples from HapMap. We then further classified the population clusters and 15 subjects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

eFigure 1: Overview of the Depression Protection Program

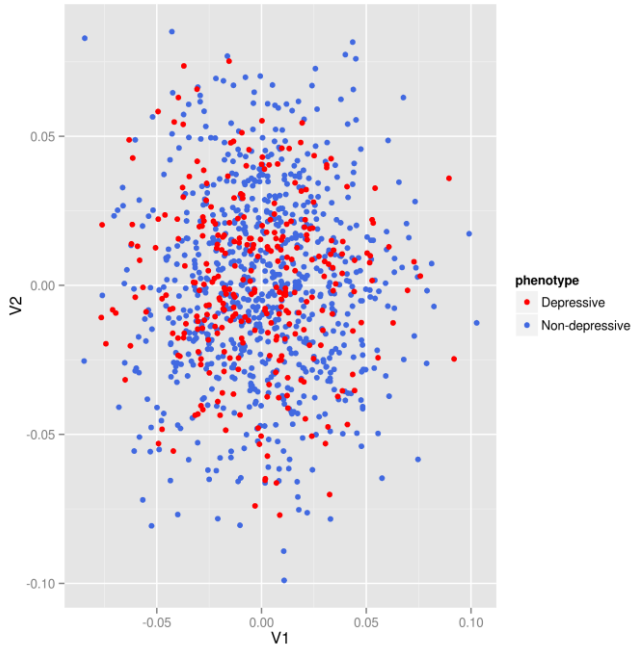


eFigure 2: Population stratification (PCA analysis: first and second Eigen vectors) (A) HapMap samples (JPT-Japanese and CHB-Chinese) and our samples (depressive and non-depressive): (B) Our samples only (depressive and non-depressive) after removing outliers based on PCA vectors from (A).

(A)

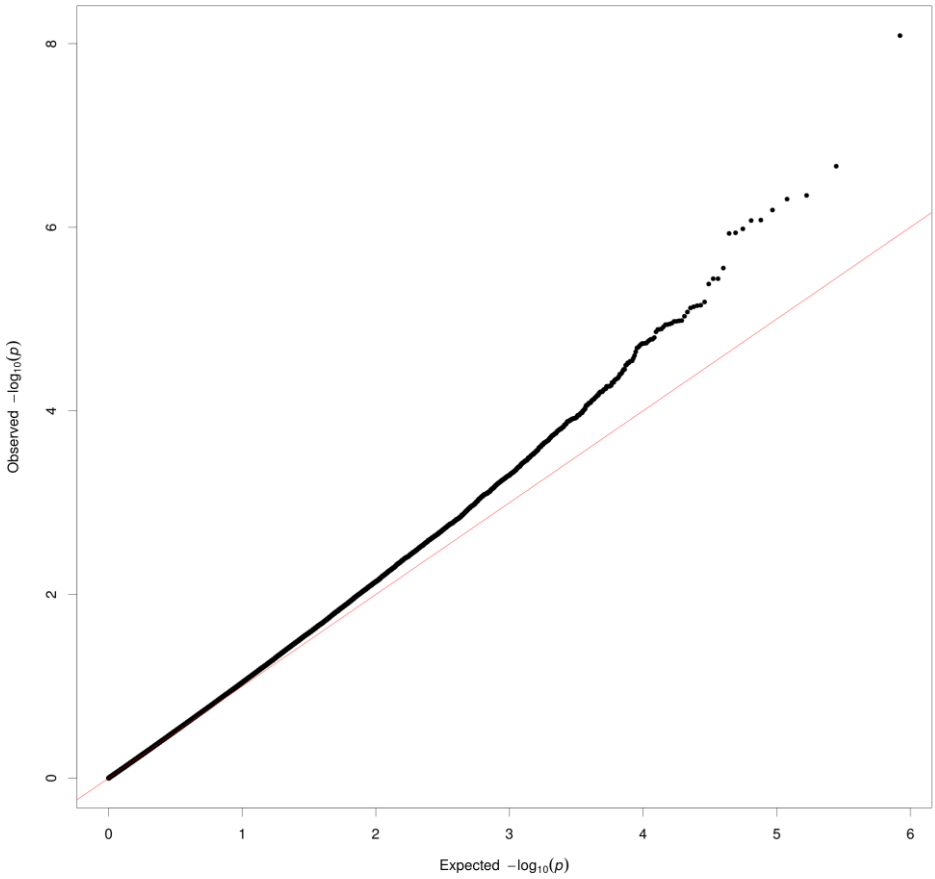


(B)



eFigure 3: Quantile-quantile plot

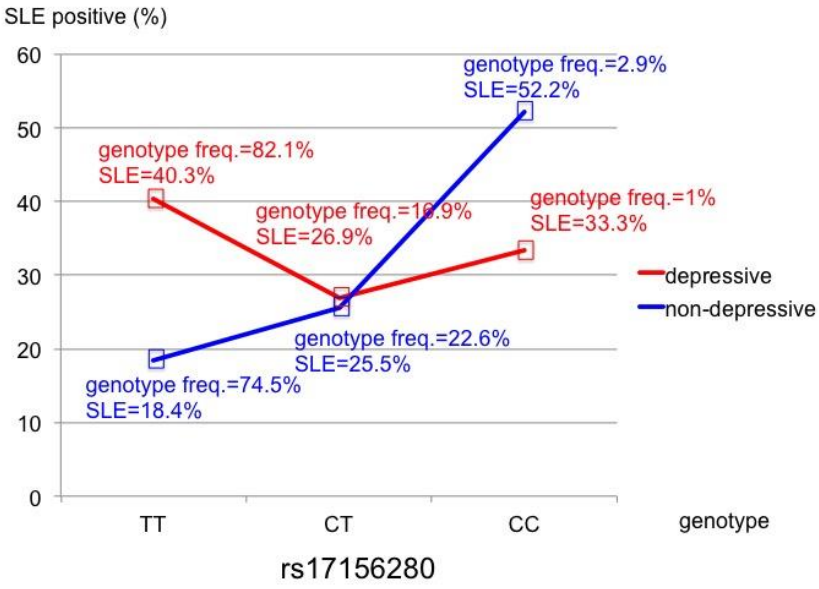
The lambda value based on -2ln(P) of chi-square distribution was 1.027.



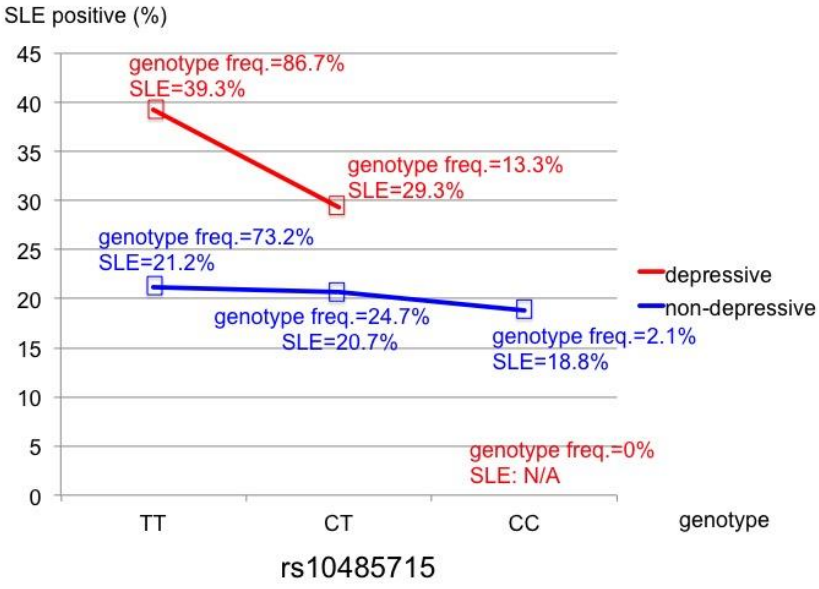
eFigure 4: Association results of *CACNA2D1* and *BMP2*

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}



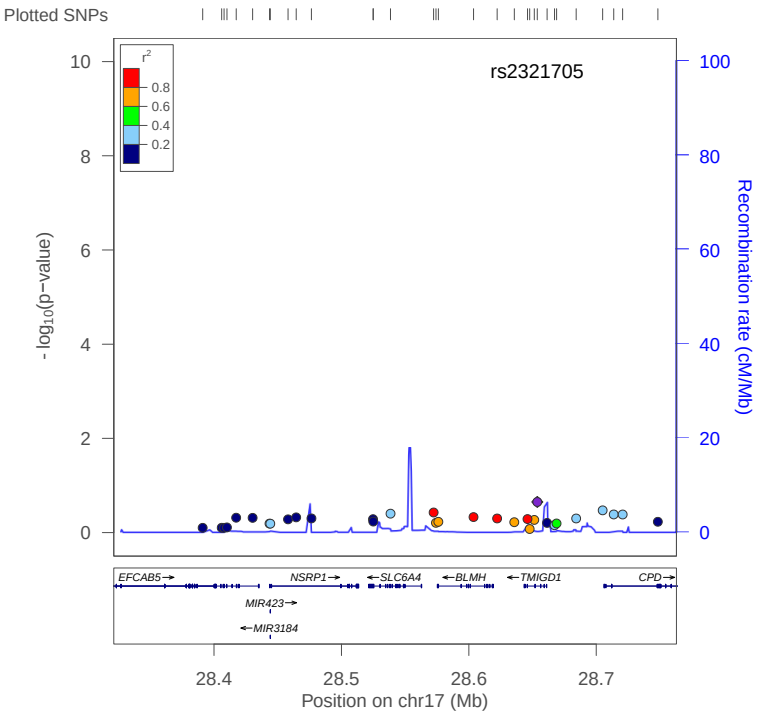
(B) *BMP2* (rs10485715): P-value (Joint test) = 8.2×10^{-9}



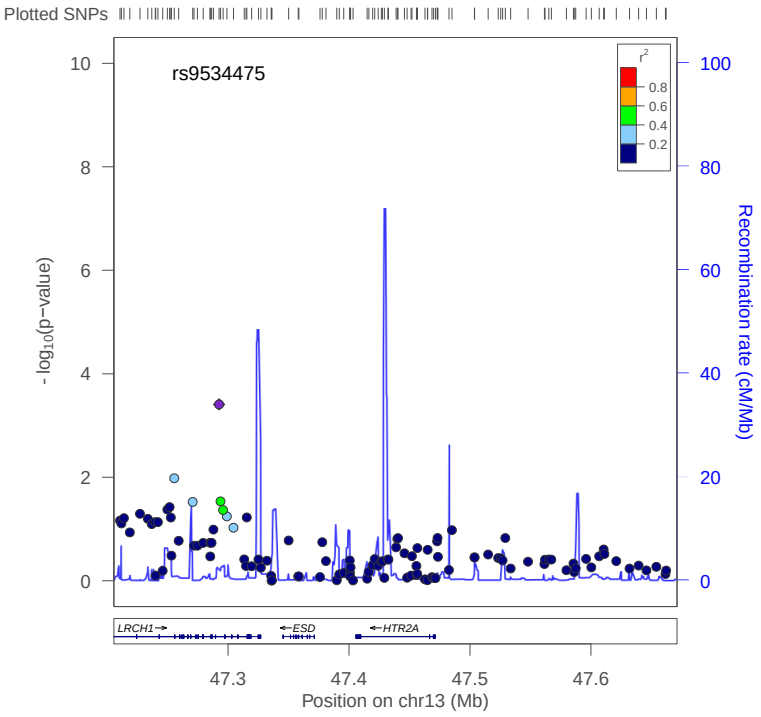
eFigure 5: Regional association plots for SNPs in the implicated loci

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

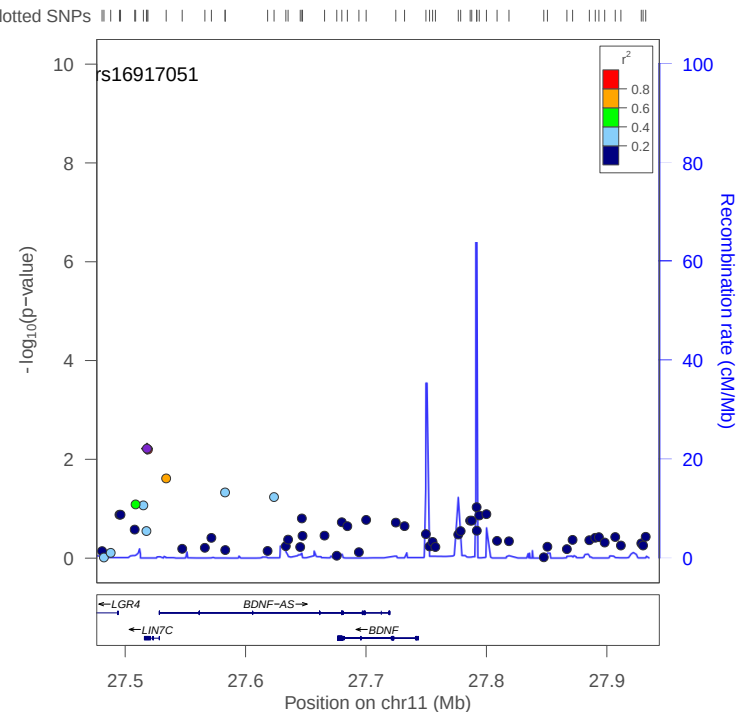
(A) Serotonin transporter gene (*SLC6A4*)



(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