Research report

Influence of serotonin transporter promoter variation on the effects of separation from parent/partner on depression.

Andrés Fandiño-Losada a,b, Yabin Wei c,d, Elin Åberg c,d, Louise K. Sjöholm c,d, Catharina Lavebratt c,d,*1, Yvonne Forsell a,1

a Department of Public Health Science, Karolinska Institute, 171 76 Stockholm, Sweden
b Public Health School/Cisalva Institute, Universidad del Valle, Cali, Colombia
c Department of Molecular Medicine and Surgery, Karolinska Institutet, 171 76 Stockholm, Sweden
d Center for Molecular Medicine, 171 76 Stockholm, Sweden

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ABSTRACT

Background: Loss of parent during childhood or loss of partner has been associated with adulthood depression. The serotonin transporter polymorphism (5-HTTLPR) has been reported to moderate stress sensitivity reflected for example in the relationship between childhood maltreatment and depression. Therefore, the effect of 5-HTT promoter variation on the relationship between the loss of parent or partner and depression was examined.

Method: 411 depressive cases and 1347 control subjects from a large well-characterized longitudinal population-based sample of adult Swedes with data on life history and life situation, including psychiatric diagnostic instruments, were studied. Their DNA was genotyped for the mini-haplotype 5-HTTLPR-rs25531.

Results: Individuals with low 5-HTT activity variants had an increased risk of depression given loss of partner last year compared to those with high activity variants. Conversely, 5-HTT activity variation appeared not to strongly influence the risk of depression given loss of parent during childhood.

Limitation: Small sample size for those with losses of both parent and partner. Limited power to detect small interaction effects.

Conclusion: The increased risk of depression given last year loss of partner appeared to be influenced by genetic variation regulating 5-HTT activity. This adds to previous findings of 5-HTT x stressful life events interactions on depression and is in agreement with stronger GxE effects when using objective environmental measures.

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1. Introduction

Parental separation, either from death of a parent, divorce or other factors, has been reported to be strongly associated with a future increased risk of psychopathology, e.g., depression (Coffino, 2009; Kendler et al., 1996; Tenant et al., 1981; Tennant et al., 1982; Tyrka et al., 2008; for review see Maughan and McCarthy, 1997). These associations might be caused by underlying variables such as a genetic liability and/or comorbid psychopathology in parents and children (Kendler et al., 1996). Also separation from partner later in life, such as death of a partner or divorce, has consistently been reported to be associated with depression (Bruce and Kim 1992; Holmes and Rahe, 1967; Kendler et al., 1995, 2006).

Research conducted with multiple species, using both observational and experimental methods, have shown evidence for that variation in the serotonin transporter (5-HTT) gene alters an organism’s stress response to their environment (for a review see Caspi et al., 2010), mechanistically modeled by Jasinska et al. (2012). Many studies have tested the hypothesis that genetic variation in 5-HTT is associated with depression given exposure to stressful life events (SLEs); the first positive association finding was reported by Caspi et al. (2003). The 5-HT transporter, which is responsible for the active clearance of synaptic serotonin (5-HT), is encoded by SLC6A4. The SLC6A4 promoter contains the well-studied biallelic serotonin transporter linked polymorphic region (5-HTTLPR), which has been reported to influence the SLC6A4 expression level. Lymphoblasts and platelets from those homozygous or heterozygous for the short ‘S’ allele had approx 40% lower SLC6A4 expression compared to those homozygous for
the long 'L' allele (Greenberg et al., 1999; Lesch et al., 1996). Also, the 'S' carriers had lower 5-HTT binding in dorsal raphe nuclei, containing highest density of 5-HTT (Lesch et al., 1996; Little et al., 1998), although genotype association to 5-HTT binding potential in human brain was not always seen (Parsey et al., 2006; reviewed in Hariri and Holmes, 2006). The less functional short 'S' allele is associated with increased amygdala activation, established by a meta-analysis (Munafo et al., 2008).

It is the 5-HTTLPR variation that has repeatedly been reported to be associated with depression given exposure to SLEs. However these reports are inconsistent and meta-analyses showed that there was no evidence for association between depression and the 5-HTTLPR variation alone, or in interaction with SLEs (Munafo et al., 2009; Reisch et al., 2009). In these meta analyses, SLEs were not stratified by type. When stratifying the analyses by the type of stressor, robust evidence was found for the 5-HTTLPR to moderate the relationship between childhood maltreatment and depression, with the short 5-HTTLPR allele strengthening the relationship (Karg et al., 2011; for review see Caspi et al., 2010).

The 5-HTTLPR association with affective disorders given environmental stressors is hypothesized to be driven by the 'S'-allele association to increased threat-related amygdala reactivity (Caspi et al., 2010).

Recently, an A→G polymorphism (single nucleotide polymorphism—SNP), rs25531, was identified in the SLC6A4 promoter (Hu et al., 2006) located 18 base-pairs of the 5-HTTLPR insertion/deletion variation (Perroud et al., 2010). The G allele induces an AF transcription factor binding site and reduces the transcriptional activity of the 5-HTTLPR long allele to that activity similar to the 5-HTTLPR short allele (Hu et al., 2006; Kraft et al., 2005). Hence, the 5-HTTLPR-rs25531 mini-haplotype implies the functional division of individuals into three expression types, each corresponding to one, two or four diplotype (diplotype is the set of 2 haplotypes of a locus in an individual): high expression type (diplotype L_A/L_A), intermediate expression type (diplotypes L_A/L_C, S_A/L_A) and low expression type (diplotypes S_A/S_A, L_C/S_A, L_C/L_C and S_C/S_C), with the S_C allele being rare in the population (0.25%) (Wendland et al., 2006). The 5-HTTLPR-rs25531 diplotype is increasingly studied and the low-intermediate expression types were recently associated with increased amygdala reactivity to angry faces (Lonsdorf et al., 2011) in line with the results from meta-analysis on 5-HTTLPR only (Munafo et al., 2008).

Self reports of SLEs may be confounded by the mental state of the respondent since it is reported that dysphoric persons show greater sub-threshold priming of depression describing words than non-dysphoric persons do, and that depression has a negative effect on the memory overall (Bradley et al., 1996, Calev, 1996, Kuyken and Dalgleish 1995). However, there is no reason to believe that there is much error in recording major objective losses such as parental separation due to death or divorce, and divorce or separation from the partner (Barraclough and Bunch, 1973). A focus on major objective losses might thus be one way to further examine the influence of genetic factors on the relationship between SLEs and later depression.

The hypothesis in the present study was that the low expression diplotypes of 5-HTTLPR-rs25531 strengthen the relationship between separation and depression.

2. Materials and methods

This study is in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The ethical committee of Karolinska Institutet approved the study and specified written consent was obtained from all the participants.

2.1. Subjects

The subjects derived from the longitudinal population based study, PART, a longitudinal study of mental health, work and relations among persons living in Stockholm County, Sweden (Hällström et al., 2002). The framework sample for the present study included data from 8613 randomly selected 20–64 year-old Swedish nationals that have responded to an extensive questionnaire twice with a three-year interval, (1998–2000 and 2001–2003). Only 11% of the PART individuals had a non-Swedish origin and, of those, the vast majority had a Nordic origin (Hällström et al., 2002). The questionnaire included childhood conditions, demographics, financial status, social network, stressful life events, somatic health, use of drugs, and screening instruments for psychiatric disorders; the Major Depression Inventory (MDI) (Bech and Wermuth, 1998), alcohol use according to Alcohol Use Disorder Identification Test (AUDIT) (Saunders et al., 1993), Sheehan Patient-Rated (Panic) Anxiety Scale (Sheehan 1983), the Yale-Brown Obsessive-Compulsive Scale (Goodman et al., 1989), symptoms of social phobia and agoraphobia according to Marks and Mathews (1979), eating disorders according to Beglin and Fairburn (1992), and the Brief Disability Questionnaire (BDQ) (Ormel et al., 1999). These instruments can be used to make diagnosis according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR®, 2000). The internal dropout rate between the waves was low since the questionnaire was completed with a telephone interview in case of missing answers. Detailed attrition analyses using official registries assured that relationships between psychiatric disorders and living conditions was likely to be identified accurately (Bergman et al., 2009; Lundberg et al., 2005). This study has a nested case-control design within the PART cohort where environmental exposures statuses were assessed concurrently in the moment of case definition (see below) for both cases and controls. Cases and controls were established according to their depressive disorder status (see definitions below). Thus, case and control status were effectively established for 4446 subjects. These subjects comprised the main study sample.

2.2. Definition of depression (cases)

Depression was defined according to DSM-IV using the Major Depression Inventory (MDI). Questions were added on the duration of symptoms and disability due to the symptoms. The symptoms during the last 14 day were scored according to the authors’ instructions (Bech and Wermuth, 1998). A validity study for MDI using interviews performed by psychiatrists to make DSM-IV diagnosis of depression in a population based setting in the PART study is provided by Forsell (2005), whereas validation of MDI for DSM-IV diagnosis of depression in clinical settings is provided by Bech et al. (2001), Olsen et al. (2003) and in outpatient settings by Cuijpers et al. (2007). For diagnosis of mixed anxiety-depression additional information was used from Sheehan Patient-Rated (Panic) Anxiety Scale (Sheehan 1983). Individuals (n=1050) characterized as having depression were those diagnosed with major depression, mixed anxiety depression or dysthymia in at least one of the two waves. A few of the cases may also have had bipolar disorder.

2.3. Definition of non-depressed (controls)

Non-depressed individuals (controls) (n=3396) were those with no pathological symptoms according to DSM-IV of anxiety,
social phobia, agoraphobia, obsessive-compulsive disorder, eating disorder, depression, or social disability due to psychological problems in any of the two waves. In addition, they reported that they had never received health care for psychiatric disorder or nervous discomfort.

2.4. DNA

All those characterized as depressed and 98% (n = 3326) of those non-depressed (controls) were asked to provide saliva for DNA extraction (Oragene®DNA, DNA Genotek Inc., Ottawa, Ontario, Canada) as described in Sjöholm et al. (2009). Among them, we successfully collected DNA from 484 (46.1%) of the depressed and 1877 (56.4%) of the non-depressed (control) individuals. For logistic reasons, genotyping was performed on 460 (95.0%) depression cases and 1500 (80.0%) non-depressed individuals randomly selected from the available DNA material. The genotyping process was successfully completed among 89.4% of cases (n = 411) and among 89.8% of controls (n = 1347), which comprised the working sample in this study, i.e., 59.7% women (1050 subjects) and 40.3% men (708 subjects).

2.5. Environmental risk factors

All genotyped individuals were classified according to their experience of separations, i.e., having been separated from any parent during the childhood, having been divorced/separated from the partner during the last 12 months or having undergone both separations.

2.5.1. Parental separation

The questionnaires contained questions on death of parent and divorce/separation of the parents that had occurred before the person was 18 old. The questions were summarized and dichotomized; thus those persons who experienced the death of any parent or divorce/separation of parents, during their childhood, were categorized as having ‘Parental separation’ in the analyses. Data from the first wave was used. Reliability in data on childhood conditions was supported by comparative analysis between data obtained in wave 1 and those obtained in wave 2 (Forssell Y and Lundberg I, unpublished). The mean age of the population sample was 44.7 (SD 12.3) years and thus the events in average took place more than 27 years ago.

2.5.2. Separation in adulthood during the last 12 months

The questionnaires contained questions (identical in the two waves) on the occurrence of death of partner and divorce/separation from partner during the last 12 months. Since separation can be experienced as negative or not (separation can be devastating to one member of a couple and a relief to the other) (Dohrenwend, 2006), there was a question on if the person experienced the event as negative or not. Only those who indicated that the separation was negative (somewhat or much) were scored as having experienced a separation last year. Thus, those persons who experienced the death of partner or a negative divorce/separation from partner, the last year, were categorized as having ‘Separation from partner’ in the analyses. For those with depression, data on separation last year were used from the same wave as they reported depression. If depression was present in both waves the first wave was used. For those within the non-depressed group, data on separation was used from the first wave.

2.6. Confounders

All stratified logistic regression and multiple logistic regressions were adjusted for sex, age, lack of social attachment, income level and educational level at wave 1 (see statistical analysis below). Availability of attachment in the social network was assessed with three questions from a Swedish modification of the Interview Schedule for Social Interaction (ISSI) (Henderson et al., 1980; Undén and Orth-Gomér, 1989); each question used a four-point Likert scale (1 = completely true to 4 = not at all) and they were added up in a single score; the higher the score the worse the social attachment. The Cronbach’s alpha for this abbreviated scale was 0.75. Income was divided into four levels: 149 K SEK or less (thousands of Swedish Crowns), 150–199 K SEK, 200–299 K SEK, 300 K SEK or more (1 € = 9.33 SEK). Education was divided into three levels depending on years of completed studies: compulsory school (≤ 9 years), uncompleted upper secondary school (10–11 years) and completed upper secondary school or more (> 12 years).

2.7. Genotyping

DNA was extracted from saliva using the Oragene Purifier. Genotyping of the 5-HTTLPR was performed using PCR conditions described in Wendland et al. (2006). PCR reactions for both SNPs were performed using TaqMan SNP genotyping assay and ABI 7900 HT instrument (Applied Biosystems (ABI), Foster City, CA); forward and reverse primer sequences were 5′-CCCTCGCGGCTCCCGCAGGGC-3′ and 5′-ATGGTGAGGAG GGCTGCTCAGC-3′ (VIC) and CTGCAACCCCGGGGATGAGC-3′ (FAM). The temperature program was 95°C for 10 min followed by 40 cycles of 92°C for 15 s, 50°C for 15 s and 60°C for 1 min. PCR reactions for both 5-HTTLPR and rs25531 were performed in 384 well format with six negative controls distributed in each plate. Twenty-nine percent of the 5-HTT-genotyped individuals were sampled at a second occasion 3 years later and those samples were genotyped for 5-HTTLPR. Of the resampled individuals, 5% had discordant genotype between sample time points. For the rs2551 assay, 20% of the original DNA samples were re-genotyped with complete concordance between runs.

The genotypes of 5-HTTLPR and rs25531 were categorized in three functional diplotype groups based on assumed expression (Kraft et al., 2005): into high expression diplotype (L/La), intermediate expression diplotypes (La/La, S/La, S/S), and low expression diplotypes (S/Lc, S/S, Lc/Lc, Lc/La, S/Lc). Based on findings from a Swedish population-based study by Aslund et al. (2009), individuals with low expression diplotypes were grouped into the low 5-HTT activity group whereas those with intermediate or high expression diplotypes comprised the high 5-HTT activity group, where activity refers to transcriptional activity. Diplotype S/Lc, implying low expression, and diplotype S/La, implying intermediate expression, could not be distinguished from each other but as S/La is previously reported to be much rarer than Lc (0.25% versus 6.5% (Wendland et al., 2006)) those diplotypes were regarded as low activity diplotypes.
2.8. Statistical analysis

Hardy–Weinberg equilibrium was tested using Pearson’s chi-square test. Crude associations between sex (women), parental separation, separation from partner, 5-HTT diplotype, age, lack of social attachment, income level, educational level and depression were explored using simple logistic regression using depression as the outcome variable. Results were expressed as Odds Ratio (OR) which indicates the relative opportunity of obtaining the outcome (i.e., the ratio depressed versus non-depressed) comparing the categories of each variable with a reference category. For example, crude ORs were calculated as the ratio depressed versus non-depressed among those exposed to separation types/ ratio depressed versus non-depressed among those not exposed to separation types (Table 1). Also, associations between the ‘5-HTT diplotype’ and separation types (parental separation and separation from partner) were explored by simple logistic regressions.

Experiences of separations among the study subjects were organized into four mutually exclusive groups: (A) those who experienced both childhood parental and last year partner separations (the ‘both separations’ group), (B) those who only experienced separation of, or death of, any or both parent (the ‘only parental separation’ group), (C) those who only experienced separation/divorce from partner the last year (the ‘only separation from partner’ group), and (D) those who did not experience any of those separations (the ‘no separations’ group), which is the reference group. To explore the heterogeneity (modification) of separation effects on depression risk due to the 5-HTT diplotype, multiple logistic regressions were run using separations (groups A, B, C and D) and the confounding variables, stratified by the 5-HTT diplotype. Thus, adjusted ORs for occurrence of depression were calculated for types of separation: (B) ‘only parental separation’, (C) ‘only separation from partner’ or (A) ‘both types of separation’ vs. the ‘no separations’ group, which is the reference group. To explore the heterogeneity (modification) of separation effects on depression risk due to the 5-HTT diplotype, multiple logistic regressions were run using separations (groups A, B, C and D) and the confounding variables, stratified by the 5-HTT diplotype. Thus, adjusted ORs for occurrence of depression were calculated for types of separation: (B) ‘only parental separation’, (C) ‘only separation from partner’ or (A) ‘both types of separation’ vs. the ‘no separations’ group, which is the reference group. To explore the heterogeneity (modification) of separation effects on depression risk due to the 5-HTT diplotype, multiple logistic regressions were run using separations (groups A, B, C and D) and the confounding variables, stratified by the 5-HTT diplotype. Thus, adjusted ORs for occurrence of depression were calculated for types of separation: (B) ‘only parental separation’, (C) ‘only separation from partner’ or (A) ‘both types of separation’ vs. the ‘no separations’ group, which is the reference group.
models was established by the Likelihood-Ratio test (Kleinbaum and Klein, 2002), using the main effects model as the reference. For all performed logistic regression models, the Nagelkerke's $R^2$ (also Cragg and Uhler's $R^2$) was calculated, indicating how much the regression model predicts the outcome, with values ranging from 0–nothing to 1–perfectly (Long, 1997). The statistical analyses were performed using IBM SPSS Statistics version 20.0 (IBM Corporation, Armonk, NY, USA) and STATA® 11.2 (StataCorp, College Station, TX; USA). A $p$-value $<0.05$ was considered to be statistically significant for the main effects; and a $p$-value $<0.10$ was considered to be statistically significant for interaction terms and interaction indices, because the epidemiologic data have limited power to detect product terms (Greenland, 1993).

Finally, given the small number of cases in the study sample (411 depressed subjects) and the small number of subjects with the low activity diplotype ($n$=430), the statistical power for obtaining a significant OR into each 5-HTT diplotype stratum (Table 2) was calculated for each separation group (A, B and C) vs. no-separation group (D, the reference). Also, the statistical power for obtaining a significant multiplicative interaction (OR of the G×E) was calculated for each separation group vs. the reference group. Power tests were performed using the QUANTO® 1.2 software [http://hydra.usc.edu/gxe/] based on methods described by Gauderman, (2002a, 2002b), Kraft et al. (2007).

### 3. Results

A sample of 411 adults with depression and 1347 adults screened to have no psychiatric symptoms, all living in Stockholm, were successfully genotyped to investigate if the 5-HTTLP-rs25531 haplotype influenced a separation–depression relationship. Thus the sampling fraction, among the population survey, was 39.1% for cases and 39.7% for controls. A description of the studied individuals is presented in Table 1. Univariable analyses (crude ORs) revealed that being female, younger, exposed to parental separation, exposed to separation from partner during the last year, having 9 years or less of education, earning less that 300 K SEK, and having less availability of social attachment were all significantly associated with being depressed (Table 1). The frequency of 5-HTTLPR allele S was 42% and the frequency of rs25531 allele G was 7% in each of the depressed group and the non-depressed group. The 5-HTT marker genotypes were in Hardy Weinberg equilibrium in both depressed and non-depressed groups ($p>0.05$). The 5-HTTLPR and rs25531 genotypes were combined into seven 5-HTTLPR-rs25531 diplotypes corresponding to putative low or intermediate-high 5-HTT expression level, here denoted low activity group (5-S/5-C, 5-S/5-S, 5-L/5-C, 5-L/S), and intermediate-high activity group (5-L/5-M, 5-L/5-L, 5-L/M). The frequency of depression did not differ between the two 5-HTT activity groups: those individuals with low activity and those with high activity ($p=0.17$) (Table 1).

There was no difference between the individuals with the low activity diplotypes and those with the high-intermediate activity diplotype in the occurrence of parental separation analysing the whole sample, i.e., 16.4% and 18.1%, respectively (OR=0.88, 95%CI=0.65–1.21). Similarly, there was no association between 5-HTT activity group and occurrence of separation from partner during the last year (low=9.2% and high=11.2%, OR=0.80, 95%CI=0.53–1.20), and there was no association between 5-HTT activity group and the occurrence of having experienced both parental separation and separation from partner during the last year (low=5.7% and high=4.8%, OR=1.19; 95% CI: 0.69–2.05) (data not shown in Tables).

Analysing the association between 'only separation from partner' (C group) and depression stratified by the 5-HTTLP-rs25531 activity groups, the risk of depression among those separated from partner during the last year was significantly increased for both 5-HTT activity groups, but it was almost two-fold higher among those in the low activity stratum compared with those in the high activity stratum (Table 2), which indicated heterogeneity of effects over the strata. In agreement, there was a significant ($p<0.10$) additive interaction between the 5-HTT activity group and the loss of partner on the risk for depression. The additive interaction indices were: RERI=2.79 (90% CI=0.15–7.68), AP=0.54 (90% CI=−0.04 to 0.71) and S=2.97 (90% CI=1.10–8.05), with RERI and S demonstrating a significant synergistic effect between 5-HTT activity group and 'only separation from partner'. However, there was no deviation from a multiplicative model of interaction, i.e., there was no '5-HTT activity' x 'only separation from partner' interaction in a multiplicative model ('5-HTT low activity' OR= 1.15, 95%CI=0.81–1.63; 'only separation from partner' OR=2.26; 95%CI=1.45–3.54; interaction term OR=2.00, 90%CI=0.91–4.36; Model Nagelkerke's $R^2$; 0.210; data not shown in tables). Moreover, the power for detecting a significant ($p<0.10$) interaction OR equal or higher than 2.00 was 60.2%, given the sample size of the study.

On the other hand, the effect (magnitude of ORs) of 'only parental separation' (B group) on the risk of depression was similar among those in the low activity stratum and those in the high activity stratum, although the effect was significant only among the latter (see Table 2). The failure to detect an effect of parental separation on depression among those having the low

### Table 2

<table>
<thead>
<tr>
<th>Diplotype (n)</th>
<th>Pseudo-$R^2$</th>
<th>No separations n/total</th>
<th>Only parental separation OR(95% CI); $n_{Depr}$/SEK</th>
<th>Only separation from partner OR(95% CI); $n_{Depr}$/SEK</th>
<th>Both separations occurred OR(95% CI); $n_{Depr}$/SEK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low activity (430)</td>
<td>0.255</td>
<td>317; 20.2%</td>
<td>1.21 (0.60–2.42); 62; 29.0%</td>
<td>4.33 (1.85–10.16); 12; 62.5%</td>
<td>2.26 (0.79–6.49); 19; 47.4%</td>
</tr>
<tr>
<td>Female (267)</td>
<td>0.238</td>
<td>180; 24.4%</td>
<td>1.43 (0.63–3.15); 46; 34.8%</td>
<td>3.68 (1.42–9.55); 27; 63.0%</td>
<td>2.20 (0.65–7.49); 14; 50.0%</td>
</tr>
<tr>
<td>Male (163)</td>
<td>0.313</td>
<td>137; 14.6%</td>
<td>0.60 (0.30–1.60); 16; 12.5%</td>
<td>3.48 (1.13–9.290); 5; 60.0%</td>
<td>2.28 (0.25–20.84); 5; 40.0%</td>
</tr>
<tr>
<td>High activity (1328)</td>
<td>0.206</td>
<td>95; 17.0%</td>
<td>1.62 (1.13–2.36); 210; 28.7%</td>
<td>2.34 (1.50–3.65); 120; 40.8%</td>
<td>2.87 (1.17–7.03); 26; 53.8%</td>
</tr>
<tr>
<td>Female (783)</td>
<td>0.158</td>
<td>547; 23.0%</td>
<td>1.38 (0.87–2.19); 124; 29.8%</td>
<td>1.98 (1.17–3.35); 86; 93.5%</td>
<td>2.27 (1.17–7.03); 26; 53.8%</td>
</tr>
<tr>
<td>Male (545)</td>
<td>0.271</td>
<td>403; 10.9%</td>
<td>2.29 (1.16–4.26); 86; 22.1%</td>
<td>2.43 (1.89–9.75); 34; 44.1%</td>
<td>4.55 (1.64–12.62); 22; 50.0%</td>
</tr>
<tr>
<td>Total (1758)</td>
<td>0.213</td>
<td>1267; 18.5%</td>
<td>1.53 (1.10–2.13); 272; 27.2%</td>
<td>2.64 (1.79–3.90); 152; 45.4%</td>
<td>3.12 (1.17–5.46); 67; 50.8%</td>
</tr>
<tr>
<td>Female (1050)</td>
<td>0.174</td>
<td>727; 23.4%</td>
<td>1.42 (0.96–2.11); 170; 31.2%</td>
<td>2.28 (1.45–3.59); 113; 45.1%</td>
<td>2.70 (1.32–5.33); 40; 52.5%</td>
</tr>
<tr>
<td>Male (708)</td>
<td>0.253</td>
<td>540; 11.8%</td>
<td>1.79 (0.99–3.25); 102; 20.6%</td>
<td>4.62 (2.16–8.39); 35; 46.2%</td>
<td>3.72 (1.52–9.12); 27; 48.2%</td>
</tr>
</tbody>
</table>

Those in ‘Only parental separation’ column were not exposed to divorce/separation from partner. Those in ‘Only separation from partner’ column were not exposed to parental separation.

Odds ratio (OR), the ratio depressed versus non-depressed among those exposed to separation/ratio depressed versus non-depressed among those not exposed to separation.

All ORs are adjusted for sex, age, lack of social attachment, income level and educational level.

Pseudo-$R^2$: Nagelkerke's $R^2$.

activity diplotypes can be explained by the limited statistical power (55.6%) to detect an OR equal or higher than 1.62 (the OR in the high activity stratum, at $\alpha=0.05$, given the sample size in that stratum (111 cases and 319 controls). Additionally, interaction analyses yielded neither multiplicative nor additive interactions between 5-HTT activity and ‘only parental separation’ (B). Thus, the logistic regression model yielded an interaction term OR of 0.82 (90%CI = 0.43–1.55; 5-HTT low activity OR = 1.16, 95%CI = 0.82–1.63; ‘only parental separation’ OR = 1.59; 95%CI = 1.09–2.32; Model Nagelkerke’s $R^2 = 0.174$; data not shown in tables). Furthermore, the power for detecting a significant ($p<0.10$) interaction OR equal or lesser than 0.82 was only 25.5%; given the sample size of the study. The hypothetical interaction should have effect size $0.52 > OR > 1.95$ in order to be detected with good power (> 80%) with our sample size.

Experience of both parental separation and separation from partner the last year (A group) appeared to increase the risk of depression given both the low activity and the high activity 5-HTT diplotypes, although the effect was significant only among those in the high activity stratum (Table 2). Again, the problem was the limited statistical power (51.9%) to detect an OR equal or higher than 2.26 (the OR in the low activity stratum), at $\alpha=0.05$, given the sample size in that stratum. However, the sample size in the low activity stratum is big enough for detecting an OR as high as that obtained in the high activity stratum (OR = 3.48), yielding a power of 85.4%. Additionally, interaction analyses yielded neither multiplicative nor additive interactions between 5-HTT activity and ‘both separations’. Thus, the logistic regression model yielded an interaction term OR of 0.72 (90%CI = 0.26–1.99; 5-HTT low activity OR = 1.16, 95%CI = 0.82–1.64; ‘both separations’ OR = 3.28; 95%CI = 1.66–6.46; Model Nagelkerke’s $R^2 = 0.202$; data not shown in tables). Furthermore, the power for detecting a significant ($p<0.10$) interaction OR equal or lesser than 0.72 was only 24.6%; given the sample size of the study.

In analyses split by sex, the 5-HTT $x$ separation interaction on depression was again statistically significant only for those who had experienced separation only from partner. Thus, among females the OR of having depression after separation/divorce from partner was 1.8-fold higher in the group with low 5-HTT activity compared with OR of those in the high 5-HTT activity group (Table 2); additive interaction indices RERI and S were significant among females (RERI = 2.48, 90% CI = 0.07–7.17; S = 3.57, 90% CI = 1.01–12.58; AP = 0.56, non-significant). Among males, the OR of having depression after separation/divorce from partner was 2.5-fold higher in the group with low 5-HTT activity compared with the OR of those in the high 5-HTT activity group (Table 2); but there was no significant additive interaction index (RERI = 10.76; S = 4.24; AP = 0.71). Multiplicative interaction was significant neither in females nor in males. The power to detect the interaction in males, given actual OR point estimate, was 52.8%.

Finally, hierarchical logistic regression models did not show any significant two-way interactions between pairs of main exposure variables (5-HTT activity, ‘parental separation’ and ‘separation from partner’). The Nagelkerke’s $R^2$s ranged from 0.213 in the ‘main effects’ model to 0.215 in the ‘all two-way interactions’ model. Furthermore, no comparative Likelihood-Ratio test was statistically significant.

### 4. Discussion

Results in the present study suggest that the 5-HTTLPR-rs25531 mini-haplotype, known to regulate expression level of the serotonin transporter, moderates the relationship between separation from partner the last year and current depression in a synergistic manner, which was evidenced by a significant additive GxE interaction (interaction indices: RERI = 2.79; S = 2.97). The diplotypes (set of 2 haplotypes) associated with a separation-depression dependence were those implying low expression of 5-HTT. Previous numerous reports on 5-HTTLPR moderation of association between last years’ SLEs (grouped together) and depression have shown inconsistent results and meta-analyses concluded that there was no detectable 5-HTTLPR influence (Karg et al., 2011; Munafò et al., 2009; Reisch et al., 2009). Our study adds to previous reports in investigating the influence of 5-HTTLPR-rs25531 on exposure to only negatively experienced separation from partner. Additionally, point estimates of additive interaction indices suggest that this GxE interaction effect could be higher among males than among females; but the lack of power for results among males (i.e., small sample size) preclude any conclusion. We further found that the 5-HTTLPR diplotype group appeared not to influence, to a large extent, the risk for depression given parental separation during childhood (neither an additive nor a multiplicative interaction was detected). Furthermore, the effect sizes (ORs) of parental separation on depression were very similar between the 5-HTT activity groups (OR-fold of 1.34 between ‘5-HTT activity’ strata). Supplementary interaction analyses were performed combining both separations (parental and partner separations) in one variable (‘any or both separations occurred’), but neither additive nor multiplicative GxE interaction was found (data not shown).

In the whole sample (both 5-HTT activity groups), the occurrence of depression was almost 2.6-fold (OR) among those exposed to divorce/separation from partner the last year compared to that among those non-exposed to any separation. Similarly, the occurrence of depression was 1.5-fold higher among those exposed to separation from any parent during childhood compared to that among those non-exposed to any separation. Similar associations have been reported in several previous studies (Kendler et al., 2006; Maughan and McCarthy, 1997).

A review by Karg et al. argued for that the 5-HTTLPR moderates the association of childhood maltreatment and specific medical conditions to adulthood depression, through the low activity allele $S$ strengthening the SLE-depression relationship (Karg et al., 2011). Also, a previous population-based study from Sweden showed that homozogosity for the low activity allele increased depression among students exposed to childhood maltreatment with an OR of 3.2 (95% CI 1.3–8.1) (Aslund et al., 2009). However, we studied specifically parental separation before age of 18 years and not childhood maltreatment; i.e., exposures such as physical violence, several changes of care givers, indifference to child performance and not enough food on the table (Karg et al., 2011). Moreover, we studied effects of 5-HTTLPR-rs25531 on exposure to only one type of separation over the life phases at a time, and exposure to both types of separation in a specific analysis following theories of significant losses (Brown et al., 1977). In this manner, exposure to both parental and last year negative partner separation appeared to increase the risk for depression regardless of 5-HTT diplotype. Further, we studied the effect of only objective life events: i.e., separations. The evidence of 5-HTTLPR moderation on stress-depression relationship was previously reported to be stronger among studies that used objective measures (Karg et al., 2011). However, in the present study separation from partner the last year was registered only if it was regarded negative by the person, implying a subjective element. Low activity allele carriers exposed to childhood maltreatment were previously shown to exhibit a bias towards perceiving negative outcomes (Williams et al., 2009). Still, no such bias appeared influential here, since the 5-HTTLPR-rs25531 diplotype was not associated with the occurrence of separations, either separation from parents during childhood or negative separation from partner the last year.
There are limitations in testing for biological/causal interaction based on statistical assessment of interaction (Wang et al., 2011). It has been argued that the calculation of statistical interaction on the additive scale is more indicative of the underlying biological mechanism (Ahlborn and Alfredsson 2005; Greenland and Poole, 1988; Koopman and Weed, 1990; Rothman et al., 2008; VanderWeele, 2009; Zou, 2008). Accordingly, we found significant values for the additive interaction indices RERI and S. It has been argued that S is the appropriate index when departure from additivity is assessed in case-control studies which include confounders (Skrdoná, 2003). To the best of our knowledge, previous reports on 5-HTX ≤ KLEGE interactions have reported analyses of multiplicative interactions.

The life trajectories of young persons from divorced families were previously shown to contain more stressful paths and more distress (Aro, 1994). Those depressed were reported to have an increased likelihood of experiencing divorce and marital difficulties (Hammen, 2003). A moderate degree of causality for dependent SLEs (SLES likely influenced by the individual’s behaviour) and previous depression episodes onto depression was demonstrated, although a large proportion of observed associations are non-causal (Kendler and Gardner, 2010).

For the individuals studied in this article, there are data from two time points three years apart. Fifty-six percent of those depressed (major depression or dysthymia or mixed-anxiety depression) at the second time point had reported depression (major depression, dysthymia, mixed-anxiety depression or mild depression) also at the first time point. Thus, a significant proportion of the depression episodes recorded seemed to be recurrent or persistent. A recent study found no effect of 5-HTTLPR on the SLE depression relationship in patients with recurrent depression but they reported no stratification by type of SLE (Fish et al., 2011).

The vast majority of 5-HTX ≤ KLEGE interaction studies scrutinized the 5-HTTLPR only. Recently, the haplotype 5-HTTLPR-rs55531 has been increasingly studied and this has provided findings regarding stress response consistent with those from only 5-HTTLPR (Grabe et al., 2011; Lonsdorf et al., 2011). Our allele frequencies of 5-HTTLPR and rs55531 were similar to those previously reported: 43% having allele S and 7% having allele G among whites (Wendland et al., 2006), and 35–40% having allele S among whites (Hu et al., 2006).

That the low activity diplotypes strengthened the separation–depression relationship is consistent with functional imaging studies that found increased stimulus-provoked stress reactivity, involving amygdala and medial prefrontal cortex, among 5-HTTLPR low activity allele carriers (Heinz et al., 2005; Munafò et al., 2008; Pezawas et al., 2005). Accordingly, serotonin transporter knockout (SLECS644) mice have increased anxiety-like behavior, increased sensitivity to stress, and an exaggerated hypothalamic-pituitary-adrenal axis activation induced by physical and psychological stressors (Li et al., 1999; Li, 2006). Further, naturally occurring low-activity SLECS644 variants in mice and non-human primates were associated with increased adrenocorticotropic hormone (ACTH) response to stress (Barr et al., 2004), and changes in dopamine signaling (Carneiro et al., 2009).

There may be a difference between the loss of a parent due to death and the loss of a parent due to divorce/separation on the risk for depression. Several studies of limited size explored this but findings are not consistent (Tyrka et al., 2008). In our sample of 94 persons with parental death and 245 persons with parental separation due to other reason showed no significant difference in the odds of depression (p = 0.6). Concerning late separation, the participants indicated whether these experiences happened to them during the past 12 months. Test–retest reliability studies of these scales indicate fair reliability (Klein and Rubovits, 1987).

The main limitation of this study is the sample size, in particular for those with exposure to both parental separation and separation from partner. Also, a participation rate of ~50% in the DNA collection may limit the generalizability of the results; although both cases’ and controls’ subsamples showed Hardy–Weinberg equilibrium, and the participation rate was similar among cases and controls. We previously reported that refusal to contribute DNA in PART was mainly explained by a lack of personal relevance and feelings of discomfort related to the DNA being used for purposes other than the respective study (Melas et al., 2010). Since participation in this study was based on self-reported questionnaire and self-administered saliva collection, persons severely ill during data collection are likely under-represented. However, using less heterogeneous environmental exposure and an ethnically and culturally quite homogeneous population we were able to detect signals in line with previous reports, and to provide novel data regarding the influence of 5-HTX promoter variation on negative effects of separation from partner.

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Conflict of interest

All other authors declare that they have no conflicts of interest.

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