# Title: Early adversity and 5-HTT-BDNF genes: New evidences of Gene-Environment interactions on depressive symptoms in a general population

**Short title:** Childhood adversity and depression is moderated by 5-HTT and BDNF genes

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**Abstract**

**Background:** Adverse childhood experiences have been described as one of the major environmental risk factors for depressive disorder. Likewise, the deleterious impact of early traumatic experiences on depression seems to be moderated by individual genetic variability. Serotonin transporter (5-HTT) and the Brain-Derived Neurotrophic Factor (BDNF) seem to modulate the effect of childhood adversity on adult depression, although inconsistencies across studies have been found. Moreover, the GxE interaction concerning the different types of childhood adversity remains poorly understood. The aim of this study is to analyse the putative interaction between the 5-HTT gene (5-HTTLPR polymorphism), BDNF gene ($Val^{66}Met$ polymorphism) and childhood adversity in accounting for adult depressive symptoms.

**Methods:** A sample of 534 healthy individuals filled in self-report questionnaires of depressive symptomatology (SCL-90-R) and different types of childhood adversities (CTQ). The 5-HTTLPR polymorphism (5-HTT gene) and the Val66Met polymorphism (BDNF gene) were genotyped in the whole sample.

**Results:** Total childhood adversity ($B=0.27$, $p<0.001$), childhood sexual abuse ($B=0.17$, $p<0.001$), childhood emotional abuse ($B=0.27$, $p<0.001$) and childhood emotional neglect ($B=0.22$, $p<0.001$) had an impact on adult depressive symptoms. Childhood sexual abuse had a greater impact on depressive symptoms in Met allele carriers of the BDNF gene than in the Val/Val group ($F=5.87$; $p<0.0001$), and in S carriers of the 5-HTTLPR polymorphism (5-HTT gene) ($F= 5.80$; $p<0.0001$).

**Conclusions:** Childhood adversity *per se* predicted higher levels of adult depressive symptoms. In addition, BDNF $Val^{66}Met$ and 5-HTTLPR polymorphisms seemed to moderate the effect of childhood sexual abuse on adult depressive symptoms.

## Introduction

Depression is a complex phenotype which involves affective, motivational, cognitive, physical and behavioural symptoms as well as complex relationships between environmental and genetic factors (Levinson, 2006).

Adverse childhood experiences have been described as one of the major environmental risk factors for adult depression (Kessler and Magee, 1993, Kendler et al., 1993, Kendler et al., 2004). Converging evidence from neurobiology and epidemiology have suggested that early disrupting adverse events during development cause enduring brain dysfunction (Anda et al., 2006, Heim and Nemeroff, 2002).

Corticolimbic circuits (hippocampus, neocortex, amygdala, cerebellum and hypothalamus) have emerged as key zones in the modulation of affectivity and emotion. Serotonin (5-HT) seems to play an important role in these corticolimbic circuits in which serotonin transporter (5-HTT) is the main reuptake mechanism. In addition, abnormalities in 5-HT functionality have classically been implicated in the origin of affective disorders (Owens and Nemeroff, 1998). Likewise, the Brain Derived Neurotrophic Factor (BDNF) has a key distribution in cerebral regions involved in emotional and behavioural regulation (Gratacos et al., 2007). Evidence suggests that it is critical for normal adaptive responses to the effects of stress (Duman, 2002). Recently, BDNF has been proposed to contribute to the genesis of depressive symptoms since it has been found i) decreased plasma levels of BDNF are found in patients with major depression (Karege et al., 2002); ii) up-regulation of BDNF in the hippocampus is produced after long-term antidepressant administration (Duman, 2002); and finally, ii) there is a down-regulation of BDNF in animal models of stress-induced depression (see review in (Angelucci et al., 2005)). Moreover, there is also recent evidence suggesting that the BDNF and serotonin systems interact with each other to regulate neural circuits

involved in affective behaviours (see review in (Martinowich and Lu, 2008)). This is, activation of 5-HT receptors can induce transcription of the BDNF gene, and conversely, BDNF can stimulate synaptic plasticity of 5-HT neuron axons (Mattson et al., 2004).

It is interesting to point out that the maturation of these areas involved in higher-order functions, such as affective regulation, continues into childhood and adolescence (Lenroot and Giedd, 2006). Thus, these brain areas may be still vulnerable to environmental insults during childhood and early adulthood.

The deleterious impact of early traumatic experiences on adult depressive symptoms and major depression seems to be moderated by individual genetic variability, specifically in those genes regulating serotonin transmission such as the 5-HTT gene (Caspi et al., 2003, Kendler et al., 2005). In that respect, two recent studies reported complex interactions between this 5-HTT gene and BDNF gene on depression in individuals who had suffered childhood adversity (Kaufman et al., 2006, Wichers et al., 2008). However, neither of these two studies differentiated between types of childhood adversity and their conclusions are restricted to the type of sample used, children and female samples, respectively.

Recent research highlighted the importance of the nature of adverse childhood experiences regarding its interplay with these genetic variants for complex phenotypes such as suicide behaviour and anxiety sensitivity (Roy et al., 2007, Gibb et al., 2006, Stein et al., 2008, Perroud et al., 2007). However, none of these studies examined the putative interactions between 5-HTT and BDNF genes and the nature of childhood adversity on adult depressive symptoms.

According to these evidences, the aim of the present study was to detect the putative gene x gene x environment interaction (GxGxE) between the 5-HTT gene (5-

HTTLPR polymorphism), BDNF gene (Val$^{66}$Met polymorphism) and childhood adversity, in relation to the presence of depressive symptoms in adulthood, in a non-clinical adult sample.

## *Methods*

### Sample

The sample consisted of 534 Spanish healthy individuals who were recruited from the campus of Jaume I University in Castelló (Spain), and from university offices and community technical schools from the metropolitan area of Barcelona (Spain). At the assessment 77 % of the participants were students.

Exclusion criteria were presence of any major medical illness affecting brain function, current substance abuse (alcohol or any illicit drug), neurological conditions, history of head injury and personal history of psychiatric medical treatment. These areas were screened by means of a short interview designed *ad hoc* for this study. Additionally, it was required to describe themselves as being of Spanish (Caucasian) ancestry to reduce the possibility of confounding by population stratification (Freedman et al., 2004, Calafell and Bertranpetit, 1994). Trained psychologists carried out the screening for the exclusion criteria.

Ethical approval was obtained from Spanish local research ethic committees. All participants provided a complete written informed consent before inclusion in the study.

### Measurements

Participants filled in the 13-item depressive scale of the revised version of the Symptom Check List (SCL-90-R; (Derogatis and Melisaratos, 1983)), a validated self-report questionnaire. This scale measures the degree of discomfort associated with each depressive symptom during the last week on a five-point scale ranging from “not at all”

to “extremely”. A continuous weighted depressive symptoms score (sum of scores on the depression items, divided by number of items filled in) was used in the analyses.

Childhood adversity was assessed by the shortened version of the Childhood Trauma Questionnaire (CTQ; (Bernstein et al., 2003)). It assessed five types of childhood adversity: emotional abuse, physical abuse, sexual abuse, emotional neglect and physical neglect. Reliability and validity of the CTQ have been demonstrated (Bernstein et al., 1994, Bernstein et al., 2003). It consists of 28 items (25 clinical and 3 validity items). The score for each item ranges from 1 to 5 according to the extent in which subjects agree with the statement. A composite index of total childhood adversity was calculated as the mean score of the 5 dimensions.

**Laboratory methods**

Genomic DNA was extracted from saliva samples using the Collection Kit BuccalAmp DNA extraction kit (Epicentre).

The 48bp insertion/delection at 5’promoter region of the 5-HTT gene (5-HTTLPR polymorphism) was analyzed using the protocol previously described by Lesch et al (1996) (Heils et al., 1997).

The SNP rs6265 (Val66Met) of the BDNF gene was genotyped using Applied Biosystems (AB) TaqMan technology. AB assay-on-demand service was used to order the probes.

Randomized individuals were re-genotyped in order to confirm the pattern reproducibility.

**Statistical Analyses**

Main effects of each type of adversity and composite index of total childhood adversity on depressive symptoms and of 5-HTTLPR genotype or Val$^{66}$Met genotype on depressive symptoms were analysed. In addition, main effects of 5-HTTLPR

genotype or Val$^{66}$Met genotype on each type of adversity and total childhood adversity were explored.

Two-way interaction effects between i) 5-HTTLPR and Val$^{66}$Met polymorphisms; ii) 5-HTTLPR and each type of adversity/total childhood adversity; and iii) Val$^{66}$Met polymorphism and each type of adversity/total childhood adversity were fitted in models of depressive symptomatology. When GxE interaction was significant post-hoc analyses were performed by means of Stata Lincom command in order to evaluate dose-response relationship (UCLA: Academic Technology Services, 2008). Differences in slope between genotypes were tested at the mean (none or minimal severity levels of maltreatment), at one standard deviation above the mean (low to moderate severity levels of maltreatment) and at two standard deviations above the mean (moderate to severe severity levels of maltreatment).

Finally, three-way interaction was performed between the measured polymorphisms and total childhood adversity on depressive symptoms.

All analyses were carried out using linear regression. Effects sizes were calculated and evaluated by the Wald test using the STATA LINCOM command. All regression analyses were controlled for age and sex. Standardized coefficient (Beta) is displayed in the results section. Analyses were performed using STATA 9.1 (StataCorp, 2005)..

Since the BDNF "Met/Met" group was small (n=29),"Met" carriers ("Met/Met" + "Val/Met") were grouped in subsequent analyses

**Results**

Sociodemographic data and allele/genotype distribution are shown in Table 1. Average score on depressive symptoms was 0.7 (SD = 0.6; range: 0-3.2).

A main effect of total childhood adversity on adult depressive symptoms was detected (B=0.27, p<0.001). Specifically, childhood sexual abuse (B=0.17, p<0.001), childhood emotional abuse (B=0.27, p<0.001) and childhood emotional neglect (B=0.22, p<0.001) are related significantly to depressive symptomatology in adulthood.

No other associations between other types of childhood adversity and depressive symptoms were found (Table 2). No significant associations were found between either 5-HTTLPR or Val$^{66}$Met genotypes on depressive symptoms. However, an association of 5-HTTLPR genotype on childhood sexual abuse was found (L/S: B=-0.33 p=0.006; S/S: B=-0.18 p=0.17; using L/L as reference category). The L/L genotype presented higher level of childhood sexual abuse compared to L/S genotype, but compared to S/S genotype the difference did not reach significant levels.

Concerning two-way interactions, a significant GxE interaction was found of 5-HTTLPR genotypes and childhood sexual abuse in the model of depressive symptoms (F= 4.36, p<0.0001). In subjects with the L/S and S/S genotypes the effect of childhood sexual abuse on adult depressive symptoms was higher than in L/L subjects (L/S x sexual abuse: B=0.25, p=0.03; S/S x sexual abuse: B=0.18, p=0.07; L/L reference category). In fact, when we compare S allele carriers (S/L + S/S) to L/L individuals, it is detected a higher effect of childhood sexual abuse on depressive symptoms (B=0.21, p=0.016) (Figure 1). Post-hoc analysis showed a dose-response relationship between 5-HTTLPR genotypes and childhood sexual abuse for depressive symptoms. That is, the interaction was only significant when childhood sexual abuse score was above average: the difference in slope between S allele carriers and L/L regression lines reach

significant level at one standard deviation above the mean (low to moderate severity levels) (B=0.28, t=2.07; p=0.039) and this difference increased at two standard deviations above the mean (moderate to severe severity levels) (B=0.49, t=2.45; p=0.015).

In addition, a two-way interaction with $Val^{66}Met$ genotype was also found (F=5.87, p<0.0001; B=0.23, p=0.015, Val/Val reference category). Childhood sexual abuse had a greater effect on adult depressive symptoms in Met carriers than in Val/Val individuals (Figure 2). Post-hoc analysis revealed also a dose-response relationship, the differences in slope between Met allele carriers and Val/Val was statistically significant only at one standard deviation above the mean (low to moderate severity levels) (B=0.32, t=2.42, p=0.016), and it increased at two standard deviations above the mean (moderate to severe severity levels) (B=0.55, t=2.62, p=0.009). Neither significant GxE interactions were found for the other sub-dimensions of childhood adversity, nor significant GxG interaction on adult depressive symptoms.

We did not detect a three-way interaction between 5-HTT gene, BDNF gene and total childhood adversity on adult depressive symptoms.

**Discussion**

Our results have shown that experiences of childhood sexual abuse, emotional abuse and emotional neglect predicted *per se* the presence of adult depressive symptoms, in accordance with previous clinical and epidemiological reports (Chapman et al., 2004, Anda et al., 2006).

From the perspective of gene-environment interaction, our results suggested that genetic variability at 5-HTT and BNDF genes moderates the effect of childhood adversity on adult depressive symptoms. Specifically, our findings showed that

childhood sexual abuse impacted more strongly on adult depressive symptomatology in S carriers of the 5-HTTLPR polymorphism (5-HTT gene) than in the LL group, and in Met allele carriers of the Val66Met polymorphism (BDNF gene) compared to the Val/Val group. Moreover, a dose-response relationship was found in both models of GxE on adult depressive symptoms.

These results are consistent with those found in adult psychiatric patients with suicidal behaviour. For example, Gibb et al. (2006) found that 5-HTTLPR polymorphism moderates the effect of childhood sexual and physical, but not emotional, abuse on suicide attempters. 5-HTT gene has also been linked to GxE interaction for other types of adversity such as emotional neglect (Roy et al., 2007). Additionally, Perroud et al. (2007) based on a large sample of suicide attempters genotyped for BNDF gene detected a GxE interaction for severity of suicidal attempt in individuals who had experienced childhood sexual abuse.

Childhood sexual abuse may differ from other types of childhood adversity on its greater capacity to disrupt the underlying neurobiological structures involved in stress response. In this sense, it has been suggested that childhood sexual abuse increases sensitivity to the depressogenic adult life experiences (Kendler et al., 2004). In fact, neurobiological studies showed i) dysfunction of hypothalamic-pituitary-adrenal (HPA) in women with a childhood sexual abuse history; ii) volumetric reductions of hippocampus and amygdala, main regulatory mechanisms of the HPA axis, in women who had experience childhood sexual abuse (Nemeroff, 2004, Teicher et al., 2002, Heim and Nemeroff, 2001). It is also likely that this dysregulation of the HPA axis may be mediated by genetic background of the individual such as 5-HTTLPR and Val66Met polymorphisms (Brown and Harris, 2008, Nestler et al., 2002).

Interestingly, it has to be noted that both polymorphisms of the analysed genes present functional consequences; the S allele of the 5-HTTLPR polymorphism (5-HTT gene) has been related to reduced transcription of the serotonin transporter (Lesch et al., 1996) while the Met allele of the Val66Met polymorphism (BDNF gene) seems to affect intracellular processing and secretion of the mature protein (Egan et al., 2003). From a gene-environment interaction point of view, the S allele has been frequently involved in risk for depressive symptoms and major depression when childhood adversity and recent life events are considered (Caspi et al., 2003, Kendler et al., 2005) see review (Uher and McGuffin, 2008)). Likewise, interaction between the Met allele of the BNDF polymorphism and childhood adversity was found by Wichers et al. (2008).

However, in the above mentioned study conducted by Perroult et al. (2007) the risk allele was the Val variant. This inconsistency between the proposed risk alleles of the BDNF Val66Met polymorphism has also been found by a meta-analysis within association genetic studies in several mental disorders (Gratacos et al., 2007). Further research is needed to better understand the pathophysiological consequences of this particular functional polymorphism in brain functioning.

Despite recent evidence that serotonin and BDNF systems may be linked at multiple intracellular and intercellular levels (Martinowich and Lu, 2008), our results did not found direct interaction between the two polymorphisms explored. Accordingly, a recent study carried out by Kim et al. (2007) in an elderly population with categorical outcome of depression did not found an interaction between these two genes. Further research may be needed to elucidate such complex relationship between BDNF and 5-HT systems in the etiology of depressive mood.

Unlike previous studies (Kaufman et al., 2006, Wichers et al., 2008) the three-way interaction between 5-HTTLPR genotype, Val66Met genotype, and childhood

adversity on depressive symptoms was not detected in our sample. However, our study has certain differences that may account for such discrepancy. Our sample, composed of healthy men and women, was collected from the general population while Kaufman et al. focused their study on children with severe maltreatment histories, and Wichers et al. on a female sample.

Although our results replicate previous published ones, there are some limitations that we would like to comment. The cross-sectional nature of the design did not permit test causal associations robustly, but *a priori* hypotheses were clearly defined and guided all analyses. Although, some studies have found high reliability of self-reports of childhood trauma (e.g., (Fink et al., 1995)), the retrospective measure of childhood adversity may be influenced by recall bias. It is important to highlight that different prevalence between each type of childhood adversity in the population may affect the power to detect other GxE interactive effects. In the present sample, following the guidelines for classification of CTQ proposed by Bernstein and Fink 1998, and in agreement with previous European surveys (May-Chahal and Cawson, 2005, Finkelhor, 1994), prevalence of i) childhood emotional abuse is 15.5 % (n=83); ii) childhood physical abuse is 4.1 % (n=22); iii) childhood sexual abuse is 9.2 % (n=49); iv) childhood emotional neglect is 27.2% (n=145); and physical neglect is 10.3% (n=55). Therefore, it seemed that absence of interactions concerning other types of adversity, except for childhood physical abuse, may not be due to a simple function of low prevalence compared to the prevalence of childhood sexual abuse. Moreover, co-occurrence of childhood sexual abuse with other forms of childhood adversity has been described (Dong et al., 2003). In order to elucidate putative confounder effect of other childhood adversities on depressive symptoms, post-hoc analyses were carried out concerning significant results. Either main effect of childhood adversity on adult

depressive symptoms or GxE interactions on depressive symptoms remained significant after controlling for other childhood adversities (results available upon request). However, other distal factors such as timing and duration in which childhood sexual abuse occurred, as well as other early environmental factors such as quality of parental care may also play a role in determining individual vulnerability to depressive symptomatology during adulthood (McCutcheon et al., 2008, Cotter, 1998, Hill et al., 2000). Other current factors not controlled in the present study, such as medical status, work and social adjustment or quality of life, may influence mood state of participants at the moment of the assessment.

Finally, it was not considered the triallelic nature of the 5-HTTLPR polymorphism (S, La and Lg). It has been shown that Lg carriers presented similar expression than S carriers. Although genotypes carrying Lg are not expected to have high frequencies in Caucasian populations (SLg=0.09, LgLg=0.03), it is clear that in the present study the attributable variance of the Lg allele is not controlled (CITA → Hu et al., 2006 Am J Hum Genet 78:815-826).

As in the study of Wichers et al (2008), gene-environment (GE) correlation next to GE interaction may play a role. A main effect of 5-HTTLPR polymorphism on childhood sexual abuse was found, that is, L/L group showed higher levels of childhood sexual abuse compared to S allele carriers. It is interesting to highlight that despite L/L group presented higher levels of childhood sexual abuse, this group presented less severe adult depressive symptoms compare to S allele carriers. However, since the GE interaction analysis was not controlled for the GE correlation between these two variables, caution must be taken interpreting the two-way interaction effect (Pak , 2006).

Nevertheless, this study contributes to this field in certain unique ways. Since depression has been described as a continuous phenotype in the population (Akiskal et al., 1997), etiopathogenic mechanisms should be detectable across a wide range of subclinical and clinical phenotypic variation in non-clinical samples such as the present one. Additionally, this study has analyzed the impact of both qualitatively distinct forms of childhood adversity and their level of global severity, which has been shown to be relevant when assessing the impact of trauma on health outcomes (Dong et al., 2004).

## Disclosure/Conflicts of interest

The author(s) declare that, except for income received from my primary employer, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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**Table 1.** Sociodemographic data and genotype/allele distribution for the 5-HTTLPR (5-HTT gene) and Val66Met (BDNF gene) polymorphisms.

| **Sociodemographic data** | | **Completed Education** | | |
|---|---|---|---|---|
| Gender distribution | Mean age (Sd) | Elementary School | High School | University education |
| 242Male/292Female | 22.9 (5.4) Range 18-50 | 3.3 % | 85.7 % | 10.9 % |

| **Genotype and allele distribution** | | | | | |
|---|---|---|---|---|---|
| **5-HTT gene** | | | | | |
| | ***Genotype distribution*** | | | ***Allele distribution*** | |
| | L/L | L/S | S/S | Allele L | Allele S |
| **5-HTTLPR polymorphism (n=475)** | 119(25.1%) | 230(48.4%) | 126(26.5%) | 468 (49.3%) | 482 (50.7%) |
| **BDNF gene** | | | | | |
| | Val/Val | Val/Met | Met/Met | Allele Val | Allele Met |
| **Val66Met polymorphism (n=470)** | 282 (60%) | 159 (33.8%) | 29 (6.2%) | 723 (76.9%) | 217 (23.1%) |

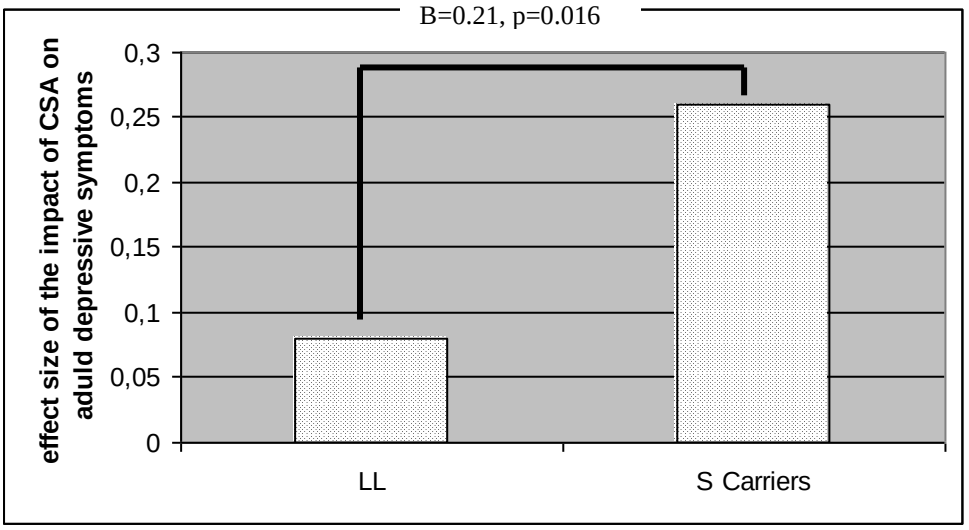
* Frequencies were found in Hardy-Weinberg equilibrium (5-HTTLPR polymorphism $\chi^2$=0.21, df=2, p=0.90; Val66Met polymorphism $\chi^2$=0.52, df=2, p=0.77)

**Table 2**. Mean scores (+SD) of types of childhood adversity/Total childhood adversity. Beta (standardized coefficient) derived from the main effects of total/types of childhood adversities on adult depressive symptoms are also shown.

| Types of Adversities (n=521) | Mean (SD) | Beta | P value |
|---|---|---|---|
| Emotional Abuse | 6.6 (2.6) | 0.27 | < 0.0001 |
| Physical Abuse | 5.4 (0.9) | 0.08 | 0.06 |
| Sexual Abuse | 5.5 (2.0) | 0.17 | < 0.0001 |
| Emotional Neglect | 8.3 (3.1) | 0.22 | < 0.0001 |
| Physical Neglect | 5.7 (1.4) | 0.07 | 0.10 |
| Total Adversity | 6.1 (1.4) | 0.27 | < 0.0001 |

* All regressions were adjusted for age and sex.

**Figure 1**. Effect size of childhood sexual abuse (CSA) on adult depressive symptoms by 5-HTTLPR genotype: the effect of childhood sexual abuse has a higher impact on adult depressive symptoms in S carriers than in the L/L group (n=452).

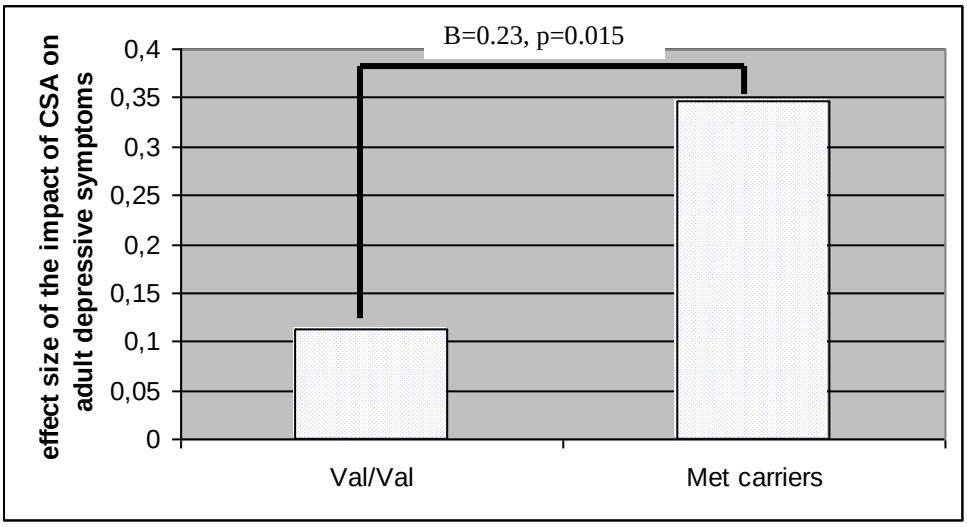



*****F= 5.80, p<0.0001

* Adjusted for age and sex.

**B=standardized coefficient

**Figure 2.** The depressogenic effect of childhood sexual abuse (CSA) is moderated by BDNF genotype: the effect of childhood sexual abuse has a higher impact on adult depressive symptoms in Met carriers than in the Val/Val group (n=456).




*F=5.87, p<0.0001

* Adjusted for age and sex.

**B=standardized coefficient