# New Evidence for the Association of the Serotonin Transporter Gene (SLC6A4) Haplotypes, Threatening Life Events, and Depressive Phenotype

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**Background:** Since the first report of the significant gene-environment interaction (G × E) in depression published by Caspi *et al.,* the literature is considerably contradictory in this field. To clarify this question, we analyzed the interaction between the serotonin transporter gene (SLC6A4) and threatening life events (TLE) on Zung Self-Rating Depression Score (ZSDS).

**Methods:** Five markers tagging the whole SLC6A4 gene (*5-HTTLPR* and 4 single nucleotide polymorphisms*: rs2020942, rs140700, rs3798908, rs1042173*) were genotyped in 567 nonclinical individuals. Generalized linear models were used to analyze single marker associations, and likelihood ratio tests and score tests were used for haplotype analysis.

**Results:** Haplotype analysis revealed a significant global effect of haplotypes on ZSDS score in high TLE subgroup ($p = .008$). Besides the *5-HTTLPR, rs140700* tagging the middle region of the gene had significant effects. Subjects carrying the A allele of *rs140700* scored lower on ZSDS independently of *5-HTTLPR* carrier status. Explained variances for depressive phenotype were 1%, 4%, and 6% when *5-HTTLPR*, *5-HTTLPR* × TLE and *5-HTTLPR* × *rs140700* × TLE were included in the model, respectively.

**Conclusions:** Our results demonstrate heterogeneity of individuals carrying S alleles of *5-HTTLPR* in association with high TLE providing possible explanation for the inconsistency of previous studies. In addition to the promoter, the middle region of the SLC6A4 gene carries the G × G × E interaction for mood, and this new model provided a higher explained variance. We report the first evidence for the significant effects of haplotypes of the SLC6A4 gene and threatening life events on depressive phenotype.

**Key Words:** 5-HTTLPR, Depression, G × E interaction, haplotype analysis, polymorphism, threatening life events

The serotonin (5-hydroxytryptamine) transporter (5-HTT) is a key protein of the serotonergic system by regulation of serotonin concentration in the synaptic cleft and extrasynaptic sites. The crucial role of 5-HTT in the pathomechanism of affective disorders is based on the hypothesis that variations in serotonergic neuronal function in the central nervous system occur in patients with major depression (1) and on the therapeutic effect of selective serotonin reuptake inhibitor antidepressants (SSRIs). Consequently, there have been several genetic studies investigating the serotonin transporter gene (SLC6A4) as a candidate for affective disorders.

One of the most frequently studied functional polymorphisms of the SLC6A4 gene related to depression is the serotonergic transporter–linked polymorphic region (*5-HTTLPR*) located in the promoter region of SLC6A4 on chromosome 17 (17q11.1–17q12). This polymorphism consists of a 44-bp insertion or deletion producing two common variants, long (L) and short (S) alleles. The short one causes reduced transcriptional efficiency of the gene, resulting in decreased serotonin transporter expression in the neuron (2). Numerous studies have reported a significant association between S carrying (SL and SS genotypes) and depression (3–8), although others have not confirmed this relationship (9,10).

Reconsideration of the multifactorial etiology of affective disorders and new methodologic approaches have resulted the idea that environmental factors should have been involved in the genetic assessments (gene-environment interactions, G × E). The majority of interaction studies in this area have investigated exposure to stressful life events (SLEs) as environmental factors. Caspi *et al.* (11) reported a significant interaction between the *5-HTTLPR* and SLEs in depression, although single marker association with depressive symptoms was not significant. S carriers showed more severe depressive symptoms and were at greater risk for a depressive episode compared with noncarriers (LL genotype) at higher exposure to SLEs. These findings were confirmed in twin-study by Kendler *et al.* (12) showing that individuals with homozygote S were more sensitive to the depressogenic effects of SLEs than were noncarriers. Wilhelm *et al.* (13) reported that the *5-HTTLPR* genotype is a significant predictor of onset of major depression following multiple adverse events, but this polymorphism was not associated with depression on its own. New results from the Spanish PREDICT-Gene cohort also have supported these data (14). SS genotype significantly modified the risk conferred by threatening life events (TLEs) for depressive episodes. Compared with SS genotypes, the same level of depressive episode occurred after significantly more TLEs in participants carrying SL or LL genotypes. Several studies replicated these associations (15–17).

In contrast, no gene × environment interactions among the *5-HTTLPR* genotype, social adversity, and major depression disorder (MDD) were observed in a replication study from a nonclinical sample designed by Surtees *et al.* (18) in which 4175 participants aged 41–80 years were investigated. In addition, increased rates of past-year prevalent MDD was associated with childhood maltreatment among LL homozygote men, which

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contrasts with previously reported findings. Certain other studies also failed to replicate these results in other models (19,20).

We hypothesized that these discrepancies may be explained by the assumption that certain other regions of the SLC6A4 interact with the functions mediated by the *5-HTTLPR* polymorphism. To test this hypothesis, haplotypes of SLC6A4 gene, TLE, and depressive symptoms were analyzed in a population genetic study. Our data provide evidence that the middle region of the gene has a significant contribution to the earlier described *5-HTTLPR* × environment interaction—namely, that individuals with the A allele of *rs140700* scored lower on ZSDS independent of the *5-HTTLPR* carrier status.

## Methods and Materials

### Sample

Five hundred and sixty-seven unrelated volunteers, 447 women and 120 men, were included in the study. Participants were recruited from general practitioners, adult students participating in a long-distance learning program, and a community-based population. The inclusion of subjects was independent of any positive psychiatric anamnesis. The mean age of participants was 30.96 ± 10.66 years. All subjects were Hungarian and of Caucasian origin, and they gave written informed consent before entering the study. The study was approved by the Central Ethics Committee. The descriptive data of the study population are shown in Table 1.

### Independent Measures

Participants completed three questionnaires: a background questionnaire, the Zung Self-Rating Depression Scale (ZSDS), and the List of Threatening Life Events. Detailed background information was obtained from all participants. The background questionnaire was adapted from the version developed by the Epidemiology Unit of the University of Manchester. This well-structured self-rating questionnaire consists of 22 items and collects detailed information about medical history including psychiatric history and medications, family psychiatric history, and socioeconomic background. The Zung Self-Rating Depression Scale (ZSDS) (21) was used to measure symptoms of depression according to the conclusion that results for the G × E interaction were similar for self-reported and interview-based measurements of depression (11,22). The ZSDS is a valid, reliable instrument that has been used in several studies to measure depressive symptoms. Higher scores correspond to more frequent symptoms, and thus this qualitative scale provides the dependent variable representing the depressive phenotype in the total sample (23–25). The number of individuals above the threshold (48 points) reflects clinically depressed persons, which is equivalent with point prevalence of depression in the Hungarian population (26).

Adverse life event experience was assessed with the List of Threatening Life Events developed by Brugha *et al.* in 1985 (27). This self-rating questionnaire contains 12 items about negative life events exposure in the previous 2 years classified in four categories: problems with intimate relationships (marital difficulties, breakup of a steady relationship); financial conditions (unemployed or seeking work, fired, financial crisis); death or serious illness of a close relative; health and other (illness, injury, problem with self or close friend, police or court, lost or stolen belongings). For statistical analysis, TLEs were coded based on the number of events as 0, 1, 2, 3, and 4 or more.

**Table 1.** Study Population Characteristics

| Sociodemographic Variables | |
|---|---|
| Gender | |
| Female | 447 (78.8%) |
| Male | 120 (21.1%) |
| Mean Age ± SD | 30.96 ± 10.66 |
| Marital Status | |
| Single | 259 (45.7%) |
| Married | 180 (31.7%) |
| Couple | 53 (9.3%) |
| Divorced | 30 (5.3%) |
| Separated | 11 (1.9%) |
| Widowed | 5 (.9%) |
| Education | |
| No qualification | 2 (.4%) |
| Technical school | 51 (9.0%) |
| High school | 403 (71.1%) |
| Degree | 147 (25.9%) |
| ZSDS Score | 38.71 ± 5.106 |
| Anamnesis of Depression | |
| Lifetime prevalence | 107 (18.9%) |
| Last year prevalence | 41 (7.2%) |
| Point prevalence (ZSDS ≥ 48) | 24 (4.2%) |
| Exposure to TLEs | |
| No TLE | 79 (13.9%) |
| 1 | 130 (22.9%) |
| 2 | 121 (21.3%) |
| 3 | 85 (15.0%) |
| 4 or more | 152 (26.8%) |
| Mean TLE score | 2.14 |

TLE, threatening life events; ZSDS, Zung Self-Rating Depression Scale.

### Genotyping

*5-HTTLPR* and four haplotype tagging single nucleotide polymorphisms (SNPs) across the gene were selected for genotyping using data from the International HapMap Project (28). These five polymorphisms cover all of the allelic variants of SLC6A4 with an $r^2 > .8$ in a Caucasian population, indicating that most of the common variations within the gene are likely to be represented in our analysis. Buccal mucosa samples were collected from each subject, and genomic DNA was extracted using a conventional phenolchloroform extraction protocol. DNA quality and quantity was determined with NanoDrop B-100 spectrophotometer, and all samples were diluted to a DNA concentration of 20 ng/mL.

For genotyping *5-HTTLPR*, the genomic region containing the polymorphism was amplified using 6-FAM labeled forward primer (Table 2). Polymerase chain reaction (PCR) was performed in a final volume of 10 mL containing .25 mmol/mL of each primer (Metabion, Martinsried, Germany), 1× $NH_4$ buffer, 1.5 mmol/mL $MgCl_2$, .2 mmol/mL of each dNTP, .2 units of Taq DNA polymerase (Bioline, London, United Kingdom) and 20 ng DNA. Cycling conditions were as follows: initial 15 min denaturation at 95°C, then 30 cycles (94°C for 20 sec, 64°C for 30 sec, and 72°C for 30 sec), and a final extension for 10 min at 72°C. PCR products (366 bp for the L allele and 322 bp for the S allele) were analyzed using an ABI3100 Genetic Analyzer and GeneScan analysis software (Applied Biosystems, Foster City, California).

Tag-SNPs were genotyped at Centre for Integrated Genomic Medical Research at the University of Manchester using Sequenom MassARRAY technology (Sequenom, San Diego, California). The iPLEX assay, based on post-PCR single base primer extension, was performed according to manufacturer's instructions.

**Table 2.** Descriptive Statistics of Genotyped Polymorphisms in the SLC6A4 Gene

| Marker | Alleles | Region | Primer Sets[a] | HWE | MAF | Success (%) |
|---|---|---|---|---|---|---|
| *5-HTTLPR* | S/L | promoter | f: 5-GCCAGCACCTAACCCCTAAT-3<br>r: 5-GTAGGGTGCAAGGAGAATGC-3 | .869 | .423 | 100 |
| *rs2020942* | A/G | intron | f: 5-ACCTGAGGTCTGTGCAAATC-3<br>r: 5-GAAGGCCATCACGAGAACAC-3<br>e: 5-AAGTTACAGTCACACTGGGTAAACC-3 | 1 | .368 | 96.2 |
| *rs140700* | A/G | intron | f: 5-GGTGAATGGATGTCAGTGTC-3<br>r: 5-GTGTGACTCCAAGGGTTGTG-3<br>e: 5-TGACCTTGAGAAAGGAGGG-3 | .767 | .087 | 98.6 |
| *rs3794808* | A/G | intron | f: 5-ATGTTTGCCATACTCACCCC-3<br>r: 5-TGAACGTAGAAGTGGAAGAC-3<br>e: 5-GGCCTAGTGCCTGAGAGA-3 | .997 | .455 | 97.7 |
| *rs1042173* | G/T | 3′UTR | f: 5-AGGTTCTAGTAGATTCCAGC-3<br>r: 5-GAACAGGGATGCTATCTCGC-3<br>e: 5-AGTAGATTCCAGCAATAAAATT-3 | .928 | .483 | 97.7 |

HWE, Hardy-Weinberg Equilibrium (*p* value of chi-square tests); MAF, minimal allele frequency; Success, rate of successfully genotyped samples (%).

[a]Primer sets mean forward (f), reverse (r), and extension primers (e) used for genotyping.

Forward, reverse and extension primers (Table 2) were designed using the Assay Design 3.0 software of Sequenom. The iPLEX reaction products were dispensed onto a 384-well SpectroChip (Sequenom), processed and analyzed in a Compact Mass Spectrometer by MassARRAY Workstation 3.3 software (Sequenom).

### Statistical Analysis

We used Haploview 4.0 software for computing Hardy-Weinberg Equilibrium, minimal allele frequency and pairwise linkage disequilibrium (LD) between genotyped polymorphisms (29). We determined the age- and sex-adjusted ZSDS in the study population applying stepwise linear regression analysis. Computed regression residuals, representing covariate-adjusted ZSDS, were used in subsequent analyses. Associations between ZSDS and independent variables were computed performing multivariable linear regression analyses, where polymorphisms coded as 0, 1, or 2 depending on the carrier status of the minor allele, TLE, and their interactions (G × E) were entered into the model. Effect of allele-carrier status was also determined. Hierarchical regression analyses were performed using the SPSS for Windows 15.0 software. Alpha level was corrected with the number of the independent variables (five polymorphisms and TLE), so that the $p$ value correction was .05/6 = .0083. Study power was computed using Quanto 1.2.3 (http://hydra.usc.edu/gxe/).

Haplotype analyses were performed in a subsample consisting of subjects with at least four successfully genotyped polymorphisms to avoid false results in haplotype estimation. Maximum likelihood haplotype frequencies in the study population were obtained based on the EM algorithm in UNPHASED 3.0.11 software (30). Rare haplotypes with frequency less than 1% were excluded from analyses. UNPHASED 3.0.11 software was used to compute global association and individual effect of haplotypes on covariate-adjusted ZSDS score in total sample and in two subgroups regarding the TLE score. The low TLE subgroup consisted of subjects with TLE score < 2 (0 or 1, $n$ = 200), and subjects with TLE score > 2 were grouped in the high TLE subgroup (3 or more, $n$ = 226). The program employs a likelihood ratio test to compute the significance level of the global association. Individual effect of each haplotype was analyzed by performing a score test for the difference in effect between a haplotype and all the others pooled together. To assess the reliability of the results, permutation procedures with 1000 random permutations were performed to generate empirical $p$ values. Permuted $p$ value < .05 was considered significant in haplotype analyses.

## Results

### Descriptive Statistics

Each genetic marker was genotyped with a success rate greater than 95%. There was no significant deviation from Hardy-Weinberg Equilibrium, and minimal allele frequency was greater than 5% in each polymorphism (Table 2). The reproducibility rate as revealed through blind duplicating was 100% in both genotyping processes. Pairwise LD D′ values are shown by Figure 1. These data are analogous with values observed for Utah residents with ancestry from northern and western Europe in the International HapMap Project (CEU) (http://www.hapmap.org). The low rate of linkage between the *5-HTTLPR* and the other polymorphisms could be explained by the recombination hotspot located in the first intron.

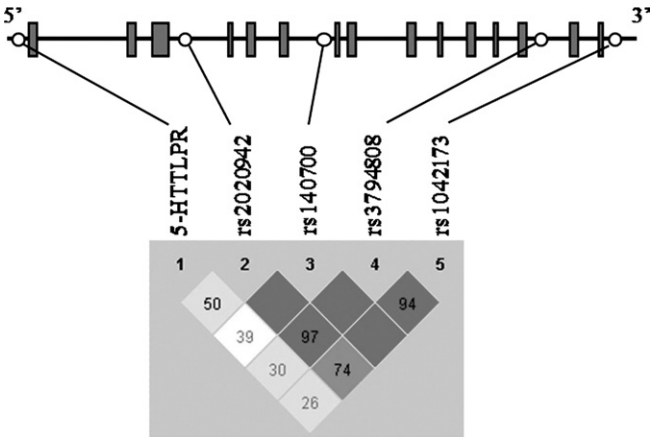


**Figure 1.** Position and linkage disequilibrium (LD) map of genotyped polymorhisms in SLC6A4. Pairwise LD statistics in examined genes were calculated with Haploview. Squares are colored darker if the |D′| value is high, that is, LD is strong. Empty dark squares mean |D′| = 1, that is, complete LD between two single nucleotid polymorphisms.

**Table 3.** Association of Study Variables with ZSDS

| Variable | Subgroup | Number | Mean ZDS ± SEM | *p* Value |
|---|---|---|---|---|
| *5-HTTLPR* | LL | 188 | 37.89 ± .35 | .0056[a] |
| | SL | 277 | 39.25 ± .31 | |
| | SS | 102 | 38.76 ± .51 | |
| *rs2020942* | GG | 214 | 38.78 ± .36 | *ns* |
| | AG | 250 | 38.64 ± .32 | |
| | AA | 72 | 38.28 ± .53 | |
| *rs140700* | GG | 460 | 38.83 ± .24 | *ns* |
| | AG | 90 | 38.29 ± .49 | |
| | AA | 3 | 34.00 ± 2.67 | |
| *rs3794808* | GG | 162 | 38.10 ± .36 | *ns* |
| | AG | 269 | 38.89 ± .33 | |
| | AA | 113 | 39.03 ± .49 | |
| *rs1042173* | TT | 144 | 38.10 ± .37 | *ns* |
| | GT | 274 | 38.65 ± .32 | |
| | GG | 126 | 39.42 ± .47 | |
| TLE | 0 | 79 | 36.67 ± .58 | .0001 |
| | 1 | 130 | 38.80 ± .40 | |
| | 2 | 121 | 38.57 ± .41 | |
| | 3 | 85 | 39.05 ± .57 | |
| | ≥4 | 152 | 39.63 ± .43 | |

TLE, threatening life events; ZSDS, Zung Self-Rating Depression Scale; ns, not significant.

[a]Comparing S carriers (SS + SL) to noncarriers (LL).

### Association of Independent Variables and Depressive Score (ZSDS) in Total Sample

One individual genetic marker was significantly related to covariate-adjusted ZSDS in our study population (Table 3). Subjects carrying the S allele of *5-HTTLPR* scored significantly higher on the adjusted ZSDS ($B$ = 1.246, SE = .448, $t$ = 2.778, $p$ = .0056, adjusted $R^2$ = .012). Adjusted mean ZSDS ± SEM was .413 ± .263 in S carriers (SS + SL) and –.833 ± .352 in S noncarriers (LL). Threatening life events were significantly associated with adjusted ZSDS score ($B$ = .579, SE = .149, $t$ = 3.883, $p$ = .0001, adjusted $R^2$ = .024).

### Interaction of TLE and SLC6A4 Genotypes

Two of five polymorphisms had significant interactions with TLE on ZSDS (Table 4). In the regression model built by *5-HTTLPR* coded as 0, 1, 2 TLE and *5-HTTLPR* × TLE, none of the independent variables had a significant conditional effect, but their interaction was strongly significant on ZSDS. The adjusted $R^2$ representing the explained variance was .042 in this model. Regarding the low frequency of the AA genotype ($n$ = 3), *rs140700* was entered into the regression model as a bivariate variable depending on the carrier status of the minor allele (variable was coded as 0 and 1 in case of GG and AA/AG respectively). In this model, the conditional effect of TLE and interaction of *rs140700* × TLE were significant, and adjusted $R^2$ was .040. Figure 2 shows the association between TLE and ZSDS in different genotype groups of the two polymorphisms. The interaction analyses demonstrated that the effect of TLE on adjusted ZSDS was significant in the SL genotype ($B$ = .618, SE = .211, $t$ = 2.921, $p$ = .0038, adjusted $R^2$ = .027), and this association was stronger in the SS genotype ($B$ = 1.299, SE = .333, $t$ = 3.903, $p$ = .0001, adjusted $R^2$ = .124), whereas it was not significant in the LL genotype of *5-HTTLRP* ($B$ = .136, SE = .263, $t$ = .517, $p$ = .6163). Regarding *rs140700* (Figure 2B), we identified that the effect of TLE on ZSDS was strongly significant in the GG genotype ($B$ = .797, SE = .166, $t$ = 4.783, $p$ < .0001, adjusted $R^2$ = .045), but there was no significant association in carriers of the A allele ($B$ = −.432, SE = .369, $t$ = −1.171, $p$ = .2452). We also tested the gene × gene interaction between the *5-HTTLPR* and *rs140700* on ZSDS as well as the three-way interaction between the two genomic markers and TLE (Table 4). The interaction between the two polymorphisms was not significant on ZSDS ($p$ = .024). The effect of the three-way interaction (G × G × E) was strongly significant ($p$ = .0005), and the explained variance of ZSDS was the highest in this model (adjusted $R^2$ = .059). These results remain significant when ZSDS was adjusted for psychiatric history, marital status, and education.

On the basis of the sample size, the power of the study was 84.3% to detect a significant effect of *5-HTTLPR* × TLE interaction on ZSDS score under additive genetic model (MAF = .423, $B_G$ = .576, $B_E$ = .552, $B_{G \times E}$ = .614). For *rs140700* × TLE interaction, there was an 87.3% power to detect the significant effect of G × E on ZSDS under the dominant model (MAF = .087, $B_G$ = .682, $B_E$ = .552, $B_{G \times E}$ = 1.251).

### Haplotype Analysis

Haplotype analyses were performed on 542 subjects. In the total sample, nine haplotypes built of the five polymorphisms were more frequent than 1%. Table 5 shows the results of haplotype analyses in which permuted $p$ value of global association ($p_{global}$) and for individual haplotype effects ($p_{effect}$) are also represented. In association analyses, the most common haplotype (SGGAG) was selected as baseline, its effect was determined as 0, and the effect of other haplotypes was represented by the software as the difference from this baseline. SLC6A4 haplotypes was significantly associated with ZSDS score only in high TLE subgroup ($\chi^2$ = 19.91, $df$ = 8, $p_{global}$ = .008). In this subgroup, three haplotypes had a significant individual effect compared with the mean of all other haplotypes (Figure 3). The common haplotype (SGGAG) was significantly related to high ZSDS, whereas two less common haplotypes (SGAGT, LGAGT) were associated with lower ZSDS. We have also compared the individual effect of the significant haplotypes with each other by applying likelihood ratio tests. The difference between the effect of SGGAG and SGAGT proved to be significant ($\chi^2$ = 9.8, $df$ = 1, $p$ < .005). The ZSDS associated to LGAGT was significantly lower than SGGAG ($\chi^2$ = 10.1, $df$ = 1, $p$ < .005), whereas the effect of SGAGT was not significantly different from LGAGT ($\chi^2$ = 1.1, $df$ = 1, $p$ > .25).

**Table 4.** Effect of TLE, *5-HTTLPR, rs140700,* and Their Interaction on ZSDS

| Dependent Variables in the Regression Model | *B* | SE | *t* | *p* Value |
|---|---|---|---|---|
| *5-HTTLPR* | −.667 | .550 | 1.213 | .2263 |
| TLE | .067 | .240 | .277 | .7821 |
| *5-HTTLPR* × TLE | .602 | .213 | 2.824 | .0049[b] |
| *rs140700*[a] | 2.085 | 1.126 | 1.851 | .0659 |
| TLE | .794 | .165 | 4.821 | <.0001[b] |
| *rs140700* × TLE | −1.227 | .419 | −2.927 | .0036[b] |
| *5-HTTLPR* | −3.540 | 2.171 | −1.631 | .1036 |
| *rs140700*[a] | −1.083 | .902 | −1.201 | .2303 |
| *5-HTTLPR* × *rs140700* | 2.635 | 1.172 | 2.249 | .0249 |
| *5-HTTLPR* | −.903 | .774 | −1.168 | .2435 |
| *rs140700*[a] | −.097 | .599 | −.163 | .8708 |
| TLE | −.094 | .253 | −.371 | .7108 |
| *5-HTTLPR* × *rs140700* × TLE | .556 | .158 | 3.512 | .0005[b] |

TLE, threatening life events; ZSDS, Zung Self-Rating Depression Scale.

[a]A carriers (AA + AG) to noncarriers (GG).

[b]*p* value less than .0083 was considered significant (see also Methods and Materials).

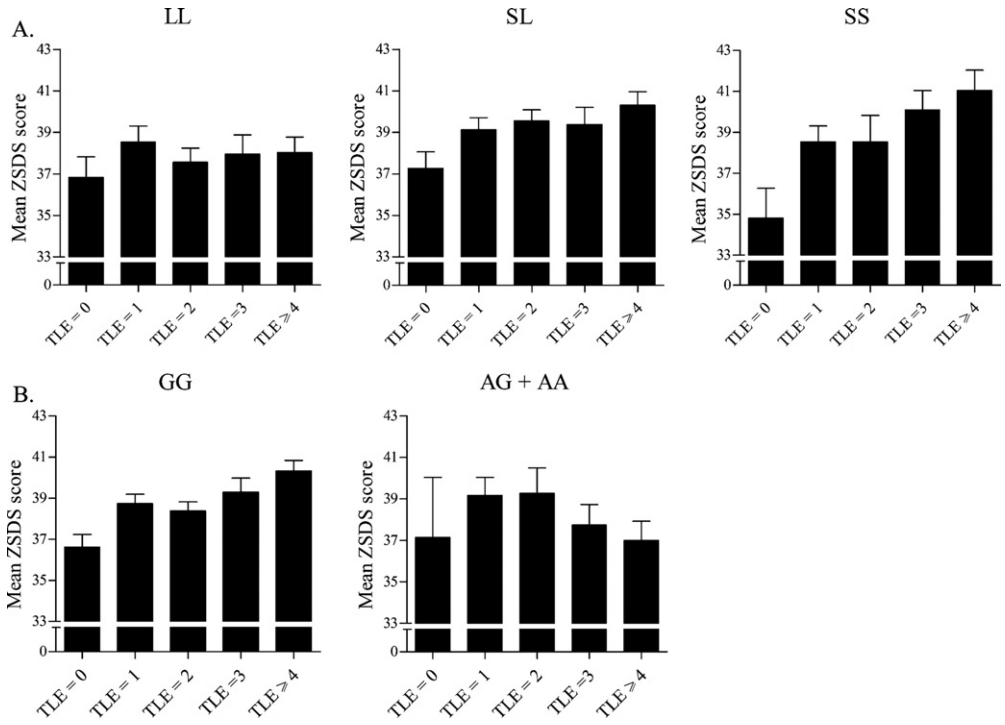


**Figure 2. (A)** Effect of threatening life events (TLE) on Zung Self-Rating Depression Score (ZSDS) in different genotype groups of *5-HTTLPR*. **(B)** Effect of TLE on ZSDS in different genotype groups of *rs140700*. TLE was significantly associated with ZSDS in individuals carrying SL and SS of *5-HTTLPR* and GG of *rs140700*. Means and standard error of means are represented.

## Discussion

Our results demonstrate heterogeneity in individuals carrying S alleles of *5-HTTLPR* in association with high TLE on the ZSDS score. Only carriers of SGGAG, the most frequent haplotype, scored significantly higher on the ZSDS, and we identified two potential protective haplotypes, one an S carrier (SGAGT, LGAGT), against vulnerability for depressive phenotype in individuals exposed to multiple TLEs. These data provide a possible evidence-based explanation of conflicting results in the literature concerning the role of SLC6A4 gene × stressful life events interaction in affective disorders.

Although certain promising data are available about haplotype analysis of other psychiatric disorders combined with environmental factor (31,32) studies reporting interaction of SLC6A4 haplotypes and stressful life events are absent. In our study, haplotype analysis revealed a significant global effect of haplotypes on ZSDS score in the high TLE subgroup but not the low TLE subgroup. Our data suggest that the effect of the S allele of *5-HTTLPR* could be modified by a functional variant in the middle region of the gene marked by the A allele of *rs140700,* and thus this region carries a significant role in G × E interaction affecting depressive phenotype. The role of *rs140700* SNP and TLEs was tested not only in haplotype analysis in conjunction with *5-HTTLPR,* but also by single marker association. Our study

**Table 5.** Analyses of SLC6A4 Haplotypes in Condition of TLE Score

| | Total Sample ($n$ = 546) $p_{global}$ = .082 | | | Low TLE Subgroup ($n$ = 200) $p_{global}$ = .117 | | | High TLE Subgroup ($n$ = 226) $p_{global}$ = .008 | | |
|---|---|---|---|---|---|---|---|---|---|
| HAPLOTYPES | Freq (%) | Add Val | $p_{effect}$ | Freq (%) | Add Val | $p_{effect}$ | Freq (%) | Add Val | $p_{effect}$ |
| SGGAG | 25.9 | 0 | .005 | 28.9 | 0 | .381 | 25.3 | 0 | <.001[a] |
| SGGGT | 5.8 | −.007 | .478 | 5.9 | −.089 | .106 | 5.9 | .024 | .116 |
| SGAGT | 2.2 | −.147 | .049 | 1.4 | −.132 | .281 | 2.6 | −.223 | .002[a] |
| SAGGT | 7.3 | −.054 | .399 | 8.0 | −.080 | .152 | 6.7 | −.061 | .723 |
| LGGAG | 18.0 | −.049 | .278 | 18.8 | −.049 | .432 | 18.5 | −.046 | .377 |
| LGGGT | 3.7 | −.068 | .173 | 3.6 | −.103 | .026 | 3.9 | −.059 | .802 |
| LGAGT | 6.6 | −.027 | .664 | 6.1 | .050 | .049 | 6.9 | −.105 | .018[a] |
| LAGGG | 3.7 | −.004 | .433 | 3.4 | .054 | .237 | 3.3 | −.159 | .486 |
| LAGGT | 24.9 | −.035 | .369 | 21.8 | .004 | .309 | 24.9 | −.050 | .331 |

Add Val, estimated additive genetic value for the haplotypes relative to the most common haplotype; TLE, threatening life events; ZSDS, Zung Self-Rating Depression Scale.

[a]Significant ($p < .05$) individual effect of haplotypes in the subgroup where global association was also significant ($p < .05$).

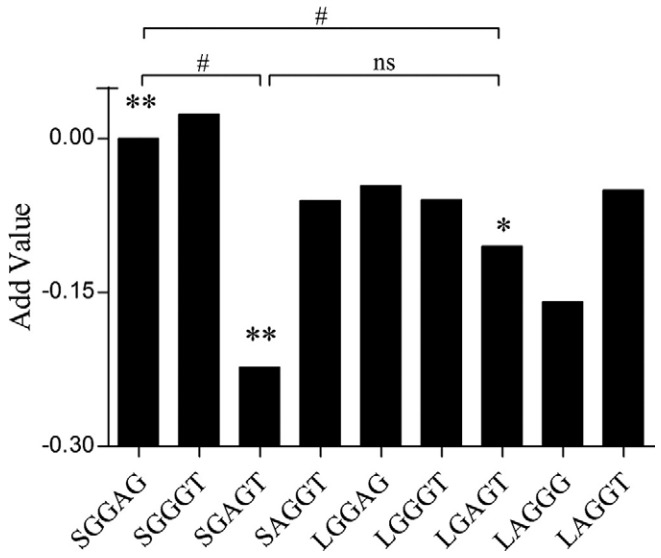


**Figure 3.** Effect of haplotypes in high threatening life events (TLE) subgroup. Individual effect of haplotypes ($^{*}p_{effect} < .05$, $^{**}p_{effect} < .005$) and difference between individually significant haplotypes are represented ($^{\#}p < .005$). Letters in haplotypes illustrate alleles for *5-HTTLPR* (S/L), *rs2020942* (A/G), *rs140700* (A/G), *rs3794808* (A/G), and *rs1042173* (G/T).

is the first report of a significant interaction between *rs140700* and TLE in the depressive phenotype. *rs140700* is a tag SNP located in the sixth intron of SLC6A4 spanning the 8-kb middle region of the gene with an $r^2 > .8$. This polymorphism may be an important marker rather than a direct contributor to the genetic functions considering that it also tags the fifth exon containing a common nonsynonymous coding variant—*rs2228673*—resulting an Asn/Lys amino acid change in position of 201 of the protein. These findings are also supported by recent studies suggesting that besides the *5-HTTLPR,* other genomic regions of SLC6A4 have role in 5-HTT function (33) as well as depression (5,34,35). However, none of these studies investigated the interaction of environmental factors.

Our data demonstrate that ZSDS was significantly influenced by TLE only in S carriers of *5-HTTLPR* in single marker association model. This is in accordance with the majority of previous studies (11–17). Certain studies had failed to replicate the association of G × E interaction with depression (18–20). Discrepancies among the results of these studies could result from several factors. In light of our results, we suggest that in addition to the promoter region, the middle region of the SLC6A4 gene has a modifying effect on gene function. Because the most frequent haplotype containing the S allele of *5-HTTLPR* is associated with the highest ZSDS score in individuals exposed to multiple TLEs, the majority of interaction studies could have replicated the significant role of the S allele and stressful life events in the pathomechanism of depression. In those studies that failed to replicate this association, there was possibly a greater number of another type of S carrier haplotype associated with low ZSDS (e.g., haplotypes containing the S allele of *5-HTTLPR* with the A allele instead of the G allele of *rs140700*).

Because of the lack of haplotype analyses, authors have discussed various reasons for conflicting data in this area (36,37). First, the study populations characterized by age, sex ratio, medical state, and recruiting conditions were different. Second, the investigation of depressive phenotype and also the stressful life events were not performed using the same methods. Third, the heterogeneity in the literature could be interpreted through the subjects' neuroticism (22). Hence, to avoid these potentially misleading factors, we have chosen the inventories circumspectly. For measuring depressive phenotype, we used a continuous scale (ZSDS) so that subclinical depression is also represented in a nonclinical sample (38,39). Negative life events were assessed by TLE in which the life events are well defined and objectively characterized by serious experiences independent of participants' neuroticism. The *5-HTTLPR* has been shown to be associated with affective temperaments that may also play a role in determining reaction to stressful life events (40). Therefore it is possible that the role of the S allele in depression is mediated by the manifestation of more extreme forms of affective temperaments leading to, for example, less adaptive coping mechanisms and thus a significant effect of stressful life events in the development of depression.

Previous studies concerning G × E interaction between *5-HTTLPR* and SLEs have discussed the results focusing only on *p* values. In addition to the *p* values, we used explained variance (adjusted $R^2$) for characterization of the associations of these models. Likewise, in cases of other multifactorial conditions, the explained variance of depressive phenotype was approximately 1% for a single marker association (*5-HTTLPR*) and 2% for the effect of TLE in itself. *5-HTTLPR* × TLE interaction revealed approximately 4% explained variance, and this was almost 6% if the regression model included *5-HTTLPR, rs140700,* TLE, and their three-way interaction. Variance of ZSDS explained by TLE was more than 12% in the SS genotype, demonstrating the relevance of G × E interaction. Our data underpin the relevance of publishing adjusted $R^2$ in the genetic studies.

In conclusion, our data indicate that there is a more sophisticated regulation of the serotonin transporter, requiring a more complex model to investigate the G × E interaction than was previously assumed—namely that *5-HTTLPR* alone does not represent the SLC6A4 gene function in this association. Our data point to the role of the middle region of this gene, tagged by *rs140700,* and its interaction with *5-HTTLPR* and TLE on depressive phenotype.

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