# GENETIC MODULATION OF COGNITIVE AND CLINICAL TRAITS ON WOMEN VICTIMS OF INTIMATE PARTNER VIOLENCE


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Biological Anthropology Master's Degree

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Section of Zoology and Biological Anthropology

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## PREÀMBUL

La violència de parella és una de les formes més comuns de violència contra les dones. Tot i que aquest tipus de violència pot ser exercida tant per homes com per dones, els perpetradors més comuns de violència cap a les dones són les seves parelles o ex-parelles masculines. Contràriament, els homes tenen moltes més probabilitats d'experimentar actes violents per part d'estranys o coneguts que per persones properes a ells. A Catalunya, d'acord amb el Dossier estadístic "Violències Masclistes 2020", un 41.4% de les dones enquestades a Catalunya ha sigut víctima de violència de gènere en l'àmbit de la parella (Institut Català de les Dones, 2020), constituint un problema social molt preocupant i greu degut a les seves conseqüències.

S'ha observat que les víctimes d'aquest tipus de violència poden tenir un major risc de desenvolupar problemes de salut a curt i llarg termini, que afecten tant la salut física (malalties cardiovasculars, diabetis o dolor crònic) com la salut mental (depressió, ansietat o síndrome de estrès post-traumàtic) i alteracions cognitives en els seus nivells d'atenció. Tot i així, el fet de patir violència de parella no implica necessàriament el desenvolupament d'aquests trastorns, ja que la resposta de cada persona pot ser molt diferent de les altres. Diversos factors de diferent índole, incloent factors biològics, semblen jugar un paper en el desenvolupament de resiliència en aquestes dones.

Aquest treball pretén evidenciar els esmentats efectes negatius que poden patir les dones sotmeses a aquest tipus de violència, i alhora investigar si aquestes diferències individuals poden estar modulades per la variabilitat genètica interindividual en determinats gens candidats. D'aquesta manera es vol indagar en els mecanismes biològics que es duen a terme un cop s'ha produït la violència i que donen lloc al desenvolupament dels símptomes clínics i les alteracions cognitives.

La finalitat última d'aquesta recerca és que els resultats obtinguts ajudin a promocionar una major investigació en aquest camp i en aquesta població vulnerable, per així poder, en un futur, millorar la prevenció i el tractament dels símptomes clínics que poden esdevenir en les dones que han patit violència per part de les seves parelles.

**SUMMARY**

**Background:** Intimate partner violence (IPV) is the most common and alarming form of violence against women. The women exposed to physical or psychological IPV have a higher incidence and severity of depressive and anxiety symptoms, post-traumatic stress disorder (PTSD). However, the biological mechanism between IPV and these mental outcomes is still not clear. One of the principal hypotheses that could explain this process involve biased attention to emotional stimuli, but the most recognized approach is the chronic stress model. There are two genes essentially involved in the stress response pathway, which are *FKBP5* and *BDNF* genes.

The present study aimed to investigate: i) the association between the different phenotypical variables (cognitive and clinical traits) ii) the impact of IPV on phenotypical variables, iii) to study the variability of the candidate genes (i.e., *FKBP5* and *BDNF*) and its association with the above-mentioned phenotypic variables, and iii) to analyse the modulating role of *FKBP5* and *BDNF* on the association between IPV and phenotypical variables.

**Methods:** IPV, Attention Bias Variability, General Attention Ability, and depressive, anxious and PTSD symptoms were assessed in 105 women. The SNPs genotyped were the rs1360780 located in the *FKBP5* and the rs6265 located in the *BDNF*. Main effects and interactions were studied using correlations and analysis of covariance (ANCOVA).

**Results:** All IPV types were associated with the clinical traits, but no with the cognitive traits. None of the analysed traits were associated with the *BDNF*-rs6265 polymorphism. Depressive symptoms were associated with the *FKBP5*-rs1360780 with CC genotype as risk factor. No gene-environment interaction was found.

**Conclusions:** The result support the role of IPV as a risk factor for developing depression, anxiety and PTSD symptoms. Further investigation is needed regarding the modulating role of *FKBP5* and *BDNF* genes in the association between IPV and cognitive and clinical symptomatology.

My contribution to this work

I participated in the DNA extraction of the sample, the DNA quantification, and the preparation of the plates for the genotyping. I genotyped the proposed SNPs (rs1360780 and rs6265) using TaqMan technology. I designed the databases and performed all the association analyses presented here.

**ACKNOWLEDGEMENTS**

The present work was supported by the Parc Taulí Foundation, the research branch of the Parc Taulí Healthcare Corporation (Corporació Sanitària Parc Taulí) as part of a larger research.

I would like to thank all the people from the Section of Zoology and Biological Anthropology (UB) who have helped me during the development of this study, especially to Natalia Azcona for her dedication and kindness. I would also like to express my sincere gratitude to Nora Peña Lozano, Dr. Araceli Rosa and Dr. Ximena Goldberg, for their support and guidance throughout the course of this project.

I am extremely grateful to all the women who have voluntarily participated in this study for their generosity in providing the samples and completing all the questionnaires, helping other women in their same situation in the future.

I would also like to thank my family and friends for their unconditional support during these years. Especially, I would wish to thank my partner, Juanma Morales, for his big heart and honesty, and my grandma, Carmen Henarejos, for her courage and resilience.

Finally, I would like to express my gratitude to all those people, men and women, who strive every day to make the world a more peaceful place.

**CONTENTS**

## 1. INTRODUCTION

**Intimate partner violence (IPV)** is the most common and alarming form of violence against women. According to the World Health Organization (WHO), IPV refers to any behaviour within an intimate relationship that causes physical, psychological, or sexual harm to those in the relationship. Such behaviour includes acts of **physical violence** (such as slapping, hitting, kicking and beating), **sexual violence** (including forced sexual intercourse and other forms of sexual coercion), **emotional or psychological abuse** (such as insults, belittling, constant humiliation, intimidation, threats of harm, threats to take away children), and **controlling behaviours** (including isolating a person from family and friends, monitoring their movements and restricting access to financial resources, employment, education or medical care) (Krug et al., 2002).

IPV is considered a **global public health problem** due to its high prevalence. It is estimated that the worldwide prevalence of physical and / or sexual intimate partner violence among all women who have ever had a partner is 30% (Garcia-Moreno et al., 2013). These results are not very distant from those obtained in Spain where, of the total number of women aged 16 and over living in Spain who have had a partner, 43.4% have suffered at least one type of violence from their current partner or previous partners (Ministerio de Igualdad, 2019). According to data published in the latest macro-survey in 2019, 41.4% of women surveyed in Catalonia have been victims of gender-based violence in the sphere of couples (Catalan Women's Institute, 2020).

Both physical and psychological IPV are associated with significant **physical and mental health consequences of the victims**. There is solid evidence that confirms that experiencing IPV is associated with increased risk of current poor health, depressive symptoms, substance abuse, developing a chronic disease (as diabetes, chronic pain, asthma, or cardiovascular disease), chronic mental illness and injury (Breiding et al., 2005) (Coker et al., 2002). Furthermore, women exposed to physical or psychological IPV have a higher incidence and severity of **depressive and anxiety symptoms, post-traumatic stress disorder (PTSD)**, and thoughts of suicide (Pico et al., 2006).

Therefore, a relationship between IPV and development of mental disorders is observed, although the biological mechanism is not yet clear. One of the principal hypotheses that could explain this process involve biased attention to emotional stimuli (Romens & Pollak, 2012). **Attention bias** is a type of cognitive bias that consist in the fact of manifest a tendency to pay more attention to emotionally salient stimuli (e.g., threatening images), than neutral ones. This attention bias has been shown in people who suffered from childhood maltreatment or stressful experiences (Bodenschatz et al., 2019) and has

been associated with a variety of mental illnesses including depression, anxiety and PTSD among others (Mennen et al., 2019, Bar-Haim et al., 2007 and Fani et al, 2012)

It has been observed that the attention bias can be produced both **towards** and **away from threatening stimuli**. In the first case, this symptom is known as hypervigilance and, in the second case, it is known as avoidance (Iacoviello et al., 2014). **Attention bias variability (ABV)** positively correlates with PTSD severity and can contribute to initiate and maintain this disorder, as demonstrated by some studies that states PTSD patients exhibit greater attention bias variability than control subjects (Naim et al., 2015).

The predisposition for developing mental diseases seems to be given by the **environment (E)** at which a person is exposed but also by the combination of **genetic variants (G)** that this person carries and the effect of their **interaction (GxE)**. GxE approaches explore how genetic risk variants in combination with environmental factors such as intimate violence partner could modulate the risk for developing the disorder *(Figure 1)*. In most of the cases, GxE studies have relied on **candidate gene** approaches examining the involvement of these genes as moderators of environmental factors due to their functional impact on relevant individual differences involved in biological stress-regulation systems and neurodevelopmental and neuroplasticity processes (Halldorsdottir & Binder, 2017). These candidate genes have a minor effect on the phenotype and the set of multiple susceptibility genes can contribute to the development of mental disorders. Generally, **single nucleotide polymorphisms (SNP)** and haplotypes (i.e., combination of SNPs) are used to analyse the association of candidate genes with the phenotype. Suitable candidate genes are those that encode proteins with a putative role in neurobiological pathways involved in attention bias, depression, anxiety, or post-traumatic stress disorder.

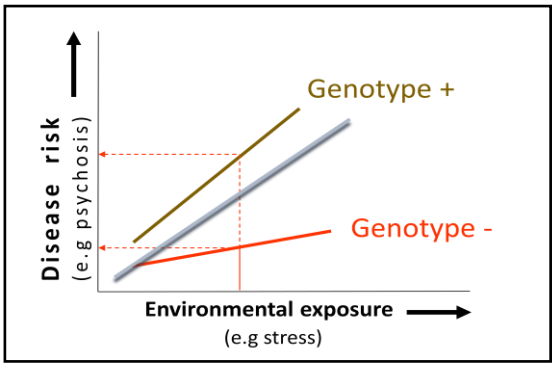



***Figure 1***. Gene-environment interaction. The effect of an environmental risk factor for a disease is modulated by the genotype. Then, with the same exposition to an environmental factor, one inividual carrying the genotype of risk (genotype +) would have higher risk than one with the non-risk genotype (genotype -).

The **chronic stress model** is the most recognized approach because exposure to IPV occurs repeatedly and can last for years (Garcia-Moreno et al., 2006). The typical neurobiological response to stressful stimuli can be activated due to IPV exposure. This response includes the activation of the **sympathetic-adrenomedullary (SAM)** and **hypothalamic-pituitary-adrenal (HPA) axis** (Chrousos & Gold, 1992), with the subsequent release of catecholamines and cortisol, respectively *(Figure 2)*. If the acute stress response is frequent and / or sustained for a long time, it can lead to pathophysiology and psychiatric illness (Chrousos, 2009). Due to this, we can define IPV as a form of chronic exposure to severe stress that alters the stress response system of exposed women, affecting their ability to cope with future everyday situations. Even so, response to stress can be very different between individuals. Knowing the cause of these differences at the molecular level is of great importance to create prevention strategies and individualized treatments against stress-related disorders.

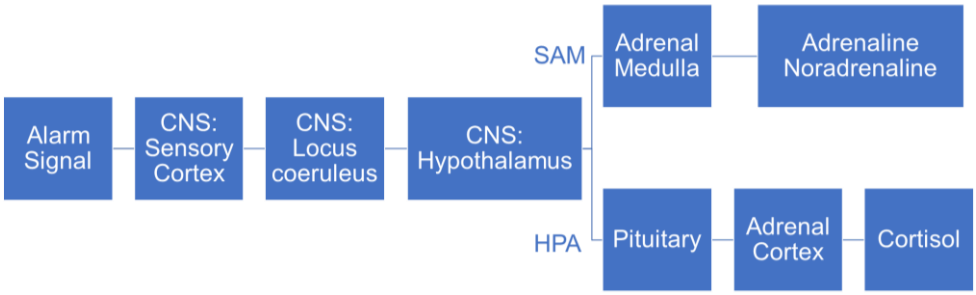



***Figure 2***. Conceptual map of the physiological response to stress. Implication of the sympathetic-adrenal-medullary (SAM) and hypothalamic-pituitary-adrenocortical (HPA) axis in the physiological response to acute stress from the activation of the central nervous system (CNS) in the event of a danger signal or alarm.

Two genes essentially involved in the stress response pathway, which could play a role in determining these differences between individuals, are *FKBP5* and *BDNF* genes, which encode for two proteins with the same names.

The *FKBP5* gene codifies for the **FK506-binding protein 5 (FKBP5)** which is an important modulator of stress responses. FKBP5 acts as a chaperone in response to stressors. Among the multitude of cellular processes that it modulates, it can regulate the activity of the glucocorticoid receptor. For its part, the regulation of the expression of the *FKBP5* gene occurs through complex interactions between environmental stressors, genetic variants of *FKBP5* and epigenetic modifications of glucocorticoid-sensitive genomic sites (*Annex 1*). In this regard, individuals carrying the T allele in the functional polymorphism rs1360780 of the *FKBP5*, that have been exposed to child abuse showed lower methylation of CpG sites located near intron 7 of the *FKBP5* gene than those individuals with the C allele. Furthermore, decreased methylation in this region is

associated with increased transcription and contributes to several aberrant phenotypes such as the risk of stress-related disorders (Zannas et al., 2016).

Regarding the *BDNF* gene, it encodes the protein **Brain-derived neurotrophic factor (BDNF)** which acts as a growth factor. BDNF binds and activates the receptor tyrosine kinase (TrkB) promoting the survival of motor neurons and the hippocampus. In addition, it has an important role in the physiological processes underlying the plasticity and development of the nervous system (Jin et al., 2019) (*Annex 2*). The *BDNF* gene contains the rs6265 polymorphism, also called Val66Met, where if a cytosine is present in position 66, the resulting codon will be translated into a valine amino acid (Val allele). In contrast, if a nucleotide change from valine to thymine occurs, the resulting amino acid will be a methionine (Met allele). Previous studies have shown that life stressful events and childhood adversity separately interacted with the Met allele of the *BDNF* Val66Met polymorphism in depression (Zhao et al., 2018). Likewise, it has been observed that individuals with at least one Met allele exhibited even higher ABV when childhood emotional abuse was present (Hori et al, 2021).

Therefore, stress produced by intimate partner violence can affect attention and the development of mental illnesses (anxiety, depression, and post-traumatic stress) and this association could be modulated by the genetic variability of two genes, *FKBP5* (involved in the stress response pathway) and *BDNF* (involved in plasticity and nervous system development). However, no previous research has studied in depth these possible associations in the area of intimate partner violence. This study aims to delve into this field with the intention of improving the understanding of the underlying biological mechanisms due to this type of violence, so that in the future the most appropriate treatments and resources can be implemented to prevent and treat the possible clinical consequences that these women may suffer.

## 2. OBJECTIVES AND HYPOTHESIS

### 2.1. General objective

The aim of this project was to test if polymorphic variants of the *FKBP5* and *BDNF* genes can interact during adulthood with an experience of IPV giving an alteration of threat-related attention bias and an increased risk of developing depression, post-traumatic stress disorder and anxiety symptoms. In order to achieve this, a sample of 105 women was used to analyse: i) the association between the different phenotypical variables, classified in cognitive trails (attention bias variability and general attention ability) and clinical traits (depressive, anxious and PTSD symptoms), ii) the impact of the assessed environmental risk variable (i.e., intimate partner violence) on the selected phenotypic variables, iii) the association between genetic variability of the candidate genes *FKBP5* and *BDNF* and the phenotypic variables and iv) the modulating role of the variability of the *FKBP5* and *BDNF* genes on the association between the phenotypical variables and the environmental risk variable.

### 2.2. Specific objectives

- To describe the scores for the intimate partner violence scale evaluated.
- To describe the scores for the cognitive trails evaluated in the analysed sample.
- To describe the scores for the scales assessing post-traumatic stress disorder (PTSD), depressive and anxiety symptoms in the participants.
- To genotype the polymorphisms rs1360780 and rs6265 in the present sample located in the candidate genes *FKBP5* and *BDNF*, respectively.
- To study the correlations between the different phenotypical variables (cognitive and clinical traits).
- To investigate the relationships between IPV and phenotypical variables (both cognitive and clinical).
- To investigate the relationships between the *FKBP5* and *BDNF* genotypes and phenotypical variables (both cognitive and clinical).
- To analyse the modulation role of the *FKBP5* and *BDNF* genotypes on the association of IPV with phenotypical variables (both cognitive and clinical).

### 2.3. Hypothesis

Intimate partner violence has been reported as a risk factor for major depressive, post-traumatic stress, and anxiety disorders, as well as alterations in the threat-related attention bias. According to that, we hypothesize that there are correlations between

Attention Bias Variability and clinical traits (depression, anxiety, and post-traumatic stress disorder).

Based on this, we also hypothesize that there are correlations between the different types of intimate partner violence and all the phenotypical variables (both cognitive and clinical).

Furthermore, genes involved in the HPA axis regulation, neuroplasticity and neurodevelopment, such as the *FKBP5* and *BDNF* genes are plausible candidates for post-traumatic stress disorder, depression, and anxiety phenotypes. Accordingly, we expect those women carrying the risk-associated genotype of the *FKBP5*-rs1360780 polymorphism (i.e., TT genotype) and *BDNF*-rs6265 (i.e., Met/Met genotype) will present higher scores for Attention Bias Variability and post-traumatic stress disorder, depressive and anxiety symptomatology scales.

Finally, we hypothesized that cognitive and psychologic symptoms are mediated by the association between intimate partner violence and the genotype of *FKBP5* and *BDNF*, adjusting to the age and educational level of the participants.

## 3. MATERIAL AND METHODS

### 3.1. Participants

The participants were women from the general population of the Barcelona area who participated in the BRAW study about "*Adaptability to acute stress among women survivors of intimate partner violence*" (Goldberg et al., 2020). Women were recruited through advertisements in the community and in social media. The interviews took place in the facilities of Parc Taulí Foundation, the research branch of the Parc Taulí Healthcare Corporation (Corporació Sanitària Parc Taulí, CSPT). Participants did not receive monetary compensation for their participation in the research.

**Inclusion criteria** of women in the study were mainly guided by their previous exposure to IPV, which defined two groups of participants: i) an IPV-exposed group with a sample size of 69 women and ii) a non-exposed IPV group with an estimated sample size of 36 women. The definition of exposure to IPV followed the WHO guidelines mentioned above. To warrant chronic exposure to stress as proposed in the rationale of the study, the minimum time of duration of the violent relationship was set at 1 year. Also, to study the long-term effects of IPV once the exposure has ceased, only women who had already ended the violent relationship for at least 1 year were included.

**Exclusion criteria** were as following: age below 21 (to allow a margin of accumulated relationship experience during adulthood) and over 50 (excluding menopause), having any pituitary and/or adrenal gland disorder, currently using steroid-based medications, being currently pregnant, lactating or menopausal and having a severe illness that may affect cognitive performance and/or consciousness. No participant was excluded based on disability, ethnicity, religion or sexual orientation.

All participants volunteered to participate in the study and gave their written informed consent in the evaluations. Some women were excluded for not completing or randomly answering the psychometric questionnaires. The total sample consisted of 105 women, of which 69 belong to the group that has suffered IPV and 36 belong to the group that has not suffered IPV, with a mean age of 35.391 (SD = 7.258, range = 22 – 50) and 32.278 years (SD = 7.7923, range = 21 – 46), respectively. Ethical approval was obtained from local research ethics committees.

### 3.2. Intimate partner violence assessment

The history of IPV lifetime was assessed with the Spanish version of the **Partner Violence Screen** (3 items) (Feldhaus et al., 1997; Garcia-Esteve et al., 2011). Whenever the woman responded “yes” to any of the three questions, the In-Depth IPV Questionnaire was administered (*Annex 3*).

The **In-Depth IPV questionnaire** is based on an adaptation of the World Health Organization Violence Against Women Instrument (VAWI) (Garcia-Moreno et al., 2005). The questionnaire was translated during the original study prior to evaluation to ensure cross-cultural comparability between countries and consists of a detailed description of IPV exposure including onset, frequency, and time since last exposure. The questionnaire is structured on 6 yes / no questions that evaluate the **control** IPV and a set of questions to evaluate the frequency (always, sometimes, many times) of the **physical** (3 questions), **emotional** (3 questions) and **sexual** (3 questions) IPV (*Annex 4*). In the present study, the four types of intimate partner violence were used for analyses, as well as an overall sum score including all items (i.e., **total intimate partner violence**).

### 3.3. Assessment of depression, anxiety, and PTSD in the sample

The Spanish adaptation of **General Health Questionnaire, 12 items version (GHQ-12)** was used to screen for current mental health disorder (Goldberg et al., 1997; Sánchez-López et al., 2008). Also, a direct question regarding current psychological and psychiatric treatment was used. When a participant presented a score of 3 or higher, or responded “yes” to the question regarding current

treatment, an in depth **Mini International Neuropsychiatric Interview (M.I.N.I.)** was administered to confirm a diagnosis (Sheehan et al., 1998; Ferrando et al., 2000).

The Spanish version of **Patient Health Questionnaire (PHQ-9)** was used, which consists of 9 items that assess the presence of **depressive symptoms** (corresponding to the DSM-IV criteria) present in the last 2 weeks (Kroenke et al., 2001; Diez-Quevedo et al, 2001). Each item has a severity index corresponding to: 0 = "never", 1 = "some days", 2 = "more than half of the days" and 3 = "almost every day", being therefore the minimum and maximum possible scores of 0 and 27, respectively (*Annex 5*).

The Spanish version of **Generalized Anxiety Disorder (GAD-7)** was used to explore **anxiety symptoms** (Spitzer et al., 2006; García-Campayo et al, 2010). This questionnaire consists of 7 items with a severity index corresponding to: 0 = "never", 1 = "some days", 2 = "more than half of the days" and 3 = "almost every day", being therefore the minimum and maximum possible scores of 0 and 21, respectively (*Annex 6*).

The Spanish version of **Post-traumatic Symptom Scale-Interview Version for DSM-5 (PSS-I-5)** was used (Foa et al., 2016). It is a 24-item semi-structured interview that assesses **Post-Traumatic Stress Disorder (PTSD) symptoms** in the past month and makes a diagnostic determination based upon DSM-5 criteria. Questions assess for frequency and intensity of 20 DSM-5 PTSD symptoms. These symptom items are rated on a 5-point scale of frequency and severity ranging from 0 (Not at all) to 4 (6 or more times a week / severe) (*Annex 7*). The sum of the 20 PTSD symptoms items yields a total PTSD symptom severity score, ranging from 0-80. An additional four items ask about distress and interference caused by PTSD symptoms as well as onset and duration of symptoms.

### 3.4. Assessment of cognition

The **dot probe task** was used to explore **attention bias in relation to threat (ABNT)**. Participants were presented with a pair of stimuli simultaneously, one emotionally salient and one neutral for 500 ms time, followed by a probe that replaces one of the two stimuli. Participants were required to respond as accurately and as quickly as possible to the probe. **Reaction times (RT)** were recorded (**threatRTmeanNT** and **neutralRTmeanNT**, respectively), and the differences in speed of responding to probe stimuli occurring in a location previously occupied by a negative stimulus, relative to locations previously occupied by neutral or positive stimuli, were contrasted (neutralRTmeanNT – threatRTmeanNT = ABNT). A decreased reaction time to probe replacing emotional stimuli compared to the neutral stimuli provide a measure of bias to be vigilant for

negative information. Positive values reflect attention toward the negative stimulus, and negative values reflect attention away from the negative stimulus. This visual probe task has shown evidence for selective processing of threat across all the major anxiety disorders as well as in nonclinical groups (MacLeod et al., 1986).

Nowadays, **attention bias variability (ABV)**, a novel index of attention bias, is used to better capture trauma-related attentional dysfunction (Alon et al, 2019). To calculate ABV, all trials were split into eight sequential bins, and attention bias scores (ABNT) were calculated for each bin. The Standard Deviation (SD) of attention bias scores across bins was calculated and divided by mean RT to correct for variance in RTs. Thus, ABV was calculated using the following equation, with greater values reflecting the instability of attention bias:

$$SD_{ABNT} = \sqrt{\frac{\sum_{i=1}^{8}(ABNT_i - \overline{ABNT})^2}{n-1}}$$

$$ABV = \frac{SD_{ABNT}}{\overline{RT}}$$

where:

*i* indicates the bin number,

*n* indicates the total number of bins (i.e., “8”),

*ABNT* indicates attention bias scores

*RT* indicates reaction time.

Although both ABNT and ABV are used to measure attention bias, ABV is a better index to capture this dysfunction. For this reason, it was determined to use only the ABV as a measure of attention bias to study the relationships with the other variables.

**General attention ability** was also examined, as it may affect attention / ABV bias. For this, the **WISC-IV digits test** was used. This test essentially measures short-term auditory memory, the ability to follow a sequence, and therefore attention and concentration. There are two tasks to perform: **direct and reverse digits**. In the first, a series of digits are said with an interval of one second between them and the participant must repeat them afterwards. In the reverse digits part, the participant is asked to repeat them but in reverse order, from back to front. It starts with two digits and increases one more digit until two consecutive faults occur.

### 3.5. Laboratory procedures

#### 3.5.1. DNA extraction

DNA was extracted from blood samples in the laboratory of the *Secció de Zoologia i Antropologia Biològica* (*Facultat de Biologia*, UB) using the **HigherPurityTM Blood and Cell Culture DNA Isolation Kit** from Canvax Biotech (AN0045) following Protocol A: DNA purification from Blood (250 µL).

A traditional DNA extraction protocol for peripheral blood samples included: 1) Cell lysis with an anionic detergent and proteinase K to solubilize the cell components and to digest the cell surface, respectively; 2) RNase treatment to remove contaminant RNA; 3) Protein precipitation to remove cytoplasmic proteins; 4) DNA precipitation with ethanol and isopropanol and 5) DNA hydration with sterile water. In the case of **silica membrane kits**, the binding solution having a specific pH is added to the lysis mixture. Before passing the lysis solution through the column, ethanol is added to the solution, removing the moisturizing layer of the DNA and exposing its phosphate groups, thereby facilitating the adsorption of the molecule to the positively charged membrane. The lipids and proteins are not related to the membrane and are removed with the help of the washing solution and a centrifugation cycle, while the genetic material remains bound to the matrix. The membrane and DNA are dehydrated with washing solutions and centrifugation cycles, then it is recommended to centrifuge the column again to evaporate the ethanol and remove excess solutions. Subsequently, water or buffer solution is added to the middle of the membrane, it is waited for the DNA to hydrate, it is centrifuged to recover it from the matrix and resuspend it (*Figure 3*). See *Annex 8* for further details on the protocols.

The remaining blood samples were stored at -20ºC.

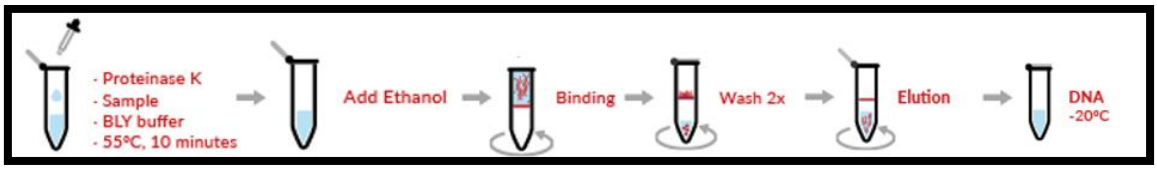



***Figure 3.*** Steps followed in the DNA extraction protocol

### 3.5.2. DNA quantification

The concentration of the extracted DNA was measured using **Qubit™ Fluorometric Quantification**. The Qubit fluorometer detects fluorescent dyes that are specific to the target of interest. These fluorescent dyes emit only when bound to the target molecules, even at low concentrations. Another type of spectrophotometers, like Nanodrop, use a different method for measuring the concentration of nucleic acids and protein, the UV-absorbance method, which measures the natural absorbance of light at 260 nm (for DNA and RNA) or 280 nm (for proteins). As so many molecules absorb light at 260 nm, this measurement is subject to inaccuracy due to potential contamination of the sample with these other molecules and is unable to distinguish between DNA, RNA, protein or free nucleotides or amino acids in the sample. Conversely, Qubit™ system is supplied with fluorescent dyes that bind specifically to analytes of interest such as double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), RNA, miRNA or protein providing more

accurate quantification. The main steps followed for using Qubit™ Fluorometric Quantification are illustrated in *Figure 4*.

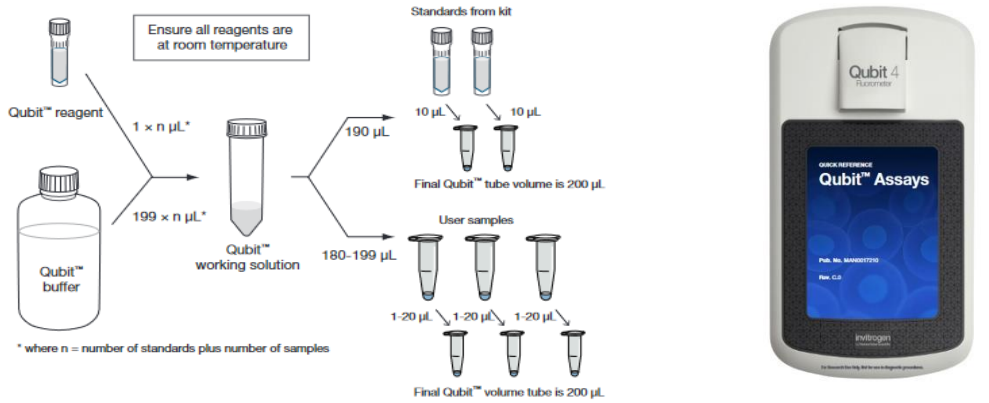



***Figure 4***. Main steps for using Qubit Fluorometric Quantification (Adapted from Thermo Fisher Scientific).

### 3.5.3. SNP genotyping

The single-nucleotide polymorphisms (SNP) genotyped in the present study were rs1360780 and rs6265 which are located in the candidate genes *FKBP5* and *BDNF* respectively. See *Table 1* for details on the analysed SNPs.

***Table 1***. *Descriptive data on the selected SNPs (reference sequence [rs], genotyping assay used, chromosome, location, alleles and Minor Allele Frequency (MAF)*

| Gene | SNP ID | Assays ID | Chromosome[1] | Location | Alleles[2] | MAF[3] |
|---|---|---|---|---|---|---|
| ***FKBP5*** | rs1360780 | C___8852038_10 | 6p21.31 | 35639794 | C/T | T=0.31 |
| ***BDNF*** | rs6265 | C__11592758_10 | 11p14.1 | 27658369 | C-Val/T-Met | T=0.20 |

[1] Data obtained from OMIM, NCBI.
[2] The less frequent allele (minor allele) is placed second.
[3] Data obtained from 1000 Genomes Project EUR population.

Genotyping was performed using the TaqMan 5'- exonuclease assay (Applied Biosystems, Foster City, CA) at the *Servei de Genòmica* of the *Universitat de Barcelona* (CCiT, http://www.ccit.ub.edu). This technology requires working with homogenized concentrations of 5 ng/µL, due to that the samples were aliquoted in 96 well plates at this concentration. The remaining DNA extractions and plates were stored at -20ºC.

Assays were run in a 384 well plate on a **QuantStudio™ 7 Pro Fast Real-Time PCR System** (ABI QuantStudio 7 Pro instrument) using standard conditions. The final volume

of the PCR was 5 µL, which contained 5 ng of genomic DNA, 2.5 µL of TaqMan Master Mix and 0.05 µL of 40x genotyping assay (C___8852038_10 and C__11592758_10). Genotype data were analysed using **QuantStudio™ Design & Analysis Software v2.5.0** (Applied Biosystems, Foster City, CA).

The **TaqMan 5'- exonuclease assay** allows the identification of genotypes using two primers (forward and reverse) that flank the SNP of interest and two allele-specific probes. Each probe is labelled with a different fluorescent reporter dye at the 5' end (VIC for one allele and FAM for the other one) and has a quencher attached to the 3' end to enable allelic discrimination.

As illustrated in *Figure 5*, when the reporter dye and the quencher are attached to the probe, fluorescence emission is quenched by proximity. However, during each extension cycle, the DNA polymerase cleaves the reporter dye from the probe, which separates it from the quencher and enables it to emit its characteristic fluorescence. Note that the 5'-exonuclease activity of the DNA polymerase only cleaves the hybridized probes.

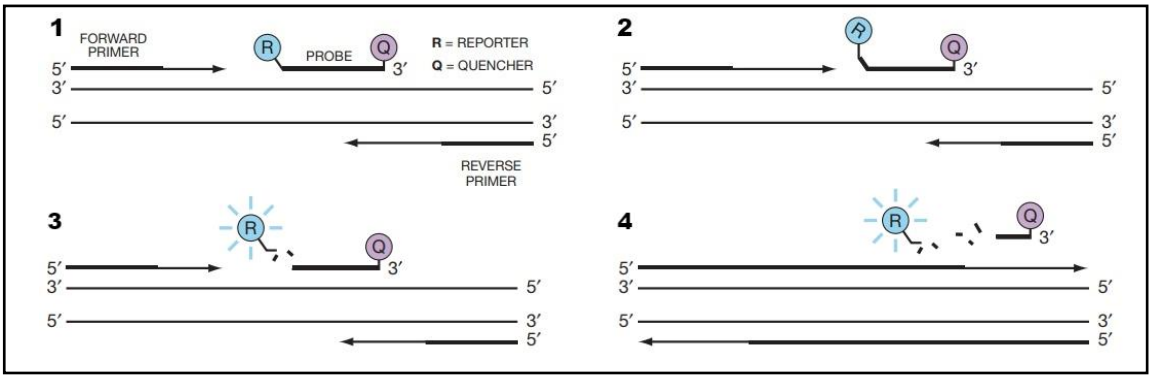
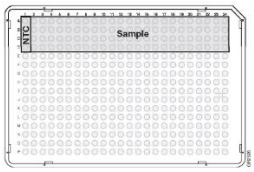



***Figure 5***. TaqMan probe-based chemistry. 1) Polymerization; 2) Strand displacement; 3) Cleavage; 4) Polymerization completed. Adapted from Applied Biosystems.

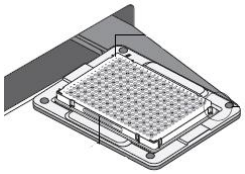
The fluorescence emitted will be different depending on the allele present. Therefore, an increase in just one fluorescence signal will indicate homozygosity whereas an increase in both fluorescence signals will indicate heterozygosity.

TaqMan assays were run by performing a file with: 1) an amplification run using a Standard Curve to generate real-time PCR data and 2) an allelic discrimination run using an Allelic Discrimination in which the QuantStudio™ Design & Analysis Software used the fluorescence measures made during the amplification run to assign allele calls. In case of any doubtful call, real-time PCR data could then be reanalysed to assign allele calls manually. The complete process is illustrated in *Figure 6*.

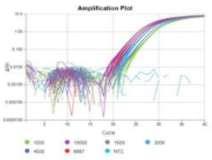



**1**. To prepare the samples and reaction plate.

**2**. To open a Standard Curve plate template and edit properties and method.

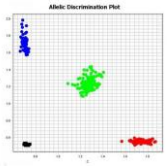


**3**. To place the prepared reaction plate into the instrument tray and perform the amplification run.




**4**. Results of the real-time PCR. Amplification plot.

**5**. To assign markers to the wells to perform the allelic discrimination post-read run.




**6**. Results of the allelic discrimination run are displayed on a scatterplot.

***Figure 6***. Main steps to run a TaqMan assay. Adapted from Applied Biosystems.

### 3.6. Statistical analyses

**Hardy-Weinberg equilibrium (HWE)** for genotypic distribution was assessed using an on-line Chi-squared HWE test calculator for biallelic markers (http://www.oege.org/software/hwe-mr-calc.shtml; Rodriguez et al., 2009). This principle states that genotypic and allelic frequencies in a population will remain constant or *in equilibrium* from one generation to the next in the absence of disturbing factors. Therefore, compliance with HWE would discard genotyping errors.

All data were processed using IBM SPSS Statistics Subscription (Compilation number 1.0.0.1447). **Chi-square test ($\chi^2$)** was used to compare allelic frequencies of European reference populations (1000 Genomes Project, www.1000genomes.org) with those observed in the present sample. We also compared the genotypic frequencies between groups by means of this test. **Descriptive statistics** were used to explore the distribution of all the variables in the total sample and between groups.

The **Kolmogorov-Smirnov (D) normality test** was performed to examine whether continuous variables (i.e., age, PTSD, depressive and anxious symptomatology scores, attention bias variability, general attention ability and IPV scores) followed a normal distribution. Normality tests will inform the decision on these of parametric or non-parametric testing. Comparisons between medians of non-normal continuous variables between groups were explored by means of the **non-parametric Mann–Whitney U test.**

The associations between IPV, attention bias variability, general attention ability, and mental health variables were explored using **correlation analysis**. The relationships

between genotypic background, attention bias variability, general attention ability, clinical traits and IPV scores were examined to explore trends of the associations between the variables. **Analysis of covariance (ANCOVA)** was used to explore **interaction effects**. In these analyses, attention bias variability, general attention ability or symptoms of depression/anxiety/PTSD was used as dependent variable and IPV scores, genotype, and the interaction between them served as the independent variables or moderators. All analyses were adjusted by age and educational level as possible confounding factors.

## 4. RESULTS

### 4.1. Descriptive of the demographic characteristics, phenotypic variables (both clinical and cognitive) and environmental risk variables.

Participants, all women, had an average age of mid-thirty and had completed a bachelor's degree. The demographic and phenotypic (both psychological and cognitive) characteristics of the sample are shown in *Table 1*. The educational level, the symptoms of depression, anxiety and post-traumatic stress disorder showed significant differences between the group of participants who suffered IPV compared to the control group, as examined by the Mann–Whitney U test (all $P < 0.05$).

***Table 1.*** Demographic, clinical, and cognitive characteristics of the sample.

| | **Total (N = 105)** Mean ± SD (Range) | **IPV group (N=69)** Mean ± SD (Range) | **NO-IPV group (N=36)** Mean ± SD (Range) | **Statistic (p-value)** |
|---|---|---|---|---|
| **Demographic characteristics** | | | | |
| **Age** | 34.32 ± 7.79 (21-50) | 35.39 ± 7.63 (22-50) | 32.28 ± 7.79 (21-46) | 1530.50 (0.05) |
| **Education Level** | 6.25 ± 1.21 (3-8) | 6.043 ± 1.30 (3-8) | 6.64 ± 0.93 (5-8) | 941.50* (0.04) |
| **Clinical characteristics** | | | | |
| **Depression (PHQ-9)** | 5.83 ± 5.02 (0-18) | 6.91 ± 4.89 (0-18) | 3.78 ± 4.65 (0-18) | 1789* (< 0.01) |
| **Anxiety (GAD-7)** | 6.28 ± 4.73 0-19 | 7.25 ± 4.85 (0-18) | 4.44 ± 3.95 (0-19) | 1664.50* (<0.01) |
| **PTSD (PSSI-5)** | 8.10 ± 10.35 (0-42) | 11.19 ± 11.10 (0-42) | 2.25 ± 5.07 (0-20) | 1849.50* (< 0.01) |
| **Cognitive characteristics** | | | | |
| **ABV** | 0.05 ± 0.02 (0.02-0.13) | 0.05 ± 0.25 (0.02-0.13) | 0.04 ± 0.02 (0.02-0.09) | 1297 (0.54) |
| **General attention (Digits direct)** | 8.67 ± 2.24 (5-15) | 8.55 ± 2.16 (5-14) | 8.89 ± 2.39 (5-15) | 1127.50 (0.43) |

*In all cases, the non-parametric Mann-Whitney U test was used because no variable presented a normal distribution. Results with statistical significance are marked with an asterisk.*

The distribution of the participants according to their educational level is shown in *Table 2*. All the participants had completed at least primary studies, and 76.2% had university studies. Age was significantly correlated with general attention. Educational level was

significantly correlated with total intimate partner violence, physical and psychological IPV, in addition to symptoms of depression, anxiety, post-traumatic stress, general attention, and ABV, as indicated by Spearman's rho.

***Table 2*.** Frequencies of the educational level of the sample.

| | **Total (N = 105)** n (%) | **IPV group (N=69)** n (%) | **No-IPV group (N=36)** n (%) |
|---|---|---|---|
| **0-No schooling (0 years)** | 0 (0%) | 0 (0%) | 0 (0%) |
| **1-Incomplete primary (<6 years)** | 0 (0%) | (0%) | 0 (0%) |
| **2-Complete Primary (6 years)** | 0 (0%) | 0 (0%) | 0 (0%) |
| **3- Incomplete secondary education (<10 years)** | 4 (3.8%) | 4 (5.8%) | 0 (0%) |
| **4- Complete secondary education (10 years)** | 3 (2.9%) | 3 (4.3%) | 0 (0%) |
| **5- Bachelor (11/12 years)** | 18 (17.1%) | 15 (21.7%) | 3 (8.3%) |
| **6- Bachelor's degree (13/18 years)** | 33 (31.4%) | 18 (26.1%) | 15 (41.7%) |
| **7- Master's degree (19/21 years)** | 32 (30.5%) | 22 (31.9%) | 10 (27.8%) |
| **8- Doctorate (>22 years)** | 15 (14.3%) | 7 (10.1%) | 8 (22.2%) |

The frequency of the four types of IPV indicated that psychological IPV was the most frequent type of violence (70.5%), followed by control IPV (60%), sexual IPV (43.8%) and physical IPV (35,2%). A description of the time elapsed since the end of the violence, the time it lasted, and the severity scores for the four types of IPV subscales and the overall sum score (i.e., Total IPV) is shown in *Table 3*. The IPV that presented the greatest severity were psychological and control, followed by physical and sexual.

***Table 3*.** Characteristics of intimate partner violence in the cases (N= 36) of the sample.

| | | **IPV group** Mean ± SD (Range) |
|---|---|---|
| **Months from end of IPV** | | 86.99 ± 56.32 (0-240) |
| **Duration IPV (months)** | | 80.99 ± 84.65 (0-360) |
| **Severity** | **Control IPV severity** | 2.42 ± 0.99 (0-3) |
| | **Psychological IPV severity** | 2.43 ± 0.78 (0-3) |
| | **Physical IPV severity** | 1.25 ± 1.25 (0-3) |
| | **Sexual IPV severity** | 1.19 ± 1.10 (0-3) |
| | **Total IPV severity** | 7.29 ± 2.90 (0-12) |

### 4.2. Correlation between phenotypical variables (cognitive and clinical traits).

Average scores of the cognitive and traits indices are presented in *Table 1*. Correlation between the two cognitive indices (General Attention Ability with Attention Bias Variability) was not significant (rho = −0.08, P = 0.40). However, correlations between the three clinical traits (symptoms of depression, anxiety, and PTSD) were all significant (all $P < 0.01$).

Regarding the relationships between clinical traits (depressive, anxiety, and PTSD symptoms) and attention indices (ABV and General Attention Ability) are shown in *Table 4*. We found that ABV was significantly correlated with the symptoms of anxiety (rho = 0.20, P = 0.04) but not with symptoms of depression or PTSD (both P > 0.05). However, General Attention Ability was significantly correlated with depression, anxiety, and PTSD symptoms (all P < 0.05), which in all cases, that correlation was negative.

***Table 4*.** Correlations between attentional and clinical traits (n=103-104).

| | **Attention Bias Variability** r (p) | **General Attention Ability** r (p) |
|---|---|---|
| **Symptoms of depression** | 0.08 (0.43) | -0.19* (<0.05) |
| **Symptoms of anxiety** | 0.20* (0.04) | - 0.20* (0.04) |
| **Symptoms of PTSD** | 0.05 (0.64) | -0.25* (0.01) |

r = Spearman/Pearson correlation coefficient; p = p value associated with the correlation coefficient
* p value < 0.05 statistically significant correlation

### 4.3. Correlation between intimate partner violence and phenotypical variables.

Correlations of the four IPV subscales and IPV total with the cognitive and clinical traits are shown in *Table 5*.

***Table 5*.** Correlations between intimate partner violence and phenotypical variables (cognitive and clinical traits) in total participants (n=104-105).

| | **Sexual IPV** r (p) | **Physical IPV** r (p) | **Psychological IPV** r (p) | **Control IPV** r (p) | **IPV Total** r (p) |
|---|---|---|---|---|---|
| **Clinical traits** | | | | | |
| **Symptoms of depression** | 0.27* (<0.01) | 0.37* (<0.01) | 0.41* (<0.01) | 0.34* (<0.01) | 0.41* (<0.01) |
| **Symptoms of anxiety** | 0.17 (0.08) | 0.32* (<0.01) | 0.30* (<0.01) | 0.23* (0.02) | 0.30* (<0.01) |
| **Symptoms of PTSD** | 0.53* (<0.01) | 0.39* (<0.01) | 0.45* (<0.01) | 0.37* (<0.01) | 0.50* (<0.01) |
| **Cognitive traits** | | | | | |
| **Attentional Bias Variability** | -0.10 (0.33) | 0.12 (0.23) | -0.03 (0.78) | -0.04 (0.65) | -0.03 (0.80) |
| **General Attention Ability** | -0.13 (0.18) | -0.10 (0.31) | -0.05 (0.59) | -0.09 (0.36) | -0.11 (0.27) |

r= Spearman/Pearson correlation coefficient; p= p value associated with the correlation coefficient; * p value < 0.05 statistically significant correlation

On the one hand, no type of IPV was significantly correlated with any of the attention indices (all P > 0.1). On the other hand, all type of IPV, except sexual IPV, were significantly correlated with depression, anxiety, and PTSD symptoms (all P < 0.05). Sexual IPV was significantly correlated with depression and PTSD symptoms (both P <

0.05) but not with anxiety symptoms, though it was close to significance level (rho = 0.17, P = 0.08). In other words, women exposed to intimate partner violence reported significantly higher scores on the depressive, anxious and PTSD symptomatology scales but they did not report significant differences in the cognitive scales.

### 4.4. Description of genetic data.

DNA was available for 112 participants and genotyping worked correctly for all the samples; the genotype call rate (i.e., the proportion of DNA samples that generated results in the total sample genotyped) was 100% for both polymorphisms. The genotypic and allelic frequencies of the SNPs of *FKBP5* and *BDNF* genes are reported in *Table 6*. Genotypic frequencies did not depart significantly from Hardy-Weinberg equilibrium ($\chi 2$ =0.88, P = 0.64; $\chi 2$ =0.13, P = 0.94, respectively) which supports the absence of genotyping artefacts. No statistically significant differences in allelic frequencies were found between the European reference populations previously reported (i.e., 1000 Genomes Project, www.1000genomes.org) and our sample ($\chi 2$ =0.78, P = 0.68; $\chi 2$ =0.30, P = 0.86, respectively).

***Table 6.*** Allelic and genotypic frequencies of the SNPs analysed, and comparison of the genotype data obtained with data of the CEU population of 1000Genome.

| | **GENOTYPIC FREQUENCIES** | | | **ALLELIC FREQUENCIES** | | **Call rate (missing)**[3] |
|---|---|---|---|---|---|---|
| | Present study n (%) | European population[1] (%) | $\chi^2$ (p)[2] | Present study (%) | European population[1] (%) | |
| ***FKBP5* gene** | | | | | | |
| **rs1360780** | | | | | | |
| CC | 56 (50%) | 47.61% | 0.78 (0.68) | C: 68.75% T: 31.25% | C: 69% T: 31% | 100% (0/112) |
| CT | 42 (37.5%) | 42.78% | | | | |
| TT | 14 (12.5%) | 9.61% | | | | |
| ***BDNF* gene**[4] | | | | | | |
| **rs6265** | | | | | | |
| Val/Val | 74 (66.07%) | 64% | 0.30 (0.86) | Val: 81.7% Met: 18.3% | Val: 80% Met: 20% | 100% (0/112) |
| Val/Met | 35 (31.25%) | 32% | | | | |
| Met/Met | 3 (2.68%) | 4% | | | | |

[1] Frequencies obtained from 1000Genome CEU-population, NCBI. [2] Calculated using the on-line Chi-Square P Value Calculator (http://www.waent.org/Chi-Square-Test.htm). [3] Frequency of individuals genotyped successfully and number of individuals missing of the total. [4] Val allele corresponds to C allele; Met allele corresponds to T allele.

All genotype data were analysed under a recessive model. In other words, we combined TT and CT genotypes (T carriers) of *FKBP5* gene and compared them with individuals with the CC genotype of this gene (non-carriers). The genotypic frequency for T carriers was 56 (50%). For *BDNF* gene, we combined Met/Met and Val/Met genotypes (Met

carriers) and compared them with individuals with the Val/Val genotype (non-carriers). The genotypic frequency for Met carriers was 38 (33.93%).

### 4.5. Relationships between the *FKBP5* and *BDNF* genotypes and phenotypical variables (cognitive and clinical traits).

To study whether there is an association between the scores of the different phenotypical variables and the variability analysed in the *FKBP5* gene (i.e., CC vs. T carriers), Mann-Whitney U test were performed. The results of these analyses reported a statistically significant effect of the *FKBP5*-rs1360780 polymorphism in the case of the depression symptomatology (U = 783, P < 0.01), where the score for depression scale of T carriers (Mean = 4.29, SD = 3.76) was lower than the score of non-carriers (Mean = 7.62, SD = 5.67). Furthermore, the effect of that polymorphism on anxiety symptoms was situated on the border of statistical significance (U = 946, P = 0.05), where the score for anxiety scale of T carriers (Mean = 5.33, SD = 4.24) was lower than the score of non-carriers (Mean = 7.27, SD = 5.08). The effect on the rest of phenotypic variables analysed were not statistically significant (*Table 7*).

***Table 7*.** Association between the variability analysed in the *FKBP5* and *BDNF* genes and the phenotypical variables (cognitive and clinical traits).

| | | ***FKBP5* gene (rs1360780)** | | | ***BDNF gene (rs6265)*** | | |
|---|---|---|---|---|---|---|---|
| | | **CC** Mean (SD) | **T-carriers** Mean (SD) | U (P) | **Val/Val** Mean (SD) | **Met carriers** Mean (SD) | U (P) |
| **Clinical traits** | **Symptoms of depression** | 7.62 (5.67) | 4.29 (3.76) | 783* (<0.01*) | 6.14 (5.21) | 5.45 (4.73) | 1026.50 (0.64) |
| | **Symptoms of anxiety** | 7.27 (5.08) | 5.33 (4.24) | 946 (0.05) | 6.14 (4.51) | 6.54 (5.25) | 1076.50 (0.93) |
| | **Symptoms of PTSD** | 9.54 (11.45) | 6.65 (8.95) | 1067 (0.25) | 7.41 (9.85) | 9.33 (11.17) | 996 (0.47) |
| **Cognitive traits** | **Attentional Bias Variability** | 0.05 (0.02) | 0.05 (0.02) | 1357 (0.44) | 0.05 (0.02) | 0.05 (0.02) | 1301 (0.76) |
| | **General Attention Ability** | 8.29 (2.07) | 8.98 (2.40) | 1036 (0.14) | 8.81 (2.47) | 8.33 (1.78) | 1014.50 (0.50) |

U= Mann-Whitney U statistic; p= p value associated with the U statistic; * p value < 0.05 statistically significant association

In the case of the association between the variability analysed in the *BDNF* gene (i.e., Val/Val vs. Met carriers), the results of the analyses did not report any statistically significant effect of the *BDNF*-rs6265 polymorphism in the phenotypic variables.

### 4.6. Analysis of the modulation role of the *FKBP5* and *BDNF* genotypes on the association of intimate partner violence with phenotypical variables (cognitive and clinical traits).

Analysis of covariance (ANCOVA) were performed to study whether the association of intimate partner violence with the phenotypical variables (cognitive and clinical traits) is modulated by the *FKBP5*-rs1360780 or *BDNF*-rs6265 genetic variability. Age and education level were included as covariates in the analysis. The ANCOVA revealed that the genotype-by-IPV interactions were not significant in any case (all P > 0.1). However, the effect of the interaction of *BDNF*-rs6265 genetic variability with IPV on anxiety and PTSD symptoms was close to the threshold of statistical significance (F= 3.42, P= 0.07; F= 3.50, P= 0.06, respectively). All results are shown in *table 8*.

***Table 8*.** Interaction between the variability analysed in the *FKBP5/BDNF* gene and intimate partner violence on the phenotypical variables (cognitive and clinical traits).

| | | ***FKBP5* gene (rs1360780) F (p)** | **Intimate Partner Violence F (p)** | ***FKBP5* x IPV F (p)** | ***BDNF* gene (rs6265) F (p)** | **Intimate Partner Violence F (p)** | ***BDNF* x IPV F (p)** |
|---|---|---|---|---|---|---|---|
| **Clinical traits** | **Symptoms of depression** | 8.78* (<0.01) | 2.60 (0.11) | 1.04 (0.31) | 1.04 (0.31) | 7.19* (<0.01) | 2.63 (0.11) |
| | **Symptoms of anxiety** | 2.76 (0.10) | 3.23 (0.08) | <0.01 (0.97) | <0.01 (0.97) | 7.65* (<0.01) | 3.42 (0.07) |
| | **Symptoms of PTSD** | 0.15 (0.70) | 12.84* (<0.01) | 0.25 (0.61) | 0.25 (0.61) | 18.07* (<0.01) | 3.50 (0.06) |
| **Cognitive traits** | **Attentional Bias Variability** | 0.02 (0.90) | 0.19 (0.67) | 0.38 (0.54) | 0.38 (0.54) | 0.42 (0.52) | 0.12 (0.73) |
| | **General Attention Ability** | 1.78 (0.19) | 0.50 (0.48) | 0.47 (0.49) | 0.47 (0.49) | 0.03 (0.85) | 0.31 (0.58) |

F= F-test of equality of variances; p= p value associated with the F-test; * p value < 0.05 statistically significant association

## 5. DISCUSSION

The present study aimed to investigate: i) the association between the different phenotypical variables, classified in cognitive indices (attention bias variability and general attention ability) and clinical traits (depressive, anxious and PTSD symptoms), ii) the impact of intimate partner violence on the attention bias variability, general attention ability and depressive, anxious and PTSD symptoms, iii) the variability of the candidate genes for mental diseases (*FKBP5* and *BDNF* genes) and its association with the phenotypical variables, and iv) the modulating role of *FKBP5* and *BDNF* on the association between intimate partner violence and phenotypical variables (i.e., gene-environment interaction).

Firstly, we found that the cognitive variables (ABV and General Attention Ability) were not correlated with each other. This was not surprising, since they evaluate different aspects regarding attention. In addition, we verified that the clinical traits (symptoms of depression, anxiety, and PTSD) were correlated with each other as indicated by other studies (Gorman, 1996; Bleich et al., 1997). Finally, we analysed whether there was a correlation between the cognitive and clinical traits. In our study, we did not obtained a significant correlation between depressive and PTSD symptoms and ABV, but we observed a positive correlation between anxiety symptoms and ABV. Our results did not replicate previous studies reporting a correlation between the increase in ABV and the severity of mental illnesses (Hori et al., 2021). As ABV is a novel index for capturing trauma-related attention dysfunction, further investigation of its possible correlations with clinical traits would be necessary. In relation to General Attention Ability, we have observed negative correlations between this index and the three types of clinic symptomatology.

Second, our results show a significant main effect of IPV on presenting PTSD and depressive, anxious traits. This association was found for the five trauma variables analysed (i.e., Psychological, Control, Physical, Sexual and Total IPV) regarding the three clinical traits. These results were in line with those provided by different studies (Bonomi et al., 2009; Campbell & Lewandowski, 1997) and further support the conception of IPV as an environmental risk factor underlying the development of mental diseases. However, the mechanism of risk is still not fully understood. The most widely accepted hypothesis is the chronic stress model. When exposure to stressors persists and the stress-induced glucocorticoid release is chronically increased, the hypothalamic-pituitary-adrenal (HPA) axis may be permanently dysregulated, which underlies the neurotransmitters abnormalities that are thought to be involved in the mental pathologies. To fully acknowledge the causal relationship between IPV and the development of mental disorders, further research is required to elucidate these mechanisms as well as to examine the differential effects of different types of traumas. In relation to the cognitive indices, we have not identified a correlation between the IPV and a greater alteration of the attention bias. To our knowledge, this is the first time that alterations in attention bias have been studied in an IPV context. We hypothesized that women who had suffered IPV would have a greater alteration in the attention bias given that in other studies a greater alteration had been perceived in people who had suffered child abuse (Hori et al., 2021). This association may not occur in adulthood. More studies in this field would be necessary to verify if there really is any association.

Third, our analyses do not reveal any significant main effect of the variability of the *BDNF*-rs6265 polymorphism on the development of cognitive or clinical alterations, but it reveals a significant effect of the variability of the *FKBP5*-rs1360780 polymorphism on the development of depressive symptomatology. Many studies support the relationship between the variability of the *BDNF* gene and an increase in depressive and anxiety symptoms (Yu & Chen, 2011; Chen et al., 2006). Furthermore, some studies indicate that the Val66Met genotype of the *BDNF* gene is associated with an increase in alterations in the attention bias variability (Hori et al., 2021). Our study does not replicate these results, possibly due to our frequency of the minor alleles being very low, which could decrease the power of our sample and hamper our analyses. To resolve this, it would be necessary to increase the sample size to have a larger frequency of participants with Met/Met genotype. In relation to the *FKBP5* gene, it has been observed in our sample that the risk genotype for depression was CC. This differs from other studies that indicate that carriers of the T allele are at increased risk of developing mental illness (Lavebratt et al., 2010). One possible explanation to these inconsistencies might involve the differential-susceptibility model, which suggests that genetic variants should be seen as "plastic variants" rather than as "risk" or "non-risk" variants (Belsky & Pluess, 2009). The term plastic variant refers to the idea that one allele can be both positive and negative for an individual's health depending on the environment. Therefore, according to this model, the same genetic variants would increase the detrimental effects of negative experiences and enhance the likelihood of benefiting from positive environments as well.

Finally, as for the rest of complex diseases, it is widely accepted that the susceptibility to developing depression, anxiety or PTSD is given by the interaction between environmental factors and genetic predisposition. In recent years, the number of groups studying the relationship between genes and environmental factors has increased. There are some studies, that following this line, investigate the variability of the *BDNF*-rs6265 polymorphism with childhood trauma as a risk factor for the development of clinical symptoms (Hori et al., 2021). There are also studies that focus on the different variants of the *FKBP5* gene and its interaction with different environmental factors, mostly focused on childhood (Zannas et al., 2016). Although in our study we have not obtained any significant interaction between one of these genetic variants and IPV, for the variants of the *BDNF*-rs6265 genotype we have obtained a value close to significance in the interaction with IPV in the development of anxiety and depression, with Met carriers being the risk genotype when interacting with IPV. It would be necessary to increase the sample size to determine if this value could cross the threshold of statistical significance.

### Future Directions

The possible involvement of the *BDNF* gene in depression, anxiety and PTSD is well considered since this gene plays a role in development, neural regeneration, synaptic transmission, synaptic plasticity, and neurogenesis, and may influence the development of these diseases. Therefore, this gene should not be ruled out as a potential candidate gene even though the results of this study do not provide evidence in favour of this role. It would be recommendable to carry out more research with larger sample sizes.

Regarding the *FKBP5* gene, it would be necessary to carry out additional studies to determine what is due to the lack of concordance in the risk genotype between the different studies.

From the results obtained in this study, the relationship between Intimate Partner Violence and clinical symptoms (depression, anxiety, and PTSD) is evident. For this reason, it is necessary to continue investigating the possible underlying biological mechanisms in this association, as well as to explore the possible differential effects of the different types of trauma.

In addition, it would be interesting to study the possible protective environments that also have the potential to be part of a gene-environment interaction and that would help prevent certain complex diseases, such as depression, anxiety, and PTSD.

Finally, to delve into the interaction mechanisms between IPV and the different genetic variables, it would be convenient to carry out studies on the levels of methylation in certain regions of these candidate genes.

## 6. CONCLUSIONS

The results of this study:

- Do not support a correlation between ABV and symptoms of depression or PTSD after IPV exposition.
- Suggest a positive correlation between ABV and anxiety and negative correlations between General Attention Ability and the three clinical traits analysed.
- Strongly supports the role of IPV as a risk factor for the development of clinical symptoms.
- Suggest an association between the variability of the *FKBP5*-rs1360780 polymorphism and depressive symptoms, being CC the genotype of risk.
- No significant interaction between the analysed genes and IPV was found, although further analysis is needed.

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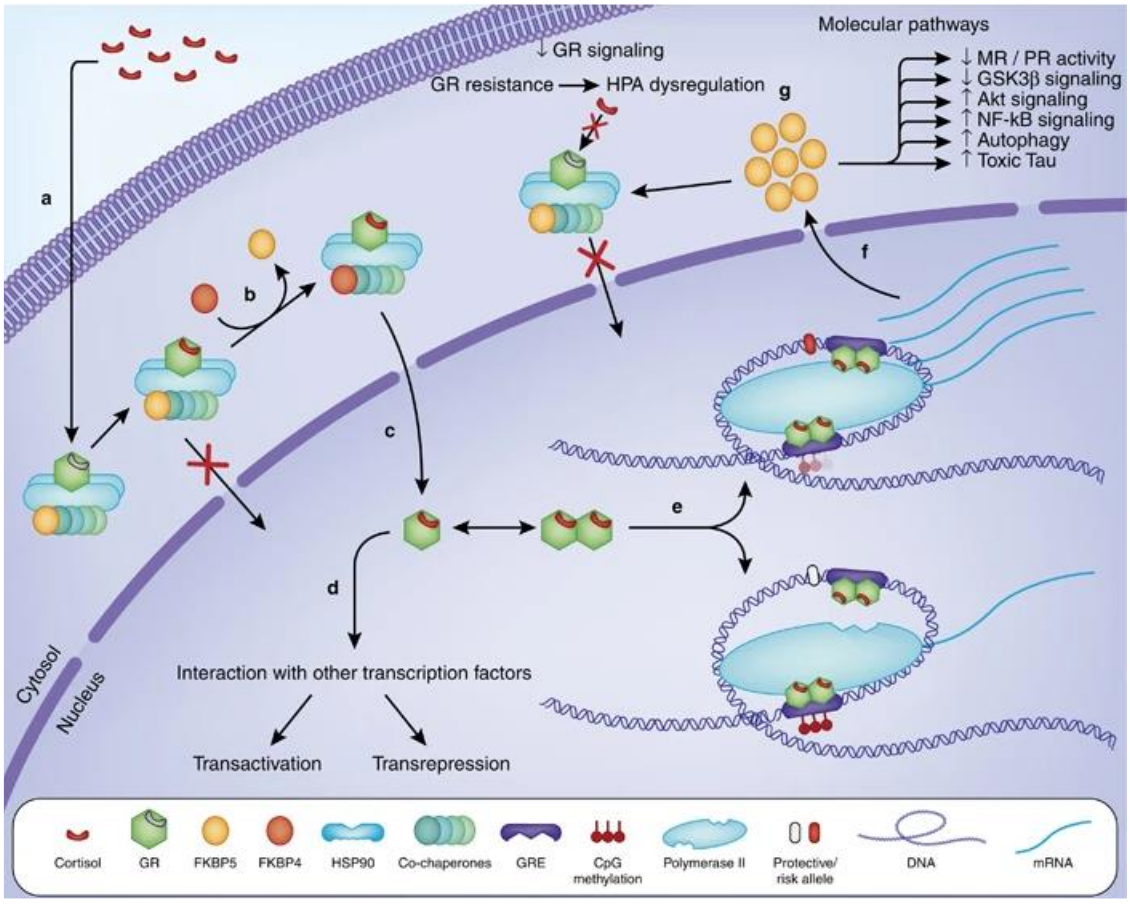
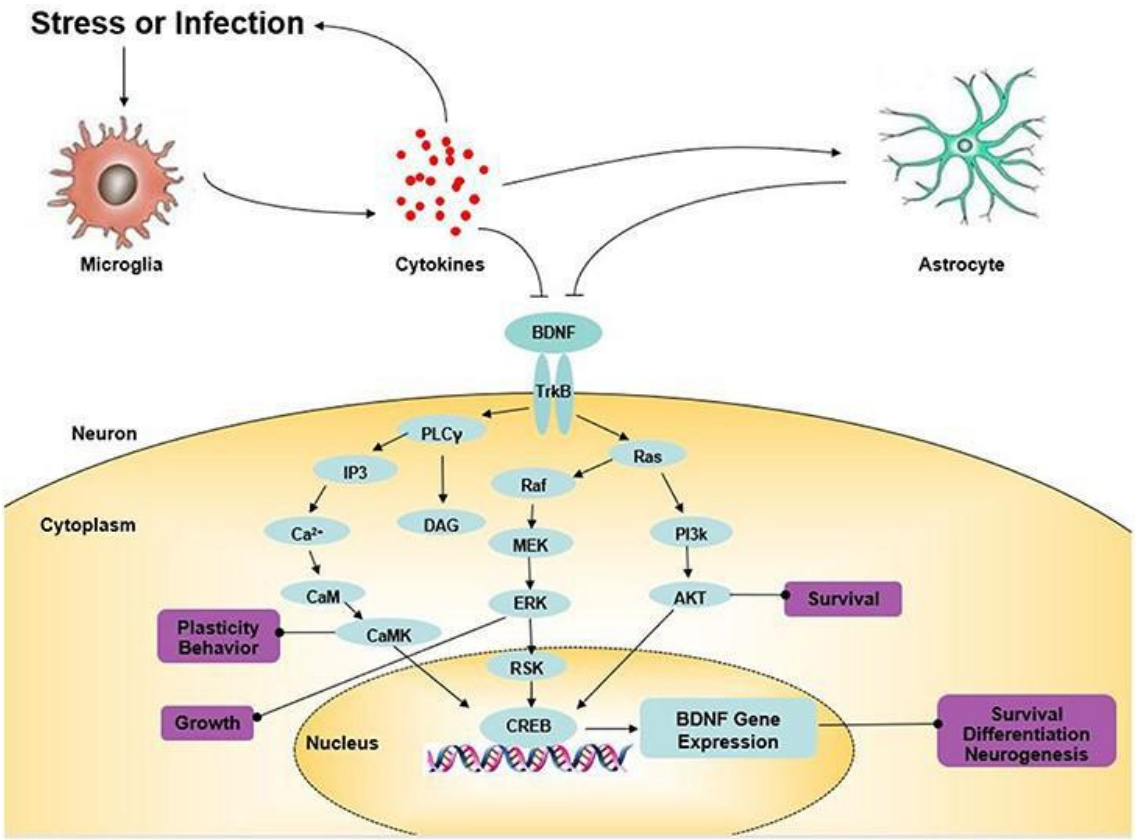
**ANNEX**

***Annex 1*. Schematic representation of the molecular events involved in glucocorticoid-mediated FKBP5 induction, the resulting intracellular negative feedback loop, and effects on other biological processes.**




***Annex 1*.** Glucocorticoids enter the cytoplasm (a) and activate the glucocorticoid receptor (GR) complex. FKBP5 binding to the complex reduces affinity of glucocorticoids to the GR and delays translocation of the GR to the nucleus. However, exchange of FKBP5 for FKBP4 (b) results in GR translocation to the nucleus (c). The GR can either interact as a monomer with other transcription factors (d) or form a homodimer that binds to DNA at glucocorticoid response elements. Overall, GR functions result in transactivation or transrepression of a large number of genes. The *FKBP5* gene is highly responsive to GR, but responsiveness depends on *FKBP5* polymorphisms and methylation status (e). The synthesized FKBP5 mRNA translocates to the cytoplasm (f) where it is translated into FKBP5 protein. FKBP5 then inhibits GR activity not only forming an ultra-short, intracellular negative feedback loop of GR signaling but also modulating several other biological pathways (g) (Zannas et al., 2016).

***Annex 2*. The role of BDNF in depression.**




***Annex 2*.** Arrows indicate activation; T-shaped arrows indicate inhibition. Akt, serine/threonine protein kinase; BDNF, brain-derived neurotrophic factor; CaM, calmodulin; CaMK, calcium-calmodulin-dependent protein kinase; CREB, cAMP response element-binding protein; DAG, diacylglycerol; ERK, extracellular signal-regulated kinase; IP3, inositol 1,4,5-trisphosphate; MEK, mitogen-activated extracellular signal-regulated kinase; PKC, protein kinase C; PI3K, PI-3 kinase; PLC-γ, phospholipase-Cγ; RSK, ribosomal S6 kinase; TrkB, tyrosine kinase B (Jin et al., 2019).

***Annex 3*. Partner Violence Screen (3 items)**

The brief PVS incorporated 2 dimensions of partner violence. It consists of 1 question that addresses physical violence, and 2 questions that address a woman's perception of her safety.

First, women were asked, "**Have you been hit, kicked, punched, or otherwise hurt by someone within the past year? If so, by whom?**"

Two questions were selected to measure the woman's perception of safety: (1) "**Do you feel safe in your current relationship?**" and (2) "**Is there a partner from a previous relationship who is making you feel unsafe now?**"

A "yes" response to the physical violence question was considered positive for partner violence if the perpetrator was a current or former spouse or other intimate partner. For the safety questions, women who reported feeling unsafe because of a current or past partner and those who were unsure about their safety were considered positive for partner violence. Women who reported feeling safe and women who had no current or past intimate relationships were considered negative for partner violence. A positive response to any 1 of the 3 questions on the PVS constituted a positive screen for partner violence.

## *Annex 4*. In-Depth Intimate Partner Violence questionnaire

| | | Sí | No |
|---|---|---|---|
| 1 | ¿Trataba de impedirle que viese a sus amigos/as o trataba de evitar que Ud. se relacionase con su familia directa o parientes? | | |
| 2 | ¿Insistía en saber dónde estaba Ud. en cada momento, o esperaba que Ud. le pidiese permiso antes de salir por su cuenta fuera de casa? | | |
| 3 | ¿Sospechaba injustificadamente que Ud. le era infiel? | | |
| 4 | ¿Se negaba a darle dinero para los gastos del hogar cuando él tenía dinero para otras cosas? | | |
| 5 | ¿Le impedía tomar decisiones relacionadas con la economía familiar y/o realizar las compras de forma independiente? | | |
| 6 | ¿No le dejaba trabajar o estudiar fuera del hogar? | | |

| | ¿Esta pareja alguna vez… | | ¿Con qué frecuencia? |
|---|---|---|---|
| 1 | … le insultó o le hizo sentirse mal con Ud. misma? ¿O le humilló delante de otras personas? | Sí<br>No | Una vez<br>Algunas veces<br>Muchas veces |
| 2 | … le asustó o intimidó a propósito (por ejemplo, gritándole o rompiendo cosas)? | Sí<br>No | Una vez<br>Algunas veces<br>Muchas veces |
| 3 | … le amenazó verbalmente con hacerle daño a Ud. o a alguien importante para Ud.? | Sí<br>No | Una vez<br>Algunas veces<br>Muchas veces |
| 4 | … le abofeteó o le tiró algo que podía hacerle daño? ¿Le golpeó con su puño o alguna otra cosa? | Sí<br>No | Una vez<br>Algunas veces<br>Muchas veces |
| 5 | … le empujó, agarró o tiró del pelo? ¿Le dio patadas, arrastró o pegó? | Sí<br>No | Una vez<br>Algunas veces<br>Muchas veces |
| 6 | … le intentó asfixiar, quemar, o le amenazó con usar algún tipo de arma contra Ud.? | Sí<br>No | Una vez<br>Algunas veces<br>Muchas veces |
| 7 | … le obligó a mantener relaciones sexuales cuando Ud. no quería? | Sí<br>No | Una vez<br>Algunas veces<br>Muchas veces |
| 8 | … mantuvo relaciones sexuales sin Ud. desearlo, porque tenía miedo de lo que podría pasar si se negaba? | Sí<br>No | Una vez<br>Algunas veces<br>Muchas veces |
| 9 | … le obligó a tener relaciones sexuales contra su voluntad, sujetándole o haciéndole daño de alguna manera sin conseguirlo? | Sí<br>No | Una vez<br>Algunas veces<br>Muchas veces |

## *Annex 5*. Patient Health Questionnaire (PHQ-9) scale

Name _____ Date _____

| Over the *last 2 weeks*, how often have you been bothered by any of the following problems? | Not at all | Several days | More than half the days | Nearly every day |
|---|---|---|---|---|
| 1. Little interest or pleasure in doing things | 0 | 1 | 2 | 3 |
| 2. Feeling down, depressed, or hopeless | 0 | 1 | 2 | 3 |
| 3. Trouble falling or staying asleep, or sleeping too much | 0 | 1 | 2 | 3 |
| 4. Feeling tired or having little energy | 0 | 1 | 2 | 3 |
| 5. Poor appetite or overeating | 0 | 1 | 2 | 3 |
| 6. Feeling bad about yourself—or that you are a failure or have let yourself or your family down | 0 | 1 | 2 | 3 |
| 7. Trouble concentrating on things, such as reading the newspaper or watching television | 0 | 1 | 2 | 3 |
| 8. Moving or speaking so slowly that other people could have noticed? Or the opposite—being so fidgety or restless that you have been moving around a lot more than usual | 0 | 1 | 2 | 3 |
| 9. Thoughts that you would be better off dead or of hurting yourself in some way | 0 | 1 | 2 | 3 |
| (For office coding: Total Score ____ = ____ + ____ + ____) | | | | |

If you checked off *any* problems, how *difficult* have these problems made it for you to do your work, take care of things at home, or get along with other people?

| Not difficult at all | Somewhat difficult | Very difficult | Extremely difficult |
|---|---|---|---|
| ☐ | ☐ | ☐ | ☐ |

PHQ-9 is adapted from PRIMEMDTODAY, developed by Drs. Robert I. Spitzer, Janet B.W. Williams, Kurt Kroenke, and colleages, with an educational grant from Pfizer Inc. For research information contact Dr. Spitzer at rls8@columbia.edu. Use of the PHQ-9 may only by made in accordance with the Terms of Use available at http:// www.pfizer.com. 

***Annex 6*. Generalized Anxiety Disorder 7-item (GAD-7) scale**

| Over the last 2 weeks, how often have you been bothered by the following problems? | Not at all sure | Several days | Over half the days | Nearly every day |
|---|---|---|---|---|
| 1. Feeling nervous, anxious, or on edge | 0 | 1 | 2 | 3 |
| 2. Not being able to stop or control worrying | 0 | 1 | 2 | 3 |
| 3. Worrying too much about different things | 0 | 1 | 2 | 3 |
| 4. Trouble relaxing | 0 | 1 | 2 | 3 |
| 5. Being so restless that it's hard to sit still | 0 | 1 | 2 | 3 |
| 6. Becoming easily annoyed or irritable | 0 | 1 | 2 | 3 |
| 7. Feeling afraid as if something awful might happen | 0 | 1 | 2 | 3 |
| *Add the score for each column* | + | + | + | |

Total Score *(add your column scores)* =

## *Annex 7*. Posttraumatic Symptom Scale-Interview Version for DSM-5 (PSS-I-5)

| Please read each statement carefully and circle the number that best describes how often that problem has been happening and how much it upset you over THE LAST MONTH. | Not at all | Once a week or less/a little | 2 to 3 times a week/ somewhat | 4 to 5 times a week/ very much | 6 or more times a week/ severe |
|---|---|---|---|---|---|
| 1. Have you had unwanted distressing memories about the trauma? | 0 | 1 | 2 | 3 | 4 |
| 2. Have you been having bad dreams or nightmares related to the trauma? | 0 | 1 | 2 | 3 | 4 |
| 3. Have you had the experience of feeling as if the trauma were actually happening again? | 0 | 1 | 2 | 3 | 4 |
| 4. Have you been very EMOTIONALLY upset when reminded of the trauma? | 0 | 1 | 2 | 3 | 4 |
| 5. Have you been having PHYSICAL reactions when reminded of the trauma (e.g., sweating, heart racing)? | 0 | 1 | 2 | 3 | 4 |
| 6. Have you been making efforts to avoid thoughts or feelings related to the trauma? | 0 | 1 | 2 | 3 | 4 |
| 7. Have you been making efforts to avoid activities, situations, or places that remind you of the trauma or that feel more dangerous since the trauma? | 0 | 1 | 2 | 3 | 4 |
| 8. Are there any important parts of the trauma that you cannot remember? | 0 | 1 | 2 | 3 | 4 |
| 9. Have you been viewing yourself, others, or the world in a more negative way (e.g. “I can’t trust people,” “I’m a weak person”)? | 0 | 1 | 2 | 3 | 4 |
| 10. Have you blamed yourself for the trauma or for what happened afterwards? Have you blamed others that did not directly cause the event for the trauma or what happened afterwards? | 0 | 1 | 2 | 3 | 4 |
| 11. Have you had intense negative feelings such as fear, horror, anger, guilt or shame? | 0 | 1 | 2 | 3 | 4 |
| 12. Have you lost interest in activities you used to do? | 0 | 1 | 2 | 3 | 4 |
| 13. Have you felt detached or cut off from others? | 0 | 1 | 2 | 3 | 4 |
| 14. Have you had difficulty experiencing positive feelings? | 0 | 1 | 2 | 3 | 4 |
| 15. Have you been acting more irritable or aggressive? | 0 | 1 | 2 | 3 | 4 |
| 16. Have you been taking more risks or doing things that might cause you or others harm (e.g., driving recklessly, taking drugs, having unprotected sex)? | 0 | 1 | 2 | 3 | 4 |
| 17. Have you been overly alert or on-guard (e.g., checking to see who is around you, etc.)? | 0 | 1 | 2 | 3 | 4 |
| 18. Have you been jumpier or more easily startled? | 0 | 1 | 2 | 3 | 4 |
| 19. Have you had difficulty concentrating? | 0 | 1 | 2 | 3 | 4 |
| 20. Have you had difficulty falling or staying asleep? | 0 | 1 | 2 | 3 | 4 |
| 21. How much have these difficulties been bothering you? | 0 | 1 | 2 | 3 | 4 |
| 22. How much have these difficulties been interfering with your everyday life (e.g. relationships, work, or other important activities)? | 0 | 1 | 2 | 3 | 4 |
| 23. How long after the trauma did these difficulties begin? | 0 | 1 | 2 | 3 | 4 |
| 24. How long have you had these trauma-related difficulties? | 0 | 1 | 2 | 3 | 4 |

## *Annex 8*. PROTOCOL A: DNA Purification from Blood (250µL)

This protocol is for purification of total DNA from whole blood, plasma, serum or buffy coat.

1. Transfer 250 µL of sample into the bottom of a 1.5 ml microcentrifuge tube (not provided).

Use up to 250 µl whole blood, plasma, serum or buffy coat. If the sample volume is less than 250 µL, add the appropriate volume of PBS. For samples larger than 250 µL, the amount of lysis buffer, proteinase K and ethanol used should be increased proportionally, while the volumes of wash and elution buffers should remain constant. For example, 400 µL sample will require 40 µL Protease K, 400 µL Buffer BLU and 400 µL Ethanol. Buffer BLU and Proteinase K can be purchased separately to supplement the Kit.

2. Add 25 µL proteinase K.

3. [Optional Step] RNA Degradation: If RNA-free gDNA is required, add 4 µL of RNase A (100 mg/ml) [not provided].

4. Add 250 µL of buffer BLU and mix by vortexing (it is important to observe a homogeneous solution).

5. Incubate in a water bath at 55 °C for 25 minutes.

6. Centrifuge at full speed for 1 minute. Transfer the mix by pipetting to a new microcentrifuge tube (not provided).

7. Add 250 µL of ethanol (96–100%) and mix by vortexing vigorously.

8. Place the minispin column in a collection tube and transfer the mix by pipetting. Centrifuge at full speed for 1 minute. Discard the flow-through solution.

9. Place the minispin column in a collection tube and add 500 µL of WB1 buffer. Centrifuge at full speed for 1 minute. Discard the flow-through solution.

10. Place the minispin column in a collection tube and add 500 µL of WB2 buffer. Centrifuge at full speed for 1 minutes. Discard the flow-through solution.

11. Place the minispin column in the same collection tube and add 800 µL of WB2 buffer. Centrifuge at full speed for 1 minutes. Discard the flow-through solution.

12. Centrifuge at full speed for 3 minutes to dry the minispin column. This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

13. Place the minispin column into a new, labelled, 1.5 microcentrifuge tube (not provided) and pipet 40 µL sterile water directly into the membrane. Close the tube and incubate for 2 minutes at room temperature.

14. Centrifuge at full speed for 1 minute to elute the DNA

15. The purified genomic DNA can be stored at 2-8°C for a few days. For longer term storage, -20°C is recommended.

**MAIN ABBREVIATIONS**

**ABNT:** Attention Bias in relation to threat

**ABV:** Attention Bias Variability

***BDNF:*** Brain-Derived Neurotrophic Factor

**CNS:** Central Nervous System

**DSM-5:** Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition

***FKBP5****:* FK506-Binding Protein 5

**GAD-7:** Generalized Anxiety Disorder, 7 items version

**GHQ-12:** General Health Questionnaire, 12 items version

**HPA:** Hypothalamic-Pituitary-Adrenal axis

**IPV:** Intimate Partner Violence

**M.I.N.I.:** Mini International Neuropsychiatric Interview

**PCR:** Polymerase Chain Reaction

**PHQ-9:** Patient Health Questionnaire, 9 items version

**PSS-I-5:** Post-traumatic Symptom Scale-Interview Version for DSM-5

**PTSD:** Post-Traumatic Stress Disorder

**RT:** Reaction Time

**SAM:** Sympathetic-Adrenomedullary axis

**SD:** Standard Deviation

**SNP:** Single-Nucleotide Polymorphism

**WHO:** World Health Organization

**WISC-IV:** Wechsler Intelligence Scale for Children-Fourth Edition