Test of the Serotonin Transporter Gene × Early Life Stress Interaction Effect on Subjective Well-Being and Loneliness Among Japanese Young Adults

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Abstract

This study investigated whether aspects of early life environment (quality of parental relationship, frequency of parental violence including disciplinary violence, amount of parental attention, and family income during childhood) would affect one's subjective well-being and loneliness later in life (i.e., during young adulthood). This study also investigated whether the negative influence of early life stress is greater among individuals with the *SS* genotype of the serotonin transporter-linked polymorphic region (5-HTTLPR) in the serotonin transporter gene. Participants were 568 university students whose genotypes were identified using nail samples. They reported their current levels of subjective well-being and loneliness as well as their recollections of their early family environments. The results showed that less stressful early life environments, such as high levels of parents' relationship quality and parental attention, were positively (negatively) associated with subjective well-being (loneliness), while frequent parental violence was negatively (positively) associated with subjective well-being (loneliness). Nevertheless, the gene × environment interaction effects were consistently non-significant—the *SS*genotype did not accentuate the effects of early life stress.

Studies exploring gene-by-environment (G × E) interactions have increased exponentially in the 21st century. According to Dick (**2011**), PubMed lists only 24 studies on G × E interactions prior to 2000; however, approximately 100 such studies were published in the first half of 2010 alone. The popularity of G × E interactions may have multifold reasons. Theoretically, they may provide an answer to the “missing heritability” problem. Several genome-wide association (GWA) studies have observed that a large set of single-nucleotide polymorphisms (SNPs) accounts for a substantially smaller portion of the variance in psychological traits than the heritability typically reported in behavioral genetics studies (e.g., twin studies). Because GWA studies typically consider only the main effects of SNPs, G × E interactions may explain at least some portion of such a “missing heritability” (Manuck & McCaffery, **2014**). The G × E interaction also has practical clinical implications. Chen et al. (**2012**), for example, found that effects of a smoking cessation treatment (i.e.,

environment) on cessation success varied among individuals with different genotypes of a cluster of nicotinic receptor genes. Given these theoretical and practical implications, it is not surprising that investigations of G × E interactions became increasingly popular during the early 2000s, when the human genome sequencing was near completion (Gibbs, **2020**).

According to Manuck and McCaffery (**2014**), G × E studies were facilitated by Caspi et al.'s (**2002**, **2003**) successful demonstrations of such phenomena. In one of these influential studies, Caspi et al. (**2003**) focused on a particular variation in the serotonin transporter gene (SLC6A4). Serotonin transporter (5-HTT) regulates serotonergic neurotransmission by removing serotonin released into the synaptic cleft. The SLC6A4 gene has two variants (short and long) in the serotonin transporter-linked polymorphic region (5-HTTLPR). The short (*S*) variant produces less 5-HTT protein than does the long (*L*) variant, thus less efficiently removing serotonin from the synaptic cleft (Canli & Lesch, **2007**). This variation has some observable effects. For example, the *S* allele is associated with attentional vigilance toward negative stimuli (see Pergamin-Hight, Bakermans-Kranenburg, van Ijzendoorn, & Bar-Haim, **2012**, for a meta-analytic review) and higher amygdala reactivity to environmental threats (e.g., Hariri et al., **2005**). These findings suggest that the *S* allele is sensitive to environmental stress. In fact, Caspi et al. (**2003**) showed that the effects of stressful life events on depression and suicidal ideation/attempts were stronger among individuals with the *S* variant than individuals with the *L* variant.

Because of the theoretical and practical importance of this finding, many replication studies were conducted but yielded mixed results. Although at least five meta-analytic reviews of this particular G × E interaction (i.e., 5-HTTLPR × life stress interaction) have been conducted, they also reached different conclusions. The first two meta-analyses found little support for the G × E interaction (Munafò, Durrant, Lewis, & Flint, **2009**; Risch et al., **2009**). Some authors have criticized these meta-analyses, arguing that their inclusion criteria were biased toward negative studies (e.g., Rutter, Thapar, & Pickles, **2009**; Uher & McGuffin, **2010**). Two more recent meta-analyses found evidence favoring this interaction (Karg, Burmeister, Shedden, & Sen, **2011**; Sharpley, Palanisamy, Glyde, Dillingham, & Agnew, **2014**). Although the most recent collaborative meta-analysis, in which each research team analyzed their data in the same manner and estimated comparable effect sizes, reached a negative conclusion (Culverhouse et al., **2018**), it was also criticized upon publication of its meta-analysis plan; Moffitt and Caspi (**2014**) expressed concern regarding the prioritization of sample size over the quality of measures and designs (see also Ancelin & Ryan, **2018**).

The mixed support for the 5-HTTLPR × life stress interaction suggests that there are some moderator variables. For example, based on their meta-analysis, Karg et al. (**2011**) suggested that childhood stress may be associated with a reliable interaction effect: the significant G × E interaction was not found in studies that assessed general stressful events but emerged upon inclusion of only those studies that narrowly focused on childhood maltreatment and specific medical conditions (see Sharpley et al., **2014**, for a different result). For example, in one study, the risk of depression in a later stage of life increased more in response to harsh childhood environments among individuals with the *SS* genotype compared to those with at least one long allele (Taylor et al., **2006**). Because early life stress has been documented as a reliable predictor of various negative outcomes in later life, such as depression and anxiety disorder (Nugent, Tyrka, Carpenter, & Price, **2011**), it may also be associated with a particularly strong G × E interaction effect.

Furthermore, Karg et al. (**2011**) noted that this G × E effect tends to be larger when continuous measures of depression are used rather than categorical measures of depression. This finding suggests that the 5-HTTLPR × environment interaction may be associated with subtle effects: although the combination of early life stress and the *S* allele may not impair mental health to the extent of a depression diagnosis, it may still reduce life satisfaction and well-being in one's later life (De Neve, **2011**; Matsunaga, Isowa, Yamakawa, & Ohira, **2013**). Subjective well-being is a broad concept that refers to people's evaluation of their own lives, reflecting the presence of positive affect and a lack of negative affect (Diener, Oishi, & Lucas, **2003**). Therefore, it is not the same as the mere absence of depression (Jahoda, **1958**). Nevertheless, it is reasonable to assume that depression and subjective well-being (and its related concepts) are inversely related. More relevant to the present study, Gärtner et al. (**2018**) found that stressful childhood environments had a negative effect on the life satisfaction of relatively young individuals (<30 years) with the *SS* genotype, but not on the life satisfaction of those with at least one *L* allele (notice, however, that this G × E interaction was reversed among an elderly subsample consisting of individuals older than 60 years).

Another subtle form of the G × E interaction may be associated with loneliness. Loneliness is defined as a negative feeling resulting from a discrepancy between one's desired and actual levels of social relations, and is typically implicated in the etiology of depression (Cacioppo et al., **2015**). Therefore, loneliness is worth investigating in the context of the 5-HTTLPR × early life stress interaction. For example, van Roekel, Scholte, Verhagen, Goossens, and Engels (**2010**) found a 5-HTTLPR × perceived maternal support interaction effect on loneliness. This longitudinal study assessed participants' loneliness five times throughout their early (12–

14 years) to late (16–18 years) adolescence. The levels of perceived parental support at Time 1 predicted loneliness, such that paternal and maternal support were negatively correlated with later loneliness. More importantly, the 5-HTTLPR × maternal support interaction was also significant: Individuals with the *SS*and *SL* genotypes were lonelier when they reported less maternal support at Time 1, whereas loneliness of individuals with the *LL* genotype was not associated with perceived maternal support at Time 1. However, the interaction with paternal support was not significant. Therefore, van Roekel et al.'s results cannot be considered as strong support for the 5-HTTLPR × *parental* support interaction effect on loneliness. In fact, Spithoven et al. (**2015**) recently failed to replicate van Roekel et al.'s results using a different dataset.

Taken together, although there may be subtle G × E effects on subjective well-being and loneliness, the evidence for such effects is mixed at best. However, if such subtle effects exist, they may have greater implications in Japan than in Western countries, where most of the previous studies on this subject have been conducted (see Goldman, Glei, Lin, & Weinstein, **2010**; Tomoda et al., **2013**, for some exceptions). It is noteworthy that the putatively susceptible allele (i.e., *S*allele) is more prevalent in East Asian countries (including Japan) than in Western countries (Goldman et al., **2010**). According to previous studies reporting the frequency of 5-HTTLPR alleles in Japan, approximately 60% of Japanese individuals have two *S* alleles, and an additional 30% have one *S* allele (see table 1 in Goldman et al., **2010**). Therefore, it is worth investigating whether early life stress is negatively associated with subjective well-being and positively associated with loneliness, especially among individuals with the *SS* genotype (vs. *L*-carriers).

This study is a part of a larger research project, an exploratory cross-cultural investigation of various G × E effects (see Ishii et al., **2018**, **2021**; Zheng et al., **2020**, for published reports on the project). We used a subset of the project's data to test for subtle effects of the 5-HTTLPR × childhood experience interaction on subjective well-being and loneliness in Japanese young adults. Accordingly, the study population consisted of ordinary university students, and no clinical assessments were conducted. Therefore, it is likely that a substantial proportion of participants were not from high-risk families. Although these sample characteristics may appear undesirable for a conceptual replication of Caspi et al.'s (**2003**) original study, they are rather appropriate for a test of subtle G × E effects. The present analysis of this dataset serves as a first step in the investigation of subtle forms of the G × E effect in Japanese young adults.

Method

Participants and Ethics Statement

This study used data from three sub-studies of the aforementioned research project (see Table 1 for a summary of demographic variables). The first sub-study, which was conducted at Kobe University in 2015, involved 213 participants. The second sub-study, which was conducted at Kobe University in 2018, involved 203 participants. The third sub-study, which was conducted at Nagoya University in 2019, involved 204 participants. Therefore, the dataset analyzed in this study included a total of 620 participants. However, the 5-HTTLPR genotype was successfully identified for 568 participants.

Table 1. Summary of demographic variables (age and gender) and genotype frequency of the three sub-studies

	Sub-study 1	Sub-study 2	Sub-study 3	Total
Gender (frequency)				
Men	100	98	94	292
Women	112	105	110	327
Unreported	1	0	0	1
Age				
Range (excluding a 33-year participant)	18–25	18–33 (18–23)	18–25	18–33 (18–25)
Mean (excluding a 33-year participant)	19.25	19.70 (19.63)	19.82	19.59 (19.57)
Standard deviation (excluding a 33-year participant)	0.99	1.42 (1.07)	1.36	1.29 (1.17)

	Sub-study 1	Sub-study 2	Sub-study 3	Total
Father's education (frequency)				
Junior high school graduate	4	1	4	9
High school graduate	50	31	36	117
College educated	14	15	15	44
University graduate	120	120	128	368
Higher than university graduate	23	33	17	73
Mother's education (frequency)				
Junior high school graduate	0	0	2	2
High school graduate	54	35	44	133
College educated	71	69	80	220
University graduate	80	94	69	243
Higher than university graduate	6	4	6	16
5-HTTLPR (frequency)				
SS	125	133	132	390
SL	56	48	50	154

	Sub-study 1	Sub-study 2	Sub-study 3	Total
LL	12	2	10	24

All participants volunteered for the study for monetary compensation. They signed an informed consent form prior to participation. After completing the study, they were debriefed and paid for their participation. This study was conducted in accordance with the ethical principles of the American Psychological Association (**2017**). All three sub-studies were approved by the ethical review boards of the institutes in which they were conducted.

Materials

The three sub-studies varied in terms of the questionnaires administered within each sub-study. However, all three studies included the Subjective Happiness Scale (Lyubomirsky & Lepper, **1999**), the UCLA Loneliness Scale (Russell, Peplau, & Cutrona, **1980**), and a childhood family environment questionnaire developed by the researchers of the project. The Japanese version of the Subjective Happiness Scale (Shimai, Otake, Utsuki, Ikemi, & Lyubomirsky, **2004**) consists of four items (e.g., "In general, what is your level of happiness?") rated on a 7-point scale (e.g., 1 = *not happy at all* to 7 = *very happy*). These four items were averaged to obtain a single score for subjective well-being (Cronbach's α coefficient = .84). The Japanese version of the UCLA Loneliness Scale (Moroi, **1992**) consists of 20 items (e.g., "There is no one I can turn to") that were rated on a 4-point scale (1 = *never feel so* to 4 = *frequently feel so*). These 20 items were averaged to obtain a single score for loneliness (Cronbach's α coefficient = .92).

The childhood family environment questionnaire contained the following items: (a) one item assessing their parents' relationship quality during childhood (1 = *very bad* to 6 = *very good*); (b) two items asking participants to compare their family incomes during childhood with the Japanese and neighborhood averages, respectively (1 = *very low* to 5 = *very high*); (c) two items assessing the amount of attention that participants had received from their fathers and mothers, separately ("How much attention did your father [mother] give to you during your childhood?"; 1 = *not at all* to 6 = *a lot*); and (d) two items assessing the frequency of violence that participants had received from their mothers and fathers, separately ("Did your father [mother] use violence, including disciplinary violence, on you during your childhood?"; 1 = *not at all* to 6 = *on a daily basis*). The two childhood income items were summed to obtain a single score for childhood income (r_{614} = .79). The two attention items (i.e., maternal and paternal attention) were summed

to obtain a single score for parental attention (r_{612} = .32). The two violence items were summed to obtain a single score for parental violence (r_{613} = .44). Participants who had a missing value for one of the two aggregated variables (one participant for childhood income, four for parental attention, and four for parental violence) were not included in the subsequent analyses. Consequently, the sample sizes involving parents' relationship quality, childhood income, parental attention, and parental violence were 559, 565, 563, and 564, respectively. These four milder forms of early life stress (quality of parental relationship, childhood income, parental attention, and parental violence) conceptually correspond to severer types of early life stress (quarrels and violence between parents, socioeconomic status, perceived parental support, and physical maltreatment) examined in previous studies (e.g., Åslund et al., **2009**; Brummett et al., **2008**; Spithoven et al., **2015**; van Roekel et al., **2010**).

The second sub-study included the Risky Family Questionnaire (RFQ), which was used in Taylor et al.'s (**2006**) G × E interaction study. Using data from the second sub-study, we confirmed that all the early life stress scores except for the childhood income score were significantly correlated with the more severe form of early life stress assessed by the RFQ (r = −.50, .57, and −.45, for parents'relationship quality, parental violence, and parental attention, respectively; see Table **S1** in the Supporting Information for more details).

Genotyping

All participants provided nail samples for genotyping. Following established procedures (Wendland, Martin, Kruse, Lesch, & Murphy, **2006**), we extracted genomic DNA from participants' nail samples using ISOHAIR kits (NIPPON GENE Co., Ltd., Tokyo, Japan). Polymerase chain reactions (PCR) were performed using the primers 5'-TCCTCCGCTTTGGCGCCTCTTCC and 5'-TGGGGGTTGCAGGGGAGATCCTG in a total volume of 25 μl solution containing 100 ng of genomic DNA, 0.4 mM of dNTPs, 0.2 μM of each primer, 1.25 U of Takara LA Taq polymerase (Takara Bio Inc., Shiga, Japan), and GC buffer I (Takara Bio Inc.). Thermal cycling consisted of 15 min of initial denaturation at 95°C followed by 35 cycles of 30 s at 94°C, 90 s at 65.5°C, and 60 s at 72°C, and the final extension step of 10 min at 72°C. The PCR products were then analyzed in a 3% agarose gel (PrimeGel Agarose LMT PCR-Sieve GAT, Takara Bio Inc.) stained with ethidium bromide. The amplification product for the *L* allele was 512 bp, while the amplification product for the *S* allele was 469 bp, as described previously (Wendland et al., **2006**). This procedure, which has been widely used in previous studies (Gadow et al., **2013**; Holmes, Bogdan, & Pizzagalli, **2010**), was used to identify each individual's genotype (either *SS*, *SL*, or *LL*).

Consistent with previous reports on the frequency of 5-HTTLPR in Japan, the majority of the participants (68% = 391/568) had the *SS* genotype, 27% (155) had the *SL* genotype, and 4% (25) had the *LL* genotype. Although this distribution contained a slightly higher number of *LL* genotype (25) than expected (18.40), it did not significantly deviate from the Hardy–Weinberg equilibrium, $\chi^2(1) = 3.52$, $p = .061$. Due to the small number of participants with the *LL* genotype, we grouped those with the *SL* and *LL* genotypes together as *L*-carriers. We think that this re-categorization (*SS* vs. *L*-carriers) is justified given the apparent additive effect of the *S* and *L* alleles. For example, Caspi et al. (**2003**) reported that the effects of stressful life events on self-reported depression symptoms, indexed by a regression slope (*b*), was 2.52, 1.71, and 0.77 for participants with the *SS*, *SL*, and *LL* genotypes, respectively; while those of childhood maltreatment were 0.60, 0.45, and − 0.01 for participants with the *SS*, *SL*, and *LL* genotypes, respectively.

Results

Hypothesis Testing

Descriptive statistics for the variables of interest are summarized in Table S2 in the Supporting Information. As can be seen in Table S2 (also in Figure 1), this sample mostly consisted of individuals from average or slightly above-average income families ($M \pm SD = 6.57 \pm 1.38$ on a scale of 2 to 10). Participants reported that they received a decent amount of parental attention ($M \pm SD = 10.04 \pm 1.52$ on a scale of 2 to 12) and did not experience frequent parental violence ($M \pm SD = 3.36 \pm 1.39$ on a scale of 2 to 12). Therefore, this sample was suitable to test the effects of non-severe forms of early life stress.

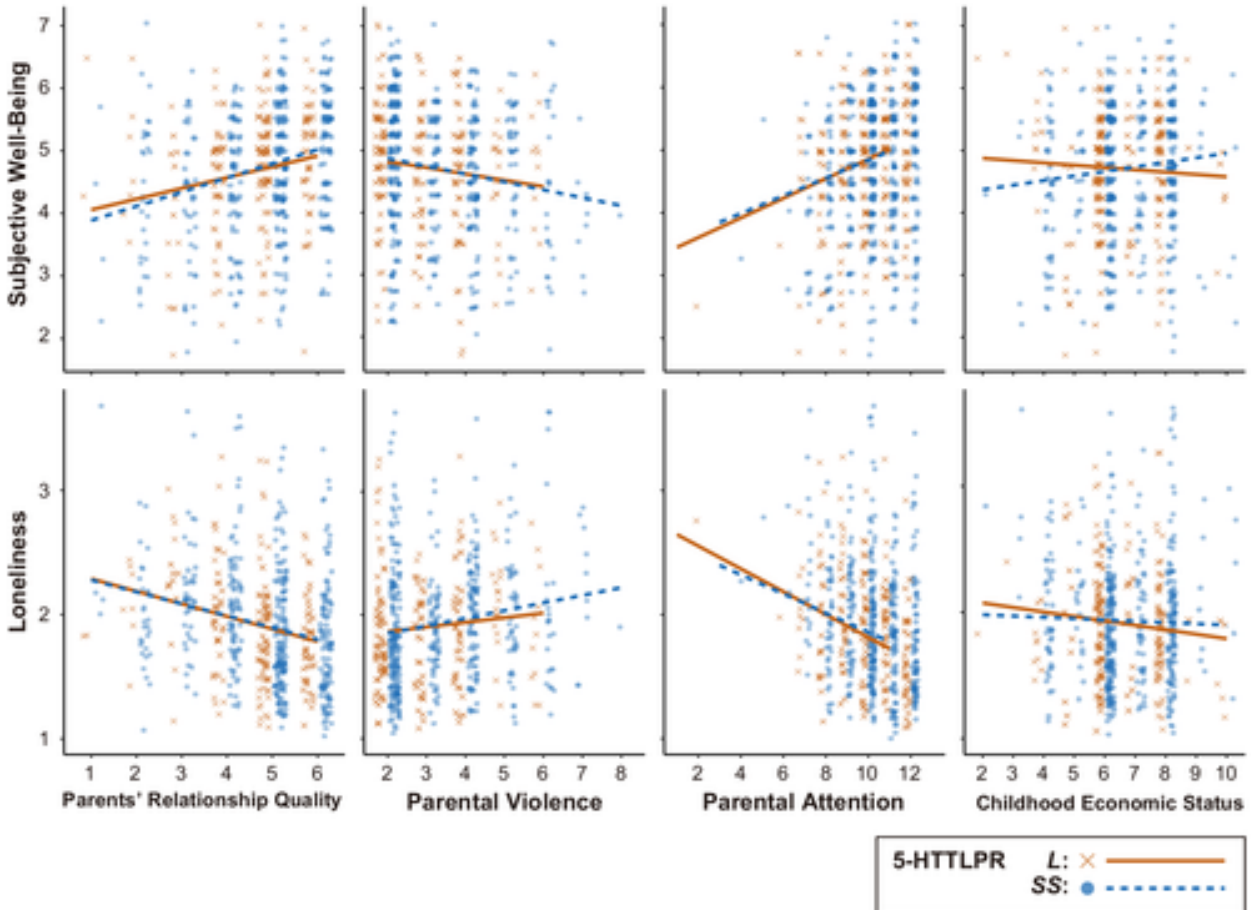

Figure 1

[Open in figure viewerPowerPoint]

Graphical illustration of G (5-HTTLPR) × E (childhood parents' relationship quality, parental violence, parental attention, and family income) effects on subjective well-being and loneliness. The solid and dashed lines correspond to *L*-carriers and individuals with the *SS* genotype, respectively. The cross marks and circles indicate *L*-carriers and individuals with the *SS*genotype, respectively. Cross-marks are distributed on the left side of the corresponding values for the environmental effects (x-axis), and the circles are distributed on the right side. The cross marks and circles are jittered in both the horizontal and vertical directions because environmental variables only take integer values and subjective well-being/loneliness only take certain values (e.g., 1, 1.25., 1.5, etc.).

We first tested the main effects of 5-HTTLPR genotype. Contrary to some previous findings, the 5-HTTLPR genotype had no main effects on well-being ($M \pm SD = 4.70 \pm 1.09$ and 4.70 ± 1.04 for *SS* individuals and *L*-carriers, respectively, $t[566] = 0.06$, $p = .955$) or loneliness (1.94 ± 0.51 and 1.91 ± 0.47 for *SS* individuals and *L*-carriers, respectively, $t[566] = 0.61$, $p = .540$).

We then tested the G × E effect by a series of eight multiple regression analyses, each involving one of the four childhood environment variables, the 5-HTTLPR genotype, and the interaction term as the independent variables and either subjective well-being or loneliness as the dependent

variable. The standardized regression coefficients, 95% confidence intervals, and *p*-values of the regression analyses are summarized in Table 2. We report standardized regression coefficients to facilitate the comparison of the effects of the four childhood environment variables because they were not measured on a common metric. The G × E effects were not significant in any of the eight regression analyses: all βs < .07 (see Figure 1 for graphical illustrations of the non-significant interaction effects).

Table 2. Standardized regression coefficients, 95% confidence intervals (in brackets), *t*-values and *p*-values of eight hypothesis-testing regression analyses

Well-being	Early environment variable			
	Parents' relationship quality	**Parental violence**	**Parental attention**	**Childhood income**
Genotype (*LL*, *SL* = 0, *SS* = 1)	.01 [−.07, .08] *t*(555) = 0.31 *p* = .759	.01 [−.07, .08] *t*(560) = 0.16 *p* = .877	−.002 [−.08, .08] *t*(559) = −0.04 *p* = .971	-3.08×10^{-4} [−.08, .08] *t*(561) = −0.01 *p* = .994
Early environment	.23*** [.15, .32] *t*(555) = 5.66 *p* = 2.39×10^{-8}	−.15*** [−.24, −.07] *t*(560) = −3.60 *p* = 3.48×10^{-4}	.21*** [−.12, −.29] *t*(559) = 5.00 *p* = 7.54×10^{-7}	.05 [.03, .13] *t*(561) = 1.19 *p* = .233
G × E interaction	.03 [−.05, .11] *t*(555) = 0.66 *p* = .510	−.01 [−.10, .07] *t*(560) = −0.35 *p* = .729	−.01 [−.09, .07] *t*(559) = −0.24 *p* = .807	.07 [−.02, .15] *t*(561) = 1.58 *p* = .116

Well-being	Early environment variable			
	Parents' relationship quality	Parental violence	Parental attention	Childhood income
Loneliness	**Parents' relationship quality**		**Parental violence**	**Parental attention**	**Childhood income**
Genotype (*LL*, *SL* = 0, *SS* = 1)	.01 [−.07, .09] $t(555) = 0.20$ $p = .838$		.02 [−.06, .10] $t(560) = 0.41$ $p = .681$	.03 [−.05, .11] $t(559) = 0.63$ $p = .527$	.02 [−.06, .10] $t(561) = 0.48$ $p = .630$
Early environment	−.23*** [−.32, −.16] $t(555) = -5.67$ $p = 2.29 \times 10^{-8}$		.15*** [.07, .24] $t(560) = 3.54$ $p = 4.36 \times 10^{-4}$	−.25*** [−.33, −.17] $t(559) = -6.10$ $p = 2.01 \times 10^{-9}$	−.05 [−.13, .03] $t(561) = -1.21$ $p = .226$
G × E interaction	.01 [−.08, .09] $t(555) = 0.14$ $p = .888$		.03 [−.05, .11] $t(560) = 0.70$ $p = .484$	.02 [−.06, .10] $t(559) = 0.54$ $p = .588$	.03 [−.05, .11] $t(561) = 0.77$ $p = .441$

- *** $p < .001$.

However, the three environmental variables (i.e., parents' relationship quality, parental violence, parental attention) were all significantly associated with both subjective well-being and loneliness. Good parental (i.e., mother–father) relationship and high parental attention during

childhood were positively associated with subjective well-being (β = .23 and .21 for parental relationship and parental attention, respectively) and negatively associated with loneliness (β = −.23 and −.23 for parental relationship and parental attention, respectively) in later years. In contrast, childhood parental violence was negatively associated with subjective well-being (β = −.15) and positively associated with loneliness (β = .15) in later years. Childhood family income was not significantly associated with subjective well-being or loneliness (all βs < .06). This pattern remained intact when the four childhood environmental variables, along with the 5-HTTLPR genotype and gender, were entered into a single multiple regression model: parental relationship, parental attention, and parental violence were significant predictors of subjective well-being and loneliness, but childhood family income was not (see Table **S3** in the Supporting Information for the detailed results of the multiple regression analyses).

Exploratory Analyses

For exploratory purposes, we conducted some follow-up analyses. Although running similar analyses multiple times would generally increase the type I error rate, none of the non-significant interaction effects in the original analyses became significant in these exploratory analyses. Nevertheless, all results reported in this section should be considered preliminary and interpreted with caution.

First, we tested for a 5-HTTLPR × maternal attention effect because van Roekel et al. (**2010**) found a G × E interaction effect on loneliness only for *maternal* support. For exploratory purposes, we tested the effects of both maternal and paternal violence/attention not just on loneliness but also on subjective well-being. The results are summarized in Table **S4** in the Supporting Information. None of the 5-HTTLPR × maternal attention effects (β = −.06 and .02 for well-being and loneliness, respectively), 5-HTTLPR × maternal violence effects (β = −.03 and .02 for well-being and loneliness, respectively), 5-HTTLPR × paternal attention effects (β = .04 and − .001 for well-being and loneliness, respectively), or 5-HTTLPR × paternal violence effects (β = .003 and .03 for well-being and loneliness, respectively) were significant.

In addition, we tested a possible moderation effect of gender. However, instead of testing a higher-order interaction (i.e., gender × G × E interaction), we repeated the same set of analyses for male and female participants separately. The results of these analyses are summarized in Tables **S5** (male) and **S6** (female) in the Supporting Information. Again, neither the G × E interaction effects nor the main effect of the 5-HTTLPR genotype was significant.

Discussion

This study tested the 5-HTTLPR genotype × early life stress interaction effect on later well-being and loneliness in a Japanese nonclinical sample. Among a nonclinical sample of Japanese young adults, there was little evidence for the hypothesized main effect of the 5-HTTLPR *S* allele, let alone the 5-HTTLPR genotype × a mild form of early life stress interaction effect—individuals with the *SS* genotype are not more susceptible to mild forms of early life stress than *L*-carriers.

Limitations and Future Directions

The results, although consistent throughout a series of analyses, have several limitations. First, the original study by Caspi et al. (**2003**) focused on more severe mental health problems, such as depression and suicidal ideation. Moreover, Nugent et al. (**2011**) noted that childhood maltreatment (but not other forms of stress) is consistently associated with the G × E effect. However, it is unlikely that many of the participants in this study had experienced severe forms of childhood stress. For example, all but four participants reported attention from both parents during childhood. This suggests that only a small portion of participants (less than 1%) had experienced parental divorce (a precursor of a severe form of early life stress) during childhood, and there was a considerable range restriction in the severity of early life stress experienced by the participants of the present study. Therefore, it is important to follow-up the current study with individuals who have experienced more severe forms of early life stress (i.e., testing whether the *S* allele accentuates the negative effect of severe childhood stress on subjective well-being and loneliness).

Second, it is possible that the phenotypes of well-being and loneliness might have been too complex, in that they are influenced by many other factors. Therefore, it may be difficult to detect subtle G × E effects on these traits, even if such effects exist (Ancelin & Ryan, **2018**; Canli & Lesch, **2007**). Nevertheless, given that the 5-HTTLPR polymorphism is associated with differential serotonin transporter expression and function, it is unlikely that this polymorphism has no effect on phenotypes. For example, Lachmann et al. (**2021**) found a significant association between 5-HTTLPR and life satisfaction in only a subset of life satisfaction domains (e.g., housing, leisure, and family) but not for overall life satisfaction. Therefore, it may be informative to assess domain-specific life satisfaction in future studies. Another possible direction for future studies is to measure endophenotypes, such as neural activation and structure, which have consistently been shown to be associated with the 5-HTTLPR genotype (Canli & Lesch, **2007**). Therefore, it is recommended that future studies include nuanced self-report measures and physiological measures to test the G × E effect. Third, it is also possible that methylation, which

is subject to environmental factors, plays an important role (Ikegame et al., **2020**). Hence, future studies investigating the effects of the G × E interaction on subjective well-being and loneliness should also investigate the potential moderation effect of methylation on these interactions.

Finally, although the reported analyses consistently supported the main effects of early life stress on later life-satisfaction and loneliness, these effects are subject to mood-congruent memory biases (Blaney, **1986**). In other words, less happy and/or lonelier individuals might have recalled their childhood environments in a more negative light than happier and/or less lonely individuals. To overcome this problem, we need longitudinal data or more objective measures of early life stress.

Concluding Remarks

Although we did not find any significant G × E effects, we consistently found the main effects of early life stress. That is, although mild forms of early life stress were negatively associated with one's well-being in later life, this negative effect is not particularly strong among individuals with the *SS* genotype of the 5-HTTLPR. This non-significant finding is still important given the prevalence of the sensitive allele (i.e., *S* allele) in Japan. However, the range restriction of environmental factors is one of the limitations of this study, especially if the purpose was a replication of Caspi et al.'s (**2003**) original finding. More severe forms of early life stress need to be included to more precisely evaluate their impact on well-being in later life. It is also informative to include more objective assessments (e.g., not relying on retrospective self-reports) of early life stress because previous reviews have reported that time-consuming but detailed assessments of environmental variables are crucial for the detection of G × E interactions (Uher & McGuffin, **2008**, **2010**). Improvements are needed to more strictly test the hypothesized G × E effect on depression, as well as on subjective well-being and loneliness, in Japan. Its reproducibility in Asian countries has not only practical implications (because of the prevalence of the sensitive allele) but also theoretical implications for the recently emerging framework of gene–culture interaction (Sasaki, LeClair, West, & Kim, **2016**). Therefore, high-quality replications in Japan and other countries would deepen both the clinically relevant knowledge and scientific understanding of the role of cultures on G × E interactions.

Conflict of Interest

The authors declare no conflicts of interest associated with this manuscript.