Research Paper

The risk for major depression conferred by childhood maltreatment is multiplied by *BDNF* and *SERT* genetic vulnerability: a replication study

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Background: There is limited evidence for a moderating role of both serotonin transporter (*SERT*) and brain-derived neurotrophic factor (*BDNF*) genes on the risk for major depression (MD) developing after childhood maltreatment. However, research on this topic remains inconclusive, and there is a lack of data from longitudinal studies with large and representative population samples. Our study aimed to clarify whether, in the presence of previous childhood maltreatment, individuals carrying low functional alleles for both *SERT* 5-HTTLPR and *BDNF* Val66Met polymorphisms had a higher risk for MD. **Methods:** We explored 2- and 3-way gene (*SERT* and *BDNF*) × environment (childhood maltreatment) interactions in a large sample of Spanish adults who were followed up over a 3-year period and assessed in person for both DSM-IV MD and exposure to childhood maltreatment. **Results:** Our study included 2679 participants. Those with both the 5-HTTLPR s allele and the *BDNF* Met allele showed the highest risk of MD if they had previously experienced emotional ($z = 2.08$, $p = 0.037$), sexual ($z = 2.19$, $p = 0.029$) or any kind of childhood abuse ($z = 2.37$, $p = 0.018$). These 3-way interactions remained significant regardless of whether the 5-HTTLPR triallelic or the 5-HTTLPR biallelic polymorphisms were included in the analyses. **Limitations:** Retrospective assessment of childhood maltreatment may have resulted in a moderate degree of recall bias. **Conclusion:** Our results confirm that the risk of depression conferred by childhood maltreatment is modified by variation at both *SERT* and *BDNF* genes.

Introduction

Experiences of maltreatment during childhood are associated with mental health disorders, possibly as a consequence of the moderating effect of specific genes on the risk conferred by such early traumatic events.[1] Thus, maltreated children with the serotonin transporter (*SERT*) genotype conferring low levels of the SERT molecule (5-HTTLPR S allele carriers) seem to have an increased risk for depression compared to maltreated children with the 5-HTTLPR L/L genotype.[1,2] Similar 2-way (gene × environment) interactions between the *BDNF* Val66Met polymorphism and childhood maltreatment have also been described in the context of other mental health outcomes,[3–5] particularly depression.[6–8]

Synergies between the serotonin (5HT) and BDNF systems have been suggested to contribute to the regulation of the development and plasticity of neural circuits involved in the origin of affective disorders.[9] Evidence of biological epistasis between *SERT* and *BDNF* has also been described, which would support the existence of a functional interconnection between serotonergic and neurotrophic systems.[10] It has been reported that BDNF influences response to mood disorder treatments[9,11–13] and also mediates the differential reactivity to environmental stress attributed to the *SERT* 5-HTTLPR polymorphism.[14] Indeed, previous reports suggest a moderating role of both *SERT* and *BDNF* on the risk for major depression (MD) in individuals who have experienced early adversity or stressful life events,[15–17] although negative findings have also been reported.[18,19]

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Five studies have focused specifically on the interaction between these 2 genes and childhood maltreatment with conflicting results on how *SERT*, *BDNF* and childhood maltreatment interact to exert their effect on depression. The studies are cross-sectional and use small heterogeneous samples of youngsters[7,8,20] or other unrepresentative selected populations,[6] a one-off outcome characterization mostly using self-reported instruments for depression[6,8,14] and broad measures of childhood maltreatment.[7,8] Moreover, none of these previous studies has yielded data about which of the tested models is most predictive of depressive outcome.

We aimed to clarify the interplay between *SERT*, *BDNF* and childhood maltreatment using a large sample of Spanish adults who were assessed repeatedly and thoroughly over a 3-year period in a longitudinal cohort study specifically designed to develop a risk index for the onset of MD in primary care.[21,22] We hypothesized that in the presence of previous childhood maltreatment, those individuals carrying low function alleles for both *SERT* 5-HTTLPR (biallelic or triallelic) and *BDNF* Val66Met polymorphisms would have a higher risk for MD. We also hypothesized that the 3-way interactive model of depression would explain our outcome better than any other model containing 5-HTTLPR, *BDNF* Val66Met and childhood maltreatment variables.

Methods

Study context and design

The PREDICT-gene study[23] is a genetic substudy nested in a larger cohort study, PredictD, whose main objective was to develop a risk algorithm for the onset of MD in general practice attendees (see the studies by King and colleagues[21,24] for a full description of PredictD–Europe and by Bellón and colleagues[22,25,26] for details on its sequel, PredictD–Spain). PredictD–Spain was designed as a 3-year prospective study with assessments at 5 different points: at the beginning of the follow-up (baseline) and 6, 12, 24 and 36 months after the first interview.

The PREDICT-gene study was approved by the Research Ethics Committee of the University of Granada.

Participants

Samples were collected in 7 different Spanish provinces and involved the participation of 41 health centres and 231 physicians throughout the country. The health centres taking part covered urban and rural settings in each province.

Our present sample consisted of individuals who were recruited for the PredictD–Spain study between October 2005 and February 2006 using a systematic random method explained elsewhere.[22] Only those participants aged 18–75 years were included. Attendees unable to understand Spanish, as well as those with an organic mental disorder and/or any terminal illness were excluded. All participants in the PREDICT-gene study were Spanish with Spanish ancestry. Participants who agreed to participate in the genetic substudy gave specific informed consent to provide a biological sample, consisting of 10 cm^3 of blood and/or up to 10 mouth swabs for saliva collection.

Assessing depression (the outcome)

The diagnosis of MD according to DSM-IV criteria was ascertained using the depression section of the Composite International Diagnostic Interview, which was designed to provide 6-month and lifetime psychiatric diagnoses.[27] Presence of depression was assessed at baseline and at each follow-up point. Our outcome variable was MD at any time over the 3-year follow-up period.

Measuring childhood maltreatment

We collected data on 3 types of maltreatment that may have been experienced during childhood, assessed using the shortened version of the Childhood Trauma Questionnaire (CTQ):[28,29] emotional, physical and sexual abuse. These questions were shown to have adequate test–retest reliability in the context of the PredictD study.[21,22] The score for each CTQ item ranges from 1 to 5 according to the extent to which participants agreed with the statement. However, in the present study we dichotomized emotional, physical and sexual maltreatment measures to facilitate the comparison of our results with those reported in previous studies. Our groups were defined as follows. The emotional group included participants who had experienced only emotional maltreatment and did not report any other kind of childhood abuse, either physical or sexual. The physical maltreatment group included those with a history of physical childhood maltreatment with or without emotional maltreatment but definitely without sexual abuse. Finally, the sexual abuse group included those who reported childhood experiences of sexual maltreatment with or without emotional or physical abuse. An "any childhood maltreatment" variable was derived from those 3 maltreatment measures and was also used in the analysis. This variable referred to participants who had experienced at least 1 of these forms of maltreatment.

Molecular analyses

Genotyping the promoter region of *SERT*

We obtained DNA from blood and saliva using standard procedures. The 5-HTTLPR (L/S) biallelic polymorphism of *SERT* was genotyped as described previously.[23]

Genotyping of the 5-HTTLPR (L_A/L_G/S) triallelic polymorphism (rs25531) was performed using a modified version of the assay followed by Grabe and colleagues[14] and Hu and colleagues.[30]

The 5-HTTLPR triallelic polymorphism analysis allowed us to recheck the 5-HTTLPR biallelic genotyping in the entire sample and to explore the distribution of the triallelic promoter polymorphism through the identification of S, L_A and L_G alleles. Based on previous reports on *SERT* expression,[30] we classified the 6 possible genotype combinations into 3 functional triallelic genotypes: *l/l* = L_A/L_A; *l/s* = L_A/L_G or L_A/S and *s/s* = L_G/L_G or L_G/S or S/S.

In this paper, we mainly report results for the *SERT* 5-HTTLPR triallelic polymorphism, although analyses were also performed for the *SERT* 5-HTTLPR biallelic variant, and we include comments on those analyses in the text.

Genotyping the *BDNF* Val66Met polymorphism

The *BDNF* Val66Met polymorphism (rs6265) was characterized using a TaqMan allelic discrimination assay from Life Technologies. Cycling was performed on a StepOne Plus termocycler with conditions recommended by Life Technologies.

Testing for the validity and accuracy of genotyping

We retested a 10% random sample for their genotype at both 5-HTTLPR and *BDNF* Val66Met (rs6265) loci to confirm the validity and accuracy of genotyping methods. In all cases patterns were reproducible.

Statistical analysis

Hardy–Weinberg equilibrium was checked in the entire sample, and both in the exposed and nonexposed cohort, using the "genhw" STATA command. Analyses were performed under the aprioristic assumption of dominant genetic models. That is, comparisons were made between s allele carriers (s/*) and l/l homozygous carriers in the case of the *SERT* 5-HTTLPR triallellic polymorphism (S/* v. L/L for *SERT* 5-HTTLPR biallelic marker) and between Met allele carriers (Met/*) and Val/Val carriers in the case of the *BDNF* Val66Met marker.

Main effects and both 2- (gene × gene and gene × environment) and 3-way (gene × gene × enrivonment) interactions on the presence of MD were examined using logistic regression analyses. Specifically, generalized linear models with the binomial distribution were used to estimate odds ratios (ORs) with 95% confidence intervals (CIs). Tests were implemented using the "binreg" STATA command. In the case of a significant interaction, we performed a detailed assessment to compare the different risk effects for MD conferred by maltreatment across different genotypes. Analyses were performed crude and adjusted for sex and age (because of their well-known associations with the onset of depression)[31] and baseline depression (depression occurred prior to the follow-up period).

We also analyzed all possible models containing 5-HTTLPR, *BDNF* and child maltreatment variables individually to determine which model had the best overall fit using Akaike information criteria (AIC).[32] Models were built up incrementally with all containing 5-HTTLPR, *BDNF* and the specific form of childhood maltreatment (sex and age were entered as covariates and retained in all models). Generally, the model with the best fit is the one that minimizes the AIC. However, models that included interactions needed to have an AIC of at least 3.84 (1.96^2) lower than the next lowest AIC for it to be accepted as the best model. This is because interactions add increased complexity to models, and accepting a difference of at least 3.84 is equivalent to requiring an additional variable to be statistically significant at the 5% level.

We calculated statistical power using QUANTO software version 1.2.4 (http://hydra.usc.edu/gxe/).[33] According to such calculations, we needed at least 2041 individuals to detect with 80% power (95% CI) a gene × environment effect in the range of 2.5 and 4.5 if we assumed dominant genetic models for both *SERT* and *BDNF* polymorphisms, a risk for depression of 0.18 (the point prevalence rate of depressed participants in our sample), a frequency of high-risk alleles ranging from 0.22 (*BDNF* Met-allele frequency) to 0.48 (5-HTTLR s allele frequency), and a 3% exposure to environmental factor (frequency of sexual abuse in our sample; estimates assuming a greater environmental exposure required smaller sample sizes).

Results

Participants

Out of 3019 general practice primary care attendees who were approached to provide a biological sample in the PredictD–Spain study, 2679 consented and were included as participants in our genetic substudy. No significant differences were found between those who were included (88.74%) and those who were not (11.26%) with regards to sex, educational level or incidence of MD based on DSM-IV criteria over the 3-year period of follow-up. Our final sample included 811 men (30%) and 1868 women (70%) with a mean age of 50.33 ± 14.91 years. Most were married or living with a partner (69.2%), and just under half had an education at the junior level (46.3%). Table 1 shows the sample's main sociodemographic, clinical and risk characteristics.

Main effects of genetic and childhood maltreatment risk factors on MD

Genotype frequencies were found to be in Hardy–Weinberg equilibrium in the whole sample (5-HTTLPR triallelic polymorphism: $\chi^2_2 = 2.46$, $p = 0.29$; 5-HTTLPR biallelic polymorphism: $\chi^2_2 = 0.27$, $p = 0.87$; *BDNF* Val66Met polymorphism: $\chi^2_2 = 0.02$, $p = 0.99$) and separately in participants with MD at any point over the follow-up period and participants without this condition.

Table 2 shows crude and adjusted main effects of *SERT*, *BDNF* and childhood maltreatment variables on MD. In brief, variation at the *SERT* 5-HTTLPR triallelic polymorphism was not found to be associated with MD. The analysis of the 5-HTTLPR biallelic polymorphism did not yield significant results either. However, the risk for MD was significantly increased among Val/Val BDNF homozygous participants (OR 1.250, 95% CI 1.034–1.512, $p = 0.019$). Regarding childhood maltreatment, MD was found to be significantly associated with all types of early maltreatment experiences (Table 2).

Two-way interactions and risk for MD

We found no significant gene × gene interactions on MD. However, concerning gene × environment interactions, we found a significant effect modification exerted by the

5-HTTLPR triallelic polymorphism on the risk for MD conferred by physical maltreatment, sexual abuse and any abuse (Table 3). These effects were present both in crude and adjusted analyses. Figure 1 shows the risk (ORs) for MD at different exposure levels according to 5-HTTLPR triallelic polymorphism and childhood maltreatment. For all types of childhood maltreatment, a significant increase of odds related to increasing levels of gene–environment exposure was found; the s/* carriers with a history of childhood maltreatment reached the highest OR values (Figure 1). Entirely equivalent results were obtained for the interaction of 5-HTTLPR biallelic polymorphism and such forms of maltreatment (Appendix, available at jpn.ca).

Concerning *BDNF* × maltreatment interactions on MD, crude analyses showed that emotional and any childhood maltreatment increased the risk for adult MD slightly differently in *BDNF* Met/* carriers (allele Met carriers) than in *BDNF* Val/Val carriers ($z = 2.17$, $p = 0.030$ and $z = 1.83$, $p = 0.07$, respectively). Although these interactions did not remain significant after adjusting for sex, age and baseline depression (Table 3), a significant increase of odds related to increasing levels of gene–environment exposure was found for emotional, sexual and any childhood maltreatment; Met/* carriers with a history of childhood maltreatment reached the highest OR values (Fig. 2).

Table 1: Demographic and clinical characteristics of the sample ($n = 2679$)

Characteristic	No. (%)*
Age, mean ± SD, yr	50.33 ± 14.91
Sex	
Male	811 (30.3)
Female	1868 (69.7)
Education†	
Elementary education	554 (20.7)
Junior school	1237 (46.3)
High school	541 (20.2)
University degree	342 (12.8)
Marital status†	
Single, never married	450 (16.8)
Married	1852 (69.2)
Divorced/separated	167 (6.2)
Widowed	208 (7.8)
Major depression over follow-up	
Without major depression	1875 (70.0)
With major depression	804 (30.0)
Childhood maltreatment	
Emotional maltreatment	406 (15.2)
Physical maltreatment	258 (9.6)
Sexual abuse	75 (2.8)
Variability in *SERT* gene 5-HTTLPR L/S biallelic polymorphism	
L allele	2746 (51.3)
S allele	2612 (48.7)
L/L	713 (26.6)
L/S	1320 (49.3)
S/S	646 (24.1)
5-HTTLPR triallelic polymorphism (rs25531)	
l allele	2526 (47.1)
s allele	2832 (52.9)
l/l	624 (23.3)
l/s	1278 (47.7)
s/s	777 (29.0)
Variability at *BDNF* gene Val66Met polymorphism†	
Val (C) allele	4032 (78.0)
Met (T) allele	1134 (22.0)
Val/Val (CC)	1574 (60.9)
Val/Met (CT)	884 (34.2)
Met/Met (TT)	125 (4.9)

SD = standard deviation.
*Unless otherwise indicated.
†$n < 2679$ because of missing values.

Three-way interactions and risk for MD

Concerning crude and adjusted 3-way (gene × gene × environment) interactions, we found that genetically vulnerable individuals of both genotypes (5-HTTLPR S/* and *BDNF* Met/*) were more susceptible to the deleterious risk effect conferred by certain forms of childhood maltreatment (i.e., emotional, sexual and any childhood maltreatment; Table 3 and Fig. 3) than carriers of other genetic profiles. Again, these effects were also detected for the 5-HTTLPR biallelic polymorphism (Appendix).

Looking for the best explicative model

The 3-way interaction model was one of the best explicative models for MD in the case of emotional, physical, sexual and any childhood maltreatment. However, in all these cases other models (i.e., those including the interaction between 5-HTTLPR and any type of childhood maltreatment plus *BDNF* as a covariate) appeared to be equally good, because their AIC values did not differ by more than 3.84 units (Table 4).

Discussion

Our study explored 2- and 3-way interactions on MD in a large and well-characterized cohort of adults ($n = 2679$) followed over a 3-year period. We found consistent 3-way gene (*SERT*) × gene (*BDNF*) × environment (childhood maltreatment) interactions demonstrating combined genetic effect modification on the risk for MD conferred by several modalities of early childhood maltreatment (i.e., sexual, emotional and any abuse).

The main strength of our study was the inclusion of participants interviewed face to face who were repeatedly assessed using objective and validated instruments, leading to thorough phenotypical characterization of both MD and childhood maltreatment. This is, to the best of our knowledge, the first study testing these interactions using no self-report measures, exploring both biallelic and triallelic forms of the *SERT* promoter genetic variance and looking for the most predictive model of depression outcome.

Main effects of childhood maltreatment and genetic risk factors on MD

We found experiences of maltreatment during childhood to be associated with MD in adults. Our results add new evidence about the well-established impact and long-term effects of childhood maltreatment on mental health.[1]

Concerning the genetic variability explored, we were not able to replicate the association between 5-HTTLPR polymorphism and MD that we had previously described in an independent study[34] and in a preliminary sample of the PREDICT-gene study.[23] This is not entirely surprising as the design and the outcome variable we used in the present study are different, allowing us to approach the analysis from a different point of view. Moreover, the fact that the effect of the 5-HTTPLR s allele on depression is expected to be small and to be associated more with emotionality and stress sensitivity than the depressive diagnosis itself could explain why we did not detect a main effect of 5-HTTLPR in the present analysis.[1,35,36]

We found an independent association between *BDNF* Val66Met variation and MD. In particular, Val/Val carriers were found to have a significantly increased risk for MD compared with those carrying the Met allele. The *BDNF* Val66Met polymorphism is a functional variant affecting intracellular processing and secretion of BDNF, with the Met allele associated with reduced BDNF activity and inefficient BDNF secretion.[37] Previous reports have associated decreased levels of BDNF with depression,[38,39] and recent studies have revealed that people homozygous for the Met allele may be at greater risk of MD than Val allele carriers.[40] Other studies suggest that this Val-to-Met mutation may increase susceptibility for MD, but only after early-life stress.[6,7,20] However, there is no consensus about which genetic profile is increasing the risk, as results are contradictory. Many previous studies report that the risk for depression is higher among those with the *BDNF* Val/Val genotype,[8,41,42] a notion supported by our own findings, whereas other studies simply fail to find any involvement of *BDNF* in depressive outcomes.[43,44] Neurobiological support for our finding comes from experimental neuroimaging-based studies reporting that those with the Val/Val genotype show reduced structural volume and diminished functionality in limbic areas key to depression, such as the subgenual anterior cyngulate gyrus[10] or the CA4/dentate gyrus.[45] However, the influence of *BDNF* variability seems to

Table 2: Crude and adjusted main effects on DSM-IV major depression

		Unadjusted		Adjusted for age, sex and baseline MD	
Variable	No.	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Genes					
SERT 5-HTTLPR L/S biallelic (S/* v. L/L)	2679	1.054 (0.871–1.276)	0.59	1.015 (0.828–1.244)	0.89
5-HTTLPR triallelic (s/* v. l/l)	2679	1.021 (0.836–1.247)	0.84	1.005 (0.813–1.244)	0.96
BDNF Val66Met polymorphism† (Met/* v. Val/Val)	2583	0.797 (0.667–0.952)	0.012	0.797 (0.659–0.963)	0.019
Type of maltreatment†					
Emotional	2674	2.683 (2.156–3.345)	< 0.001	2.096 (1.659–2.650)	< 0.001
Physical	2676	3.128 (2.394–4.087)	< 0.001	2.502 (1.881–3.326)	< 0.001
Sexual abuse	2676	2.561 (1.601–4.097)	< 0.001	1.801 (1.099–2.952)	< 0.001
Any abuse	2676	2.569 (2.096–3.148)	< 0.001	2.013 (1.621–2.500)	< 0.001

CI = confidence interval; MD = major depression; OR = odds ratio.
†*n* < 2679 because of missing values.

Table 3: Interactions between 5-HTTLPR triallelic, *BDNF* Val66Met and childhood maltreatment on adult major depression (adjusted for sex, age and baseline MD)

	Type of childhood maltreatment, OR (95% CI), *z* score, *p* value			
Interaction	Emotional	Physical	Sexual	Any
Gene × environment				
Maltreatment × 5-HTTLPR	1.445 (0.838–2.491), *z* = 1.32, *p* = 0.19	2.744 (1.387–5.428), *z* = 2.90, *p* = 0.004	5.611 (1.511-20.838), *z* = 2.58, *p* = 0.010	2.017 (1.217–3.341), *z* = 2.72, *p* = 0.006
Maltreatment × *BDNF*	1.576 (0.960–2.586), *z* = 1.80, *p* = 0.07	0.842 (0.457–1.550), *z* = –0.55, *p* = 0.58	1.080 (0.386–3.018), *z* = 0.15, *p* = 0.88	1.418 (0.898–2.239), *z* = 1.50, *p* = 0.13
Gene × gene × environment				
Maltreatment × 5-HTTLPR × *BDNF*	1.733 (1.033–2.909), z = 2.08, *p* = 0.037	1.187 (0.625–2.254), z = 0.53, *p* = 0.60	2.249 (1.088–4.647), z = 2.19, *p* = 0.029	1.783 (1.106–2.875), z = 2.37, *p* = 0.018

CI = confidence interval; MD = major depression; OR = odds ratio.

shift to Met allele carriers when important environmental risk factors for depression (e.g., childhood maltreatment) are taken into account.[3] A plausible explanation for these incongruent results could be that BDNF may not be associated with depression itself but rather with the modulation of activity-dependent plasticity within emotional processing networks.[46] How such plasticity produces functional changes or the extent to which it is modulated could rely on genetic and environmental factors that may determine the magnitude and direction of the effect of *BDNF* on mood regulation.

Two-way interactions and risk for MD

Our data provide further evidence supporting the modulating effect of both *SERT* and *BDNF* on the risk for depression conferred by childhood maltreatment experiences.

Concerning 2-way interactions and in agreement with previous reports,[6,7,20] we found that 5-HTTLPR s allele carriers (s/*) who had experienced physical, sexual or any childhood maltreatment were at higher risk for MD than L/L carriers. Moreover, *BDNF* Met allele carriers (Met/*) who had experienced emotional or any kind of childhood abuse were more prone to depression developing in adulthood (although the interaction did not reach significance after adjusting for possible confounders). As mentioned previously, we also explored the putative effect of both the 5-HTTLPR L/S biallelic polymorphism and the 5-HTTLPR l/s triallelic (rs25531) marker. Only 1 previous study has reported partial results for these 2 *SERT* promoter polymorphisms.[14] In accordance with these results,[14] our findings for the triallelic 5-HTTLPR form paralleled those for the biallelic 5-HTTLPR form. Although the genotyping of the rs25531 is supposed to yield an improved characterization of the functional variants at the *SERT* promoter region, our results and those of Grabe and colleagues[14] seem to suggest that the classical way of genotyping this polymorphic region (using the 5-HTTLPR L/S biallelic variant) might be good enough to capture the main functional differences associated with each 5-HTTLPR genotypic combination.

Three-way interactions and risk for MD

We also detected significant 3-way interactions on MD. These effects were especially robust for childhood sexual abuse but were also prominent and significant for emotional and any childhood maltreatment. These results go in the same direction as those reported by Kaufman colleagues[20] in

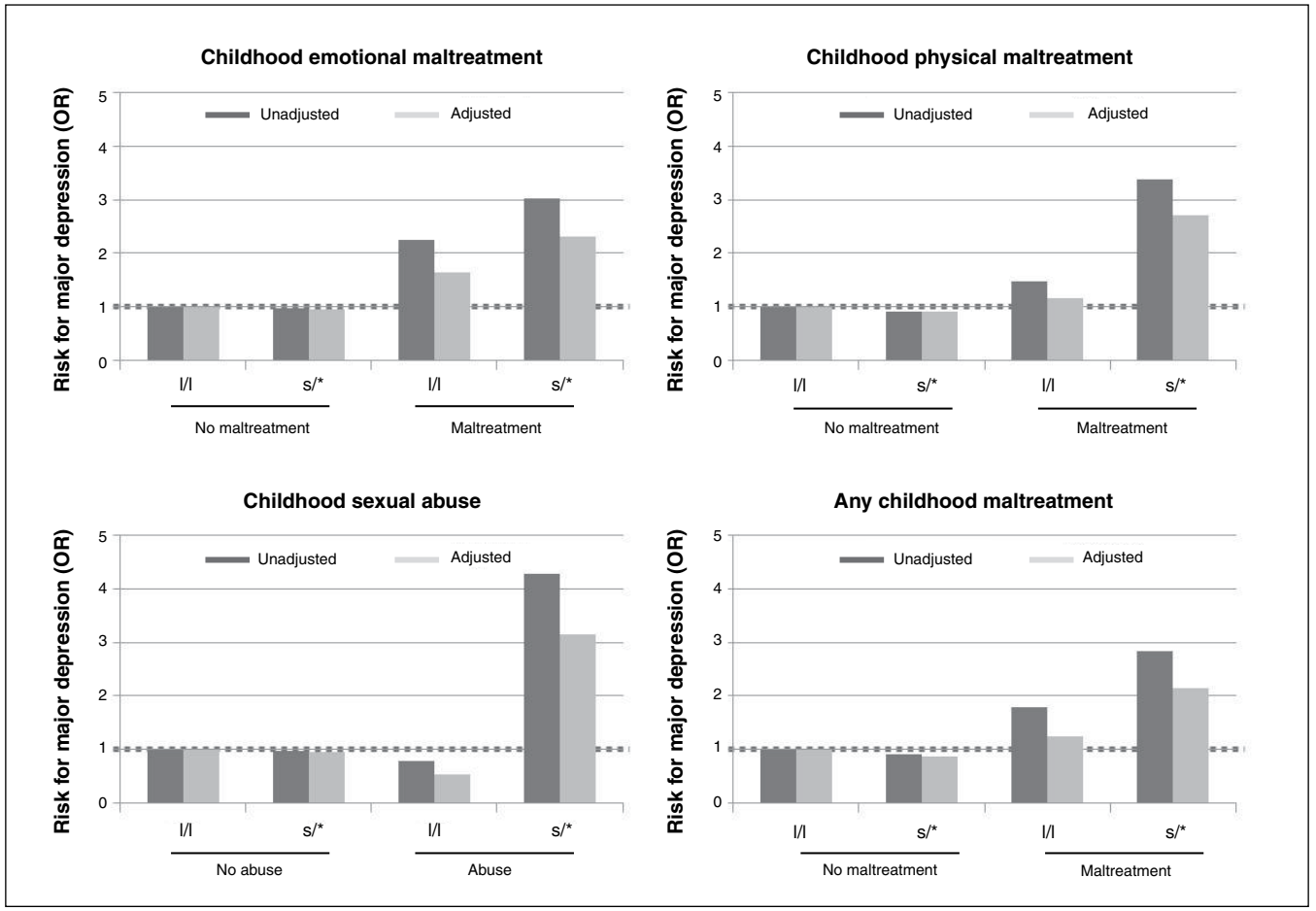

Fig. 1: Risks (odds ratios [ORs]) for major depression conferred by childhood maltreatment and the 5-HTTLPR triallelic polymorphism.

children but in the opposite direction to those described by Grabe and colleagues[14] and Comasco and colleagues,[8] who found that depending on the *BDNF* genotype, the S/S 5-HTTLPR genotype could show either risk (S/S + Val/Val) or protective (S/S + Met/*) properties with regard to depression. These apparently contradictory findings across studies concerning the genetic profile of risk (the Val/Val genotype associated with vulnerability to depression in maltreated individuals according to some authors[8,14] and the Met/* genotype designated as the risk genotype by our study and by Kaufman and colleagues[20]), may be attributable to the heterogeneity across studies in terms of sample type, size, race, study design and reliability of measurements. Inconsistencies in findings may also be explained by the fact that children and adolescent samples would necessarily estimate short-term effects of both genetic profile and childhood maltreatment, whereas adult samples would provide evidence on the longer-term effects of both genetic and environmental exposures. Alternative explanations for such conflicting results in the field are also possible. Functional changes in response to childhood abuse might involve epigenetic mechanisms occurring early in life. Although such epigenetic changes may be enhanced in the context of the putative risk variant, they could also occur in individuals bearing the protective allele under other circumstances.[47] Future studies are needed to understand how these or other kinds of molecular links could apply to the effects of *SERT*, *BDNF* and childhood maltreatment interactions on depression.

Looking for the best explicative model

Unlike previous reports, our study included a detailed comparative analysis between different explanatory models showing a significant association with MD. Our main results suggest that a variety of such different models could explain how *SERT, BDNF* and childhood maltreatment interact with each other to modulate the risk for MD. Consistent with previous studies, the 3-way interactive model was in all cases among the best fitting models explaining our outcome. Nonetheless, given that other explanatory models were also found to be valid (e.g., a 2-way *SERT* × childhood maltreatment interaction model adjusted by *BDNF*), the interpretation of our findings should be taken with caution as they need external replication. Future analyses in independent and well-characterized samples would be welcome to understand the nature of *SERT, BDNF* and childhood maltreatment interactions in depression. In the absence of such new studies, our

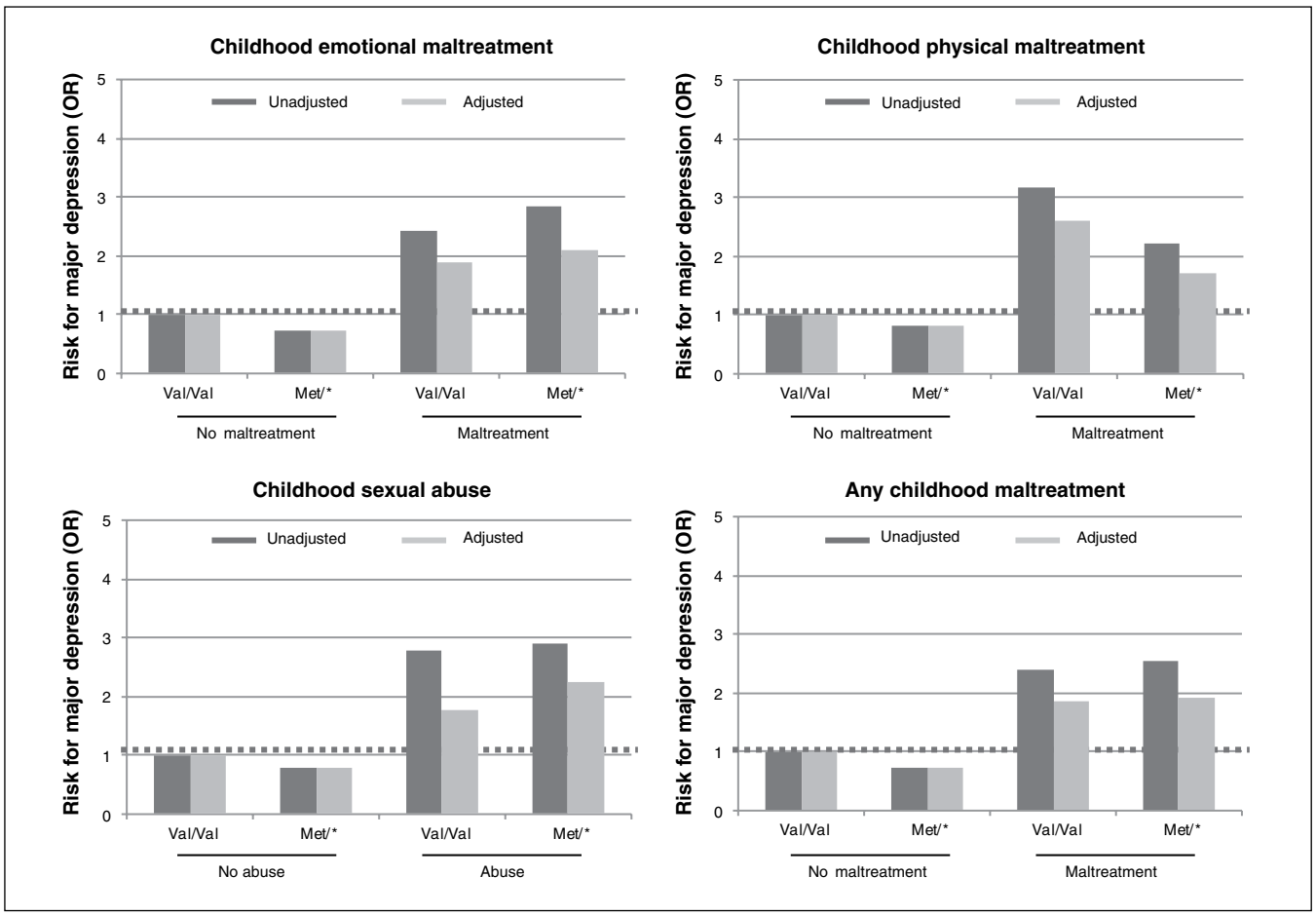

Fig. 2: Risks (odds ratios [ORs]) for major depression conferred by childhood maltreatment and the *BDNF* Val66Met polymorphism.

findings constitute further evidence in support of the notion that these gene × gene × environment interactions increase the risk for depression. Such findings seem not only neurobiologically plausible, but also consistent with prior evidence from human observational, experimental neuroscience and animal model studies.[1]

Limitations

One of the limitations of the present study is the retrospective assessment of childhood maltreatment, which may have resulted in a moderate degree of recall bias, including a potential "effort after meaning" effect. However, maltreatment measures

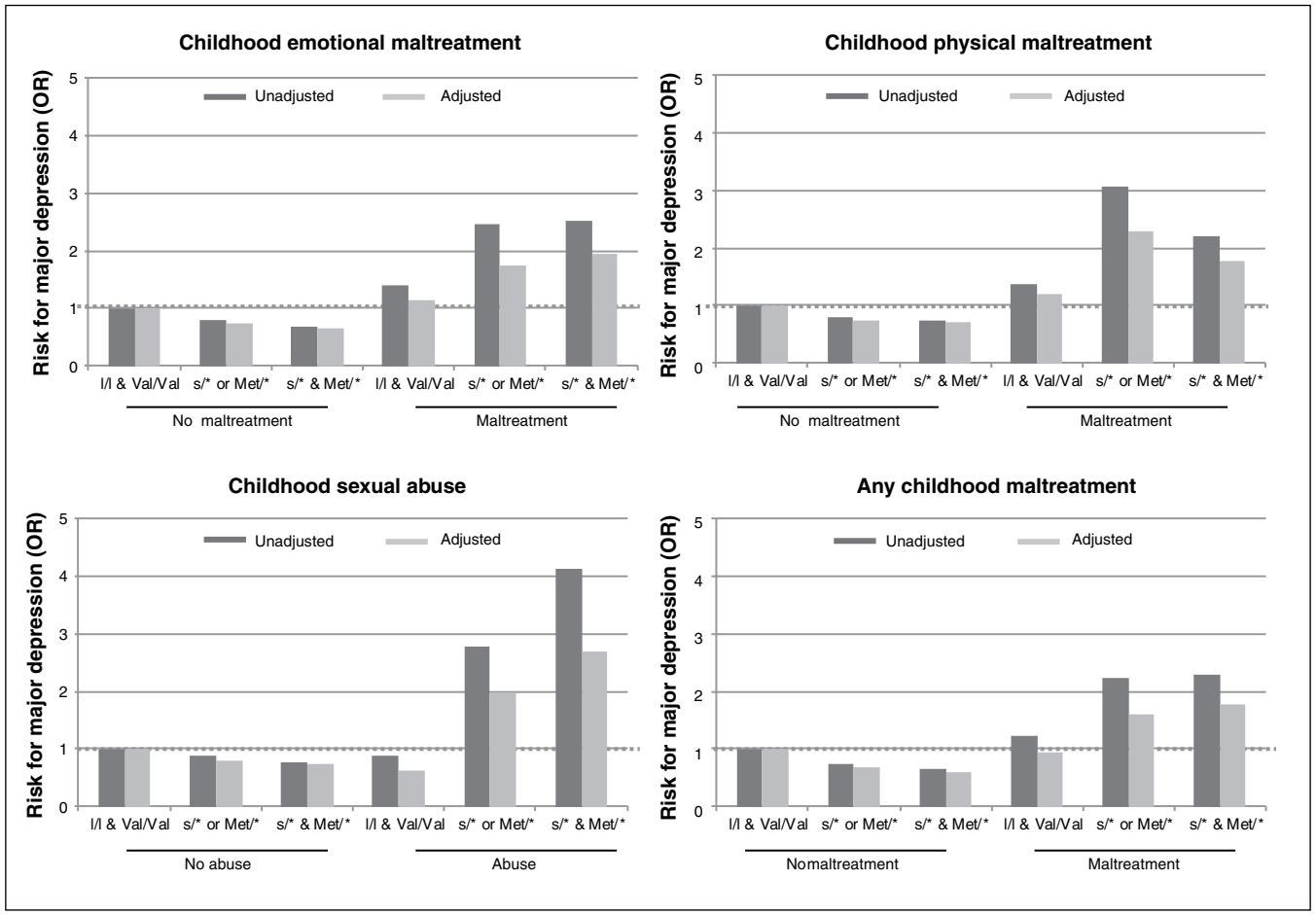

Fig. 3: Risks (odds ratios [ORs]) for major depression conferred by any type of childhood maltreatment according to the genetic profile for both *SERT* 5-HTTLPR triallelic and *BDNF* Val66Met polymorphisms.

Table 4: Overall fit of putative explicative models for major depression

		Emotional		Physical		Sexual		Any	
Model*	df†	AIC	AIC–minAIC‡	AIC	AIC–minAIC‡	AIC	AIC–minAIC‡	AIC	AIC–minAIC‡
A, B, C	7	2701.228	2.438	2700.694	6.717	2735.968	5.711	2700.653	7.813
A × B, C	8	2699.909	1.119	2699.165	5.188	2734.061	3.840	2698.979	6.139
A × C, B	8	2699.796	1.006	2693.977	0	2730.257	0	2693.232	0.392
A, B × C	8	2699.981	1.191	2702.390	7.205	2737.947	5.696	2700.409	7.569
A × B × C	11	2698.790	0	2695.185	1.208	2732.251	1.994	2692.840	0

AIC = Akaike information criteria; df = degrees of freedom; MD = major depression.
*All models include (A) 5-HTTLPR triallelic, (B) *BDNF* Val66Met and (C) childhood maltreatment as risk factors and sex, age and baseline MD as covariates.
†Degrees of freedom are associated with the analysis of every AIC model.
‡AIC-minAIC shows the difference between every AIC with respect to the lowest AIC value in each category. Differences lower than 3.84 (1.96^2) make the model statistically as good as the model with the minAIC value.

were repeatedly assessed at each follow-up point using validated instruments that allowed a thorough phenotypical characterization of the history of childhood maltreatment.

Another potential limitation could emerge from the limited controlling for confounders derived from not including in the models both the covariate × environment and the covariate × gene interaction terms, as recently suggested by Keller.[48] That acknowledged, we decided a priori to follow the most common approach used by most previous studies in the field so that our results were more comparable with previous ones. This more conservative approach is also supported by other authors, who suggest that inclusion of covariables can entail overadjusting and disminish the power to detect real genetic associations.[49]

Conclusion

Our results confirm that the increased risk for major depression conferred by childhood maltreatment is modified by variation at both *SERT* and *BDNF* genes. In particular, individuals who carry both the *SERT* S allele and the *BDNF* Met allele appear to be more vulnerable to the impact of childhood maltreatment on mental health. Our findings strongly support the *SERT*/*BDNF* stress-sensitivity hypothesis for depression.

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