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Associations Between Self-Reported and Objectively Recorded Early Life Stress, *FKBP5* Polymorphisms, and Depressive Symptoms in Midlife

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ABSTRACT

BACKGROUND: FK506-binding protein 51 is involved in hypothalamic-pituitary-adrenal axis regulation. Single nucleotide polymorphisms (SNPs) in the *FKBP5* gene have been shown to interact with retrospectively self-reported early life stress (ELS) in patients with psychiatric disorders. We examined interactions between three selected *FKBP5* SNPs and self-reported and objectively recorded ELS in relation to depressive symptoms in midlife.

METHODS: This study comprised 1431 Helsinki Birth Cohort Study participants genotyped for *FKBP5* SNPs shown to alter cortisol metabolism (rs1360780, rs9470080, and rs9394309). Participants completed the Beck Depression Inventory (BDI) at ages 61.5 years (time 1) and 63.4 years (time 2); 165 and 181 participants were separated from their parents in childhood as a result of evacuations during World War II as indicated by self-reports and the Finnish National Archives registry, respectively.

RESULTS: Associations between self-reported and objectively recorded ELS, but not stressful events in midlife, and the mean BDI score (average of time 1 and time 2) or mild to severe BDI scores (10–63 points at time 1 and time 2), or both, were moderated by the *FKBP5* variants (p values for interactions $< .05$; p values between self-reported and objectively recorded ELS in these interactions $> .18$). Mean BDI scores or odds for having mild to severe BDI scores, or both, increased according to number of minor alleles and haplotypes derived from these alleles in the separated groups, but not in the nonseparated groups.

CONCLUSIONS: *FKBP5* variations in combination with self-reported and objectively recorded ELS predict more pronounced depressive symptoms in midlife. Our findings confirm previous retrospective findings in a prospective epidemiologic study setting.

Keywords: Cortisol, Depression, Early life stress, FKBP5, HPA, Longitudinal study, Separation

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Major depressive disorder (MDD) and self-reported depressive symptoms are increasingly prevalent psychiatric phenotypes (1) resulting from a complex interplay between genetic factors and environmental adversities (2). Family and twin studies suggest heritability estimates of 30%–40% (3,4). However, more recent genome-wide association studies and mega-analyses have failed to uncover a single genetic polymorphism that both survived correction for multiple testing and was replicated in subsequent samples (5,6). Although the lack of consistent genome-wide findings may reflect a lack of sufficiently large sample sizes, it may also reflect constraints of the methodology of genome-wide association studies to account for environmental factors.

Because stressful events occurring early in life (early life stress [ELS]), including maltreatment, separation from parents because of divorce or death, parental or own illness, war, severe accident or injury, and natural disaster, are among the most consistently demonstrated early environmental adversities associated with risk of depression and its recurrence (7–10), and because dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis stress response is a frequently demonstrated neurobiological abnormality in depression (11), a few gene-environment (G × E) interaction studies in depression have focused on genes involved in the regulation of the HPA axis stress response. In these studies, increasing attention has been devoted to a gene that encodes the FK506-binding protein 51 (*FKBP5*) located on chromosome 6 (6p21.31). *FKBP5* is a heat shock protein 90 co-chaperone that participates in inhibition of glucocorticoid receptor activity, the main regulator of the HPA axis (12,13). Glucocorticoid receptor activation participates in the induction of *FKBP5* transcription (13,14). These *FKBP5* functions of inhibition and induction create an intracellular, ultrashort feedback loop that regulates glucocorticoid receptor sensitivity (15). According to Zannas

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and Binder (16), *FKBP5* is in a "prime position" to mediate G × Es relevant for mood and anxiety disorders because it is responsive to stressor exposure, it is responsive to concomitant increase in the levels of glucocorticoid stress hormones, and it participates in the regulation of glucocorticoid receptor sensitivity.

However, findings from the few existing studies focusing on *FKBP5* × ELS interactions in depression do not form an entirely consistent picture. A study reported that in homozygous minor allele carriers, but not in heterozygous or homozygous major allele carriers, of *FKBP5* single nucleotide polymorphisms (SNPs; rs1360780, rs3800373, rs4713916, rs9296158, and rs9470080), exposure to traumatic events and experience of severe trauma early in life were associated with a higher risk of incident MDD in adulthood (17). These findings were replicated in one sample but not in another sample (17). Another study reported that in homozygous minor allele carriers but not in heterozygous or homozygous major allele carriers of *FKBP5* SNPs (rs1360780, rs4713899, and rs9368881), exposure to physical abuse in childhood was associated with a higher risk of MDD and with a higher level of self-reported depressive symptoms in adulthood (18). One other study reported null interactions between *FKBP5* SNPs (rs1360780, rs3800373, rs4713916, rs9296158, rs9470080, rs992105, rs737054, and rs1334894) and exposure to traumatic events early in life in relation to self-reported depressive symptoms in adulthood (19).

In all the previous studies, ELS was retrospectively self-reported in adulthood using various instruments covering different adversities. We are not aware of any previous study that tested *FKBP5* × ELS interactions with ELS obtained from objective records or of studies that allowed comparisons between self-reported and objectively recorded ELS in G×E interactions. Such comparisons would be valuable to overcome problems with the validity of retrospective reports by adults on their early life adverse experiences (20). Comparison between the effects of self-reports and objective records in the context of G×E was possible because ELS in our study arose from one distinct event—temporary separation from both biological parents as a result of child evacuations during World War II. In this study, we examined whether the associations of ELS, as self-reported and objectively recorded, and depressive symptoms in midlife were moderated by three selected *FKBP5* SNPs (rs1360780, rs9470080, and rs9394309) and haplotypes based on these SNPs. We chose these SNPs because previous research linked them with altered HPA axis responsiveness (21,22). We previously reported that effects of these separations on depressive symptoms and HPA axis stress responses vary according to their timing and duration (7,23). Therefore, we also tested if the G×E interactions were specific to age at and length of the exposure to the ELS.

METHODS AND MATERIALS

Participants

The study was carried out in accordance with the Declaration of Helsinki, and the study protocol was approved by the Institutional Review Board of the National Public Health Institute. Written informed consent was obtained from all participants. The original Helsinki Birth Cohort Study comprised 4130 women and 4630 men ($N = 8760$) who were born as singletons at the Helsinki University Central Hospital between 1934 and 1944, had birth and child welfare records, and were living in Finland in 1971 when a personal identification number was allocated to each member of the Finnish population. Details of the Helsinki Birth Cohort Study can be found elsewhere (24,25).

Between 2001 and 2004 (time 1), a random subset of 2902 individuals was invited to participate in a clinical examination during which blood samples for DNA were obtained and a survey including depressive symptoms was administered. Of the invited individuals, 1075 women and 928 men ($N = 2003$; 69%) with a mean age of 61.5 (SD 2.9) years participated (24,25). In 2004 (time 2), a psychological survey including a retest of depressive symptoms was mailed to participants of the time 1 clinical examination who were still traceable ($n = 1975$; 98.6%). The survey was returned by 1667 (57.4% of the original invited random subset; 84.4% of the traceable invited subset) participants with a mean age of 63.4 (SD 2.9) years, on average 1.9 (SD .7) years after the time 1 clinical examination (26).

Data for the genotype (283 individuals of the time 1 participants were not included in the genetic analyses because of sex discrepancy, relatedness, failure in DNA sampling, or refusal to participate in the genotyping) and data for depressive symptoms at time 1 and time 2 were available for 574 men and 857 women, and they formed the analytic sample of this study. Compared with the time 1 sample, women were overrepresented in the analytic sample ($p < .001$), but there were no differences in father's occupational status in childhood ($p > .08$), own maximum attained level of education in adulthood ($p > .08$), or depressive symptoms scores or recent stressful life events at time 1 ($p > .37$).

Measures

Depressive Symptoms. The participants completed the Beck Depression Inventory (BDI) (27) at time 1 and time 2. The BDI includes 21 items, each consisting of four statements rated on a 0–3 scale. A higher BDI sum score (range, 0–63) reflects higher symptom severity in the past 2 weeks.

The mean-level (mean difference between time 1 and time 2 BDI scores = .14, $p = .24$) and rank-order stability of the BDI was high (time 1 and time 2 intraclass $r = .68$, $p < .001$). In the analyses, we used a mean BDI score averaged across time 1 and time 2 sum scores with square root transformation to attain normality and defined mild to severe depressive symptoms as a BDI sum score of 10–63 (27) at time 1 and time 2 ($n = 159$; 11.1%) .

Early Life Stress. Using the Finnish National Archives registry, which was kept by the Ministry of Social Affairs and Health between 1939 and 1946 and which includes detailed data on separations of 48,628 children, we identified 181 (12.6%; 58.6% women) participants who were separated temporarily from their parents in childhood. These participants formed the "objectively separated" group. The median age at separation was 3.8 years (range, .6–10.1 years), and the median length of separation was 1.4 years (range, .2–4.7

years). Data on age at and length of separation were available on a subset of 165 and 161 participants, respectively. The separations occurred during World War II when nearly 80,000 Finnish children were evacuated to Sweden and Denmark without their biological parents (28).

Although the Finnish National Archives registry is highly reliable (7), some additional children were evacuated either abroad or within Finland through personal contacts of the families: it has been estimated that this group may include >20,000 children (28). A question addressing wartime separation from both parents was embedded in the psychological survey at time 1. An additional 165 (11.5%; 66.1% women) participants self-reported being separated from both parents during World War II. These participants formed the "self-reported separation" group. If the participant was identified in the Finnish National Archives registry and self-reported separation, he or she was classified as being objectively separated. Because of constraints that relate to remembering events occurring at the earliest stages in life and because events rather than sequences of timing of the same events are recalled more accurately (20), we did not use self-reported information on age at and length of separation.

Stressful Life Events in the Last 12 Months. At time 1, 15 life events in the last 12 months (e.g., ongoing financial strain, threat of unemployment or personal bankruptcy, ongoing difficulties in close relationships, concern over health of a family member or a close friend, difficulties in housing) known to be major stressors were measured (29-31). The participants evaluated the occurrence and stressfulness of these events (0, not occurred; 1, not at all stressful; 2, mildly stressful; 3, moderately stressful; and 4, extremely stressful) during the past 12 months. For the analyses, the measurement scale was dichotomized by contrasting moderately and extremely stressful events with events that were not at all or mildly stressful or had not occurred at all (29,30).

SNP Genotyping. Genotypes of intronal SNPs rs1360780, rs9470080, and rs9394309 were taken from the modified Illumina 610k array (Illumina, San Diego, California). These SNPs were chosen because of their location in the *FKBP5* gene and effect on HPA axis reactivity (21,22) as suggested previously (32). Genotyping was conducted at the Wellcome Trust Sanger Institute, Cambridge, United Kingdom, according to standard protocols. We conducted multidimensional scaling (MDS) analyses with PLINK and derived the first three components to control for population stratification. The genotyping success rate was >99% in all three SNPs. Observed genotype frequencies did not deviate from the Hardy-Weinberg equilibrium ($p > .39$). Minor allele frequencies were 21.6% for rs1360780, 25.6% for rs9470080, and 24.3% for rs9394309. SNPs were in high linkage disequilibrium in this sample ($r^2 = .72–.94$), and belonged to the same haploblock according to solid spine algorithm with default values in Haploview 4.1 (33) (Supplemental Figure S1).

Statistical Analyses

We used multiple linear and logistic regression analyses to examine if the three selected SNPs in *FKBP5*, separation status, and their interaction predicted the mean BDI and mild to severe BDI scores, respectively. Comparable haplotypic association analyses were performed with the Haplo.stats 1.6.11 package (34) of the R 3.0.2 statistical software, using the Haplo.glm function. Haplo.glm infers haplotype frequencies with the expectation-maximum algorithm and calculates for each haplotype regression coefficient and p value, comparing each haplotype (TTG haplotype with frequency of .20) with the most common haplotype (CCA haplotype with frequency of .74). Haplotypes with frequency < .05 were discarded.

G×E interactions were tested by each SNP (rs1360780, rs9470080, and rs9394309) and haplotypes based on these SNPs in separate models assuming additive genetic model and by using the following separation status variables: we first combined 1) the objectively recorded and self-reported separated groups and contrasted them with the nonseparated group; we then contrasted 2) the objectively recorded separated group with the nonseparated group and 3) the self-reported separated group with the nonseparated group; finally, we contrasted 4) the objectively recorded and self-reported separated groups with each other. We also investigated whether associations between stressful life events in the last 12 months and depressive symptoms were moderated by the three selected SNPs in *FKBP5* or haplotypes based on these SNPs. Lastly, we tested whether associations between age at (median split at 3.8 years; dummy coded with nonseparated group as referent) and length of (median split at 1.4 years; dummy coded with nonseparated group as referent) separation and mean BDI and mild to severe BDI scores were moderated by the three selected *FKBP5* SNPs in the objectively separated group. These analyses were not conducted with haplotypes because of the limited sample size.

In all analyses, we adjusted for age at testing (average age of time 1 and time 2), sex, father's occupational status in childhood (manual worker, junior clerical, and senior clerical as extracted from birth records), own maximum attained level of education in adulthood (basic/primary or less, upper secondary, lower tertiary, or upper tertiary recorded at 5-year intervals during the period 1970–2005 by Statistics Finland), stressful life events in last 12 months (except when this variable was dependent), and the first three MDS components to control for population stratification. In addition, in the models pertaining to interaction tests, we also made adjustments for interactions of all covariates with separation status and with *FKBP5* variants as suggested by Keller (35). Because of high linkage disequilibrium, assumptions for Bonferroni correction were not met, and no correction for multiple testing was applied. Two-tailed $p < .05$ is considered statistically significant.

RESULTS

Characteristics of the sample by separation status are listed in Table 1. As previously reported (7,23), separated individuals were slightly older, had attained a lower maximum level of education in adulthood, and reported higher mean BDI scores in midlife than nonseparated individuals.

Mean BDI scores or odds for mild to severe BDI scores, or both, were higher for women, older individuals, individuals whose fathers had lower occupational status in childhood,

Table 1. Characteristics of the Sample

	Nonseparated Control Subjects (n = 1085)	Objectively Recorded Separation (n = 181)	Self-Reported Separation (n = 165)	
Study Variables	n (%) or Mean (SD)	n (%) or Mean (SD)	n (%) or Mean (SD)	p
Sex, Men	443 (40.8%)	75 (41.4%)	56 (339%)	.23
Age, Years	61.8 (2.6)	64.6 (2.9)	64.4 (2.8)	< .001
Father's Occupational Status in Childhood				.10
Manual worker	690 (64.7%)	129 (72.1%)	107 (66.9%)	
Junior clerical	236 (22.1%)	37 (20.7%)	39 (24.4%)	
Senior clerical	140 (13.1%)	13 (7.3%)	14 (8.8%)	
Maximum Attained Level of Education in Adulthood				.005
Basic/primary	340 (31.4%)	71 (39.2%)	64 (39.0%)	
Lower secondary	192 (17.7%)	35 (19.3%)	38 (23.2%)	
Upper secondary	311 (28.7%)	37 (20.4%)	26 (15.9%)	
Tertiary	240 (22.2%)	38 (21.0%)	36 (22.0%)	
Mean BDI Score[a]	5.7 (5.1)	6.0 (5.5)	6.8 (5.5)	.04
Mild to Severe BDI Score[b]	111 (10.2%)	27 (14.9%)	21 (12.7%)	.14
Stressful Life Events in the Last 12 Months	1.2 (1.7)	1.3 (1.7)	1.5 (1.5)	.29
FKBP5 Polymorphisms				
rs1360780				.45
CC	667 (61.9%)	113 (63.1%)	97 (59.1%)	
CT	361 (33.5%)	62 (34.6%)	62 (37.8%)	
TT	50 (4.6%)	4 (2.2%)	5 (3.0%)	
rs9470080				.43
CC	594 (55.2%)	103 (57.5%)	84 (51.5%)	
CT	419 (38.9%)	71 (39.7%)	69 (42.3%)	
TT	64 (5.9%)	5 (2.8%)	10 (6.1%)	
rs9394309				.50
AA	620 (57.2%)	107 (59.1%)	88 (53.7%)	
AG	404 (37.3%)	69 (38.1%)	66 (40.2%)	
GG	60 (5.5%)	5 (2.8%)	10 (6.1%)	

BDI, Beck Depression Inventory.
[a]Mean BDI score refers to mean of BDI scores at age 61.5 years (time 1) and 63.4 years (time 2).
[b]Mild to severe BDI score refers to BDI scores 10–63 at age 61.5 years (time 1) and 63.4 years (time 2) years.

individuals who themselves had attained lower maximum level of education in adulthood, and individuals with greater number of stressful life events in the last 12 months (all p values < .013). After adjusting for covariates, none of the SNPs or haplotypes predicted mean BDI scores (unstandardized regression coefficients < .03, 95% confidence intervals = −.09 to .12, p values > .60) or mild to severe BDI scores (odds ratios < 1.1, 95% confidence intervals = .84–1.53, p values > .31).

G×E Interactions

G×E interactions on mean BDI and mild to severe BDI scores are presented in Table 2. After adjusting for main effects of covariates and interactions between these covariates with the *FKBP5* variants and separation status, the following interactions were significant: rs1360780, rs9470080, rs9394309, and TTG haplotype interacted with combined self-reported and objectively recorded separation versus nonseparation in relation to mean BDI score (p values for interaction < .05) and to mild to severe BDI score (p values for interactions < .003). The same variants interacted with self-reported separation versus nonseparation in relation to mild to severe BDI score (p values for interaction < .024). Moreover, these variants interacted with objectively recorded separation versus nonseparation in relation to mean BDI and mild to severe BDI scores (p values for interactions < .05), with one exception: rs9394309 showed a trend in the G×E analyses of mild to severe BDI scores (p = .072).

Across all significant interactions, mean BDI scores or odds for mild to severe BDI scores, or both, increased according to the number of minor alleles or TTG haplotypes in the separated groups but not in the nonseparated group (Table 3). Figure 1 displays the interactions with the mean BDI scores, and Figure 2 displays interactions with the mild to severe BDI scores.

Table 2 also shows that interactions between *FKBP5* SNPs and haplotypes with self-reported versus objectively recorded separation were not significant in relation to either mean BDI or mild to severe BDI scores (p values > .13). Next, we found that neither the three *FKBP5* SNPs (p values for interaction > .19) nor haplotypes based on these SNPs (p values for interaction > .07) moderated associations between

Table 2. *FKBP5* Selected SNP and Haplotype × Early Life Stress Interactions in Relation to Depressive Symptoms in Midlife

	Mean BDI Score[a]				Mild to Severe BDI Score[b]			
	Nonseparated vs.				Nonseparated vs.			
FKBP5 SNP	Combined SR and OR Separated, $p_{interaction}$[c]	SR Separated, $p_{interaction}$[c]	OR Separated, $p_{interaction}$[c]	SR Separated vs. OR Separated, $p_{interaction}$[c]	Combined SR and OR Separated, $p_{interaction}$[c]	SR Separated, $p_{interaction}$[c]	OR Separated, $p_{interaction}$[c]	SR Separated vs. OR Separated, $p_{interaction}$[c]
rs1360780 (CC/CT/TT)	.042	.540	.020	.240	.002	.020	.026	.509
rs9470080 (CC/CT/TT)	.016	.238	.014	.247	.001	.005	.047	.771
rs9394309 (AA/AG/GG)	.019	.247	.016	.225	.003	.012	.072	.704
TTG haplotype[d]	.031	.348	.018	.181	.001	.024	.005	.805

BDI, Beck Depression Inventory; OR, objectively recorded; SNP, single nucleotide polymorphism; SR, self-reported.

[a]Mean BDI score refers to mean BDI scores at age 61.5 years (time 1) and 63.4 years (time 2).

[b]Mild to severe BDI score refers to BDI scores 10–63 at age 61.5 years (time 1) and 63.4 years (time 2).

[c]$p_{interaction}$, *p* values for *FKBP5* SNP/haplotypes × early life stress interactions in relation to depressive symptoms after adjusting for age at testing, sex, father's occupational status in childhood, own maximum attained level of education in adulthood, stressful life events in the last 12 months, the first three multidimensional scaling components derived from genome-wide data, and interactions of these covariates/confounders with *FKBP5* SNPs and with separation status.

[d]TTG haplotype is haplotype containing T allele in rs1360780, T allele in rs9470080, and G allele in rs9394309.

stressful life events in the last 12 months and mean BDI or mild to severe BDI scores.

Finally, we tested if the *FKBP5* SNPs interacted with age at and length of separation. For these analyses, groups homozygous and heterozygous for minor alleles were combined because of small cell sizes (these analyses were restricted to the objectively separated group, and data on age at and length of separation were available in a smaller subset within this group). We found that rs9470080 and rs9394309 interacted with age at separation, and rs9470080 interacted with length of separation in relation to mean BDI scores (*p* values for interactions $< .04$). Figure 3 shows that individuals with any minor alleles compared with individuals homozygous for major alleles had higher mean BDI scores if their separation occurred at an age older than the median age at separation in this sample (Figure 3A, B) and if their separation lasted longer than the median length of separation in this sample (Figure 3C).

DISCUSSION

We show in this study that three selected *FKBP5* polymorphisms and a haplotype derived from these SNPs interacted with ELS but not with recent stressful life events in predicting self-reported depressive symptoms in midlife. Mean depressive symptoms score averaged across two measurement points 2 years apart or odds for having a mild to severe depressive symptoms score at both of these two time points, or both, increased according to the number of minor alleles in rs1360780, rs9470080, and rs9394309 in the *FKBP5* locus and haplotypes derived from these alleles in individuals who were exposed to ELS and not in individuals who were not exposed to ELS. These associations were not due to several confounders. Although these interactions were slightly more pronounced when we used objectively recorded rather than self-reported information on the ELS exposure in childhood, the difference in interaction effects according to the source of information was not statistically significant. We did not find either any genetic main effects or indication of correlation between *FKBP5* polymorphisms and separation status that might bias the significant interactions. Our findings suggest that *FKBP5* polymorphisms in combination with ELS predict more pronounced depressive symptoms in midlife. Our findings also add to the previous literature by showing in a prospective epidemiologic study setting that objective documents and self-reports offer valuable information on ELS. This finding suggests that although studies on the long-term effects of ELS should aim at using data of objectively recorded traumas, retrospective studies have a worthwhile place in research providing a "second best option" (20).

Our findings are in agreement with two previous studies (17,18) but in disagreement with one previous study (19) that tested *FKBP5* × ELS interactions in relation to MDD and depressive symptoms. Apart from differing in whether ELS was retrospectively reported or objectively recorded, all these studies differ from each other in events captured by the various ELS instruments. Our study is unique in that ELS was defined by one distinct adverse separation event that was the same for all exposed study participants. In the previous studies, ELS was defined by the number of various traumatic events, which resulted in contradictory findings. Although significant *FKBP5* × ELS interactions were obtained for retrospectively reported physical and sexual abuse, rape, natural disaster, serious accident, or kidnapping (17,18), there were no significant *FKBP5* × ELS interactions when ELS was defined by self-reports of parental death or divorce (17), experiences of maltreatment, emotional or sexual abuse, or emotional or physical neglect (18) or by the number of childhood physical or sexual abuse trauma events and nonabuse trauma events, which included exposures to a natural disaster, serious accident or injury, serious life-threatening illness, and military combat (19). These studies suggest that the statistically significant and nonsignificant *FKBP5* × ELS interactions are not specific to a single adverse event, and hence in this context no single event can be categorized as more adverse

Table 3. *FKBP5* Selected SNP and Haplotype Effects in Participants Who Were and Who Were Not Exposed to Early Life Stress in Relation to Depressive Symptoms in Midlife

	Mean BDI Score[a]		Mild to Severe BDI Score[b]	
FKBP5 SNP Effect Among	Unstandardized Regression Coefficient (95% CI)	*p*	Odds Ratio (95% CI)	*p*
rs1360780 (CC/CT/TT)				
Combined self-reported and objectively recorded separated[c]	.22 (.019, .42)	.03	2.6 (1.5, 4.8)	.001
Self-reported separated[d]	.06 (−.25, .37)	.70	2.6 (1.01, 5.5)	.05
Objectively recorded separated[e]	.33 (.06, .60)	.02	2.7 (1.2, 6.1)	.02
Nonseparated[f]	−.01 (−.11, .10)	.92	.88 (.60, 1.3)	.49
rs9470080 (CC/CT/TT)				
Combined self-reported and objectively recorded separated[c]	.23 (.04, .41)	.02	2.7 (1.6, 4.7)	.000
Self-reported separated[d]	.12 (−.15, .39)	.39	3.0 (1.3, 6.9)	.01
Objectively recorded separated[e]	.31 (.05, .58)	.02	2.5 (1.1, 5.7)	.03
Nonseparated[f]	−.04 (−.13, .06)	.48	.81 (.57, 1.2)	.27
rs9394309 (AA/AG/GG)				
Combined self-reported and objectively recorded separated[c]	.21 (.03, .40)	.03	2.5 (1.5, 4.3)	.001
Self-reported separated[d]	.11 (−.16, .38)	.44	2.7 (1.2, 6.1)	.02
Objectively recorded separated[e]	.30 (.04, .57)	.03	2.2 (.97, 5.1)	.06
Nonseparated[f]	−.04 (−.14, .06)	.38	.85 (.59, 1.2)	.40
TTG haplotype[g]				
Combined self-reported and objectively recorded separated[c]	.227 (SE .1027)	.028	.110 (SE .033)	.001
Self-reported separated[d]	.071 (SE .154)	.645	.1095 (SE .0489)	.027
Objectively recorded separated[e]	.338 (SE .141)	.019	.336 (SE .141)	.019
Nonseparated[f]	−.02 (SE .053)	.67	−.009 (SE .016)	.565

Genomic effects are adjusted for age at testing, sex, father's occupational status in childhood, own maximum attained level of education in adulthood, stressful life events in the last 12 months, and the first three multidimensional scaling components derived from genome-wide data.

BDI, Beck Depression Inventory; CI, confidence interval; SNP, single nucleotide polymorphism.

[a]Mean BDI score refers to mean of BDI scores at age 61.5 years (time 1) and 63.4 years (time 2).

[b]Mild to severe BDI score refers to BDI scores 10–63 at age 61.5 years (time 1) and 63.4 years (time 2).

[c]n = 334–337.

[d]n = 157–159.

[e]n = 176–179.

[f]n = 1057–1063.

[g]TTG haplotype is haplotype containing T allele in rs1360780, T allele in rs9470080, and G allele in rs9394309.

than another. Apart from the methodologic measurement-related differences, variations in the findings may arise from different distributions of specific ELS events and age at measurement of ELS (range, 14–90 years) in the studied samples; variations in accuracy of memory, reinforced memory, nondisclosure, and perceived stressfulness of the ELS event; variations in age at and recurrence of ELS exposure; and variations in resources available to cope with the ELS event. Other differences in these studies relate to mental health of the samples, including MDD diagnosis (17,18); severity of depressive symptoms (18,19) and presence of other psychopathology, such as posttraumatic stress disorder, in the sample (19); and individual differences in personality and intelligence (36,37)—factors that may introduce additional bias in the retrospective reports.

Our finding that associations between stressful events in midlife and depressive symptoms were not moderated by genetic variation in *FKBP5* are in line with earlier findings related to posttraumatic stress disorder (16,19) and point toward a sensitive period in childhood. However, constraints relating to recalling infancy and early childhood events will hamper studying age and recurrence effects of ELS in any future study pertaining to retrospective recall. Hence, a novel aspect of our study was that we were able to explore the age at which ELS exposure occurred and its duration. Individuals with any minor alleles had higher depressive symptoms scores than individuals homozygous for the major allele, but only if their separation from their biological parents took place at an age older than the median age at separation in this sample, which was 3.8 years, and if their separation lasted longer than the median length of separation in this sample, which was 1.4 years. These interaction effects were not found across all three *FKBP5* SNPs, but with rs9470080 (age at separation) and rs9394309 (age at and length of separation) SNPs. Therefore, findings from these interactions should be interpreted with some caution, even though they may point to a critical time window for stress neuromodulation in individuals genetically vulnerable to HPA axis dysregulation.

Epigenetic modifications may underlie the detected associations: Some studies, albeit not all (38,39), reported methylation differences in *FKBP5* between individuals who reported ELS and individuals who did not (13,40). *FKBP5* rs136780 moderated these effects such that demethylation was significantly higher in the minor allele carries reporting ELS (13).

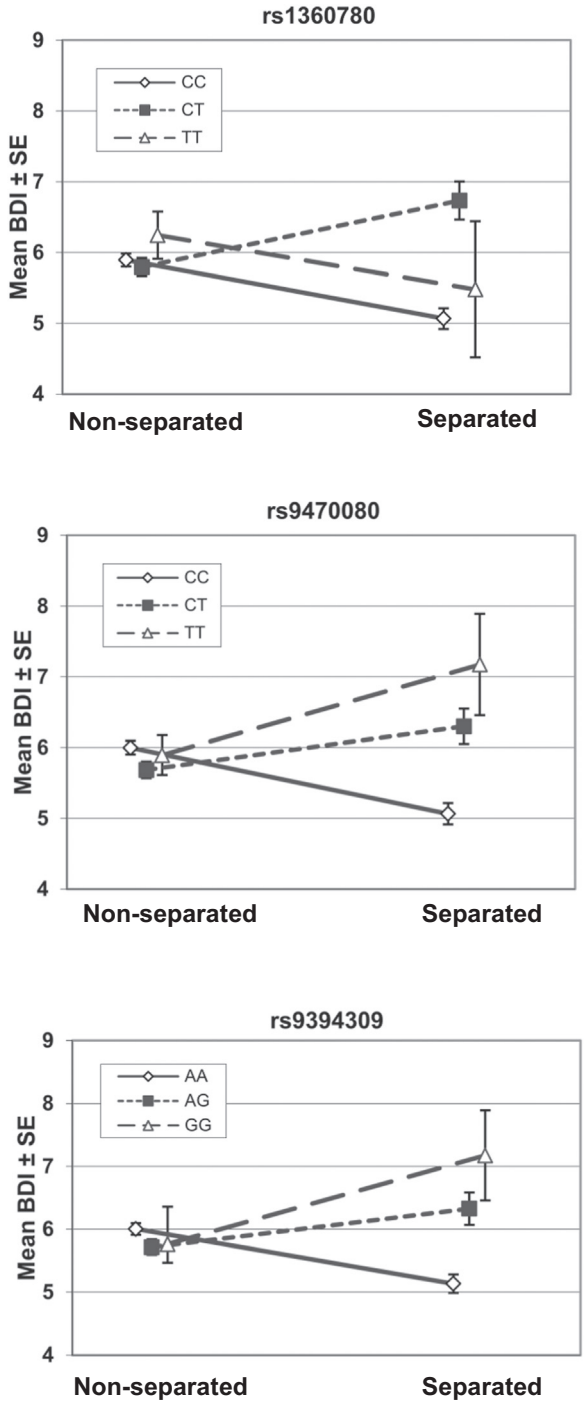

Figure 1. Estimated mean depressive symptoms scores for different genotypes in three selected *FKBP5* single nucleotide polymorphisms in participants who were exposed to early life stress (separated group) and participants who were not exposed to early life stress (nonseparated group). Mean Beck Depression Inventory (BDI) score refers to mean of BDI scores at age 61.5 years (time 1) and 63.4 years (time 2). Error bars reflect SEM. The BDI scores were adjusted for age at testing, sex, father's occupational status in childhood, own maximum attained level of education in adulthood, stressful life-events in the last 12 months, and the first three multidimensional scaling components.

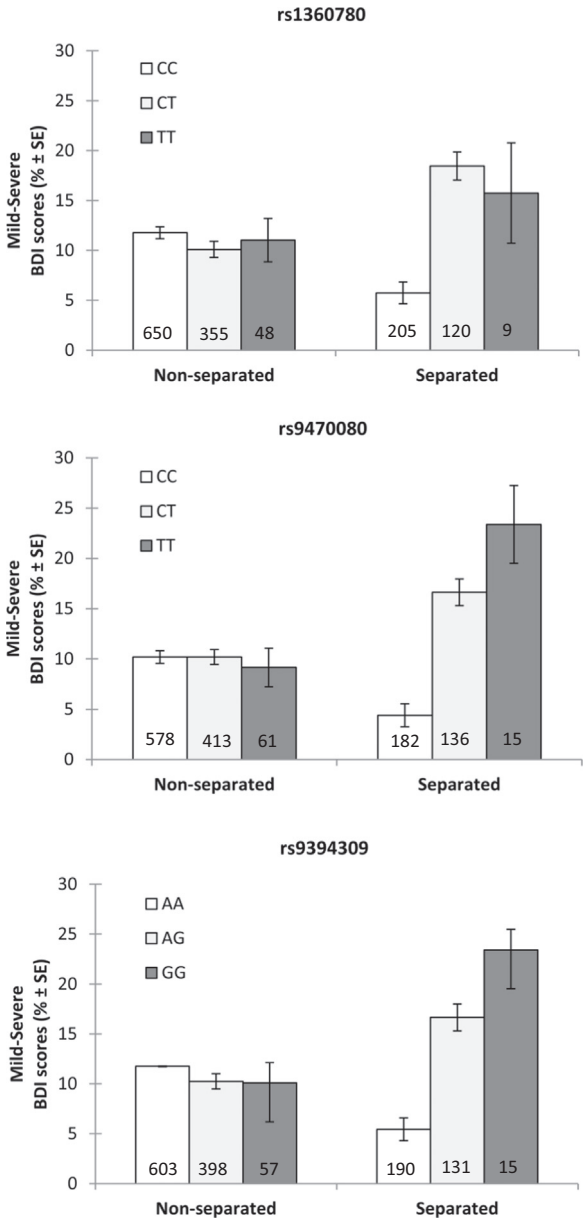

Figure 2. Estimated percentage of individuals with mild to severe depressive symptoms scores for different genotypes in three selected *FKBP5* single nucleotide polymorphisms in participants who were exposed to early life stress (separated group) and participants who were not exposed to early life stress (nonseparated group). Mild to severe Beck Depression Inventory (BDI) score refers to BDI scores 10–63 at age 61.5 years (time 1) and 63.4 years (time 2). Error bars reflect SEM percent, and numbers in columns indicate number of participants. Percentages were adjusted for age at testing, sex, father's occupational status in childhood, own maximum attained level of education in adulthood, stressful life events in the last 12 months, and the first three multidimensional scaling components.

Differences in the findings may be explained by differences in sample tissues and measured CpG sites or by relatively small sample sizes. Further studies in larger samples are warranted.

Strengths of this study relate to a relatively large sample size, self-reported and objectively recoded single measure of

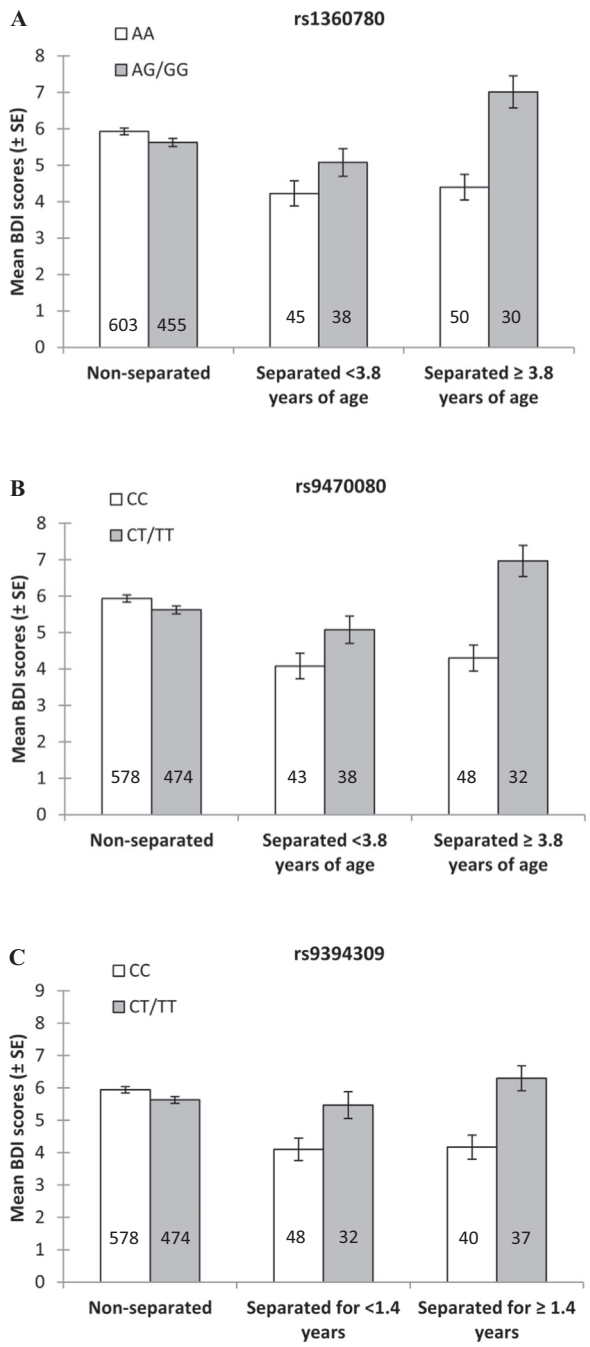

Figure 3. Estimated mean depressive symptoms scores for different genotypes in selected *FKBP5* single nucleotide polymorphisms in participants who were exposed to early life stress younger or older than the median age at exposure in the sample **(A, B)** and for shorter or longer than the median duration of exposure in the sample **(C)**. Mean Beck Depression Inventory (BDI) refers to the mean of BDI scores at age 61.5 years (time 1) and 63.4 years (time 2). Error bars reflect SEM, and numbers in columns indicate number of participants. The BDI scores were adjusted for age at testing, sex, father's occupational status in childhood, own maximum attained level of education in adulthood, stressful life events in the last 12 months, and the first three multidimensional scaling components.

ELS providing more standardized and objective estimate of stressful environment, and follow-up over several decades. Our data also allowed us to investigate the impact of timing and length of separation. Moreover, our design allowed us to exclude the possibility that the environmental stressor was affected by the gene.

Our study also has some limitations. Although our findings suggest that self-reports and objective records of ELS offer valuable information in this study context, validity and measurement error in self-reported ELS are questionable. Also, we have no data on stressfulness of the separation. Some of the separated children also may have experienced another separation from foster parents when they returned to Finland. Furthermore, we cannot determine how much more stressful the separation from both parents was compared with the level of stress in the nonseparated participants who stayed with their parents during the war. However, the separated and the nonseparated participants were exposed to war, as none of the children were evacuated before the war broke out, and some of the separated children returned home during the war. Hence, our findings may offer a conservative estimate of the differences between the separated and the nonseparated groups. Other study limitations relate to generalizability of the findings of self-reported depressive symptoms to mood disorders and lack of data on childhood psychopathology precluding analyses pertaining to continuity. Also, our sample was overrepresented by women, although the associations were not moderated by sex; it was homogeneous in age testing, and all study participants were Finns, precluding generalizability of our findings to groups varying in age and ethnicity. However, the genetic structure of individuals born in South Finland, such as the participants of this study, has been shown to be closer to individuals born in Central Europe than to individuals born in Northern Finland (41).

In conclusion, our study shows that *FKBP5* polymorphisms in combination with ELS predict more pronounced depressive symptoms in midlife. Our findings confirm previous retrospective findings in a prospective epidemiologic study setting and suggest that retrospective reports and objective records of ELS provide valuable information in this G×E context.

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