Candidate genes for psychosis and their interaction with environmental factors: impact on psychosis-proneness

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Marta de Castro i Català

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Doctoral Thesis

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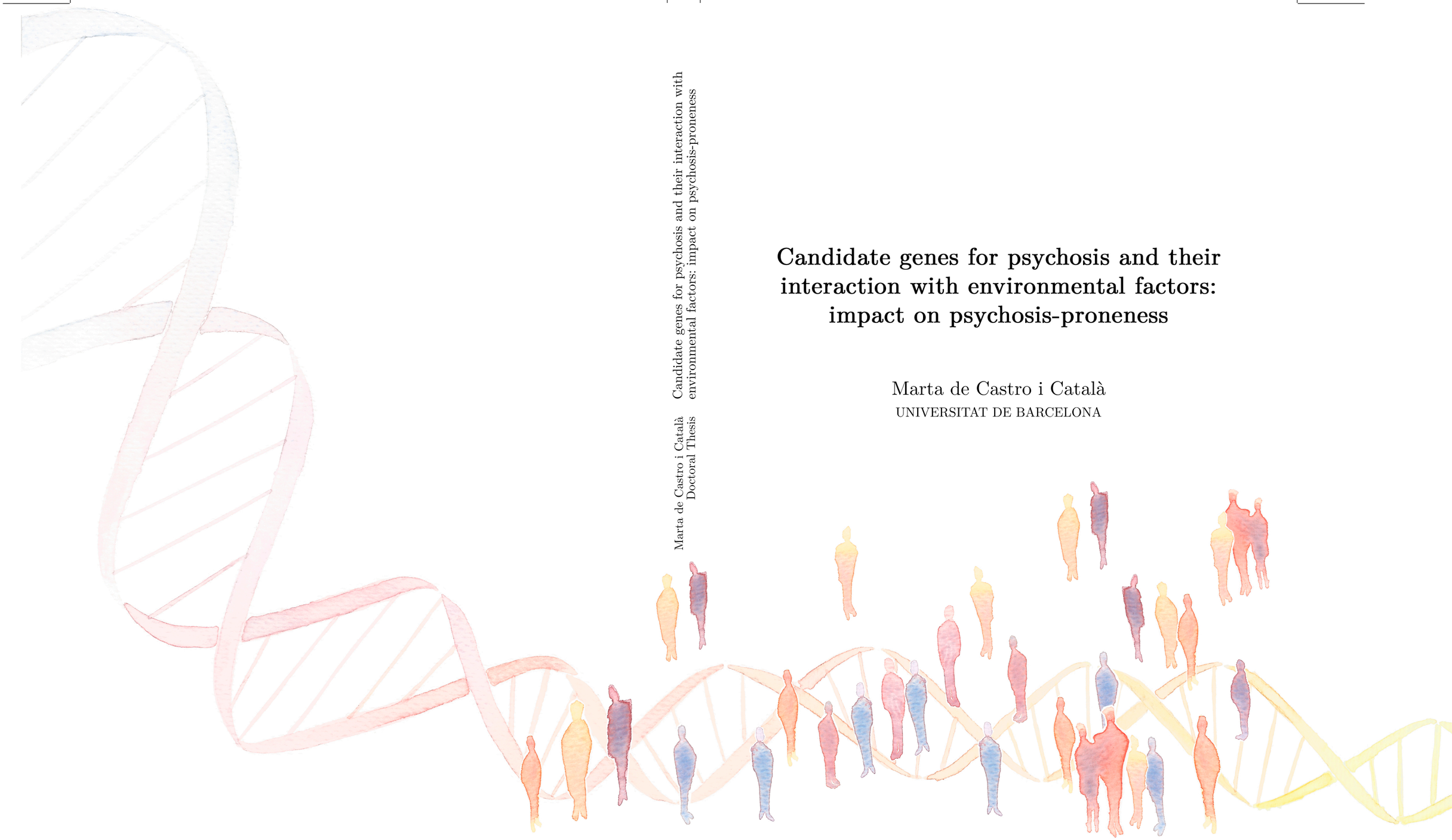
UNIVERSITAT DE BARCELONA



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Memòria presentada per

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Doctorand

Marta de Castro i Català

a tu, que ets aquí sempre

The only thing greater than the power of the mind is the courage of the heart.

- John Nash, in *A beautiful mind* -

If you hear nothing else today, please hear that there are not schizophrenics, there are people with schizophrenia. And each of these people may be a parent, may be your sibling, may be your neighbour, may be your colleague.

- Elyn Saks -

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1. Introduction

1.1. The human brain

The brain is the most complex of all organs. It controls nearly all the activities of the body by processing, integrating and coordinating all the information that receives from the sense organs, interpreting and analysing all these data and taking decisions that will be transmitted to the rest of the organism.

The human **brain development** is a protracted and complex process that begins in the first weeks of gestation and extends through postnatal periods and late adolescence (Figure 1). The major events that contribute to the development of the human brain from its early embryonic state through adolescence have been thoroughly reviewed by Stiles and Jernigan (2010). Although basic brain structures and neural connections are completely developed by late adolescence as a result of both gene expression and environmental input, the brain remains malleable and susceptible to modifications throughout life. This **plasticity**, or changes in the organization or properties of the neural circuitry, is a result of learning, experience, memory formation and also brain damage (e.g. as a result of a stroke). These changes play an incredibly important role in our brain development (or decline) and in shaping our distinct personalities and, additionally, is what allows humans to adapt and respond adequately when facing different situations during our lives.

One essential variable that highly influences the brain and has often been ignored is the sex of the individual. In fact, developmentally, the brain is programmed by default to originate a female phenotype (as the gonads) and only when the sex-determining region Y (SRY) gene on the Y chromosome is expressed, a male brain (and gonads) will be developed. The largely reported brain **differences between males and females** (Cahill, 2006; Cosgrove *et al*, 2007) are mostly established early in development by the programming effects of sex hormones (i.e. gonadal steroids). These hormones will determine the neuroanatomy and/or neurophysiology particular of each sex and will create different predispositions or weighted valences for responding to particular stimuli (i.e. particular behavioural response in one sex versus the other). Additionally, the presence of sexual hormones during puberty may have long-lasting behavioural effects (Berenbaum and Beltz, 2011). These differences in brain can be reflected also in gender-biased risk for early onset disorders, such as autism, schizophrenia, attention deficit/hyperactivity disorder, Tourette syndrome or dyslexia, among others (Aleman *et al*, 2003; McCarthy, 2016). All of these are either more frequently diagnosed in males, with earlier age of onset or more severe in symptomology, both clinically and when modelled in rodents, being in autism the most robust gender bias (diagnosed four to five times more frequently in boys). As mentioned in McCarthy (2016), there are several hypothesis for this differences, but the exact mechanisms causing them are still unknown.

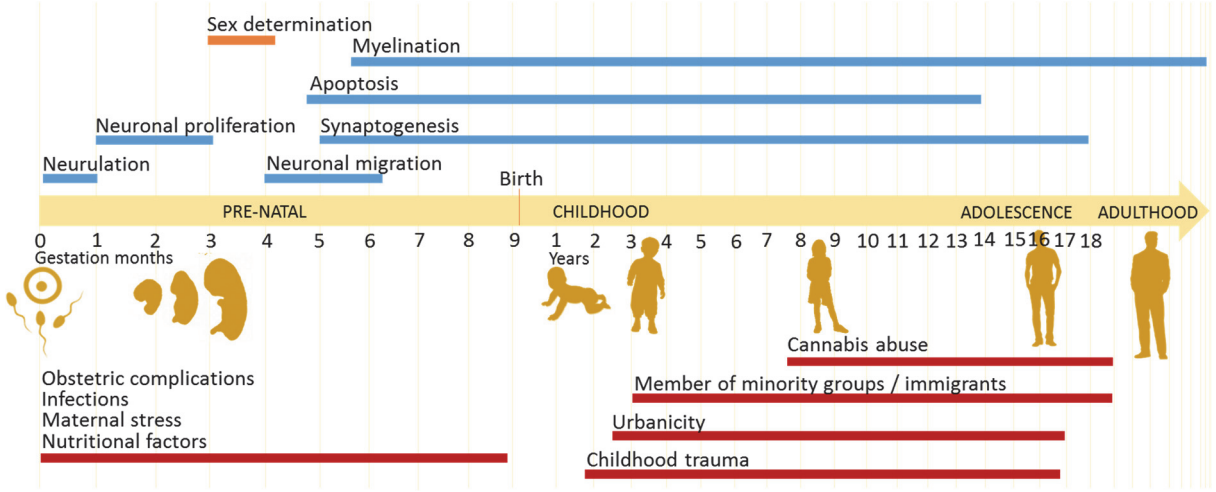

Figure 1 | Stages of brain development and windows of vulnerability. Developmental periods occurring in phases (blue and orange lines) set a stage for putative vulnerability periods (red lines). Pre-natal insults would produce important modifications on fundamental innervation patterns, and later pre-pubertal insults may cause more functional changes.

1.2. Schizophrenia

Schizophrenia is a severe mental disorder ranked among the top leading causes of disability worldwide. Despite its low prevalence, estimated to be about 1% of the population worldwide (Jablensky, 2000), its health, social and economic burden has been tremendous, not only for patients but also for families, caregivers and the wider society in general.

This illness is characterized by distortions in thinking, perception, emotions, language, sense of self and behaviour, which results in an inability to cope with life's ordinary demands and routines. It usually appears in late adolescence or early adulthood, two to three times more frequent in males than in females (Iacono and Beiser, 1992), and developing earlier in men (in the early twenties) than in women (in the mid-to-late twenties) (Shtasel et al, 1992; Szymanski et al, 1995).

Several hypothesis of the pathophysiology of schizophrenia have been proposed, attributing schizophrenia to an abnormal dopaminergic state, but also implicating altered serotoninergic and glutamatergic neurotransmission, among others (Davis *et al*, 1991; Javitt and Zukin, 1991; Matsubara and Meltzer, 1989). However, as all these systems interact, these hypotheses are not mutually exclusive, suggesting a complex picture that comprises different types of neurotransmission (e.g. glutamatergic, serotoninergic, dopaminergic, etc.) involved in the development of such illness and related traits. The fundamental causes underlying schizophrenia appear to act during the neurodevelopment rather than simply at the onset of the disorder (Lewis and Murray, 1987). In fact, many epidemiological findings show that

children who subsequently develop schizophrenia have higher rates of neuropsychological and motor deficits, and reductions in size of some brain structures that could, in principle, be of **neurodevelopmental origin** (Cannon *et al*, 2002). This neurodevelopmental origin seems reasonable taking into account that the maturation of most brain areas starts early in the embryonic stages and continues into childhood and adolescence (Lenroot and Giedd, 2006), with genetic and environmental factors determining the pathways involved in this process. Thus, disruption of these factors and pathways may cause modifications in neuronal structure, function or connectivity (Lewis and Levitt, 2002), leading to a final brain that, probably, will be more susceptible to develop psychotic or psychotic-like symptoms.

One of the major concerns of most clinicians and researchers regarding schizophrenia is that patients with this diagnosis show high diversity of symptoms between them. Moreover, one patient may present different symptoms along the course of the illness. As an example, in the case of two patients diagnosed both with schizophrenia, whereas one may have paranoid schizophrenia, characterized by positive symptoms, such as delusions and auditory hallucinations, the other one may show negative symptoms such as apathy, social withdrawal or anhedonia. It is believed that different biological mechanisms are underlying each type of symptoms (for example, hyperactivity of subcortical dopamine transmission has been related specifically with positive symptomatology (Laruelle, 2014)). In this sense, this variability intra- and inter- individual within the same diagnostic makes the exploration of the aetiology and biological pathways underlying the development and effects of the illness, as well as its treatment, substantially difficult and suggests that alternative strategies may be more helpful for the study of such disease. This is also applicable to other mental disorders, such as personality disorders.

1.3. The psychosis continuum

The psychotic phenotype has traditionally been thought as a dichotomous entity, classifying subjects as “healthy” or “ill”. However, compelling evidence shows that psychosis exists as a distribution of symptoms expressed across a dynamic continuum ranging from nonclinical (e.g. schizotypy, psychotic-like experiences), subclinical (e.g. at risk mental states for psychosis) to a range of full-blown clinical manifestations with discontinuous degree of impairment and need for care (Johns and Van Os, 2001; Kwapil and Barrantes-Vidal, 2015) (Figure 2).

This dimensional or continuum model hypothesises that the same etiological and developmental processes are underlying the continuum psychotic phenotype with varying degree of severity and dysfunction across nonclinical and clinical manifestations. Thus, the clinical definition of psychosis (e.g. schizophrenia) represents the extreme manifestation of

a continuum of cognitive, affective and behavioural features, and non-psychotic individuals with a high number of such characteristics will present an increased vulnerability for the disease and, probably, a heightened degree of shared overlapping risk factors with the illness (van Os and Kapur, 2009).

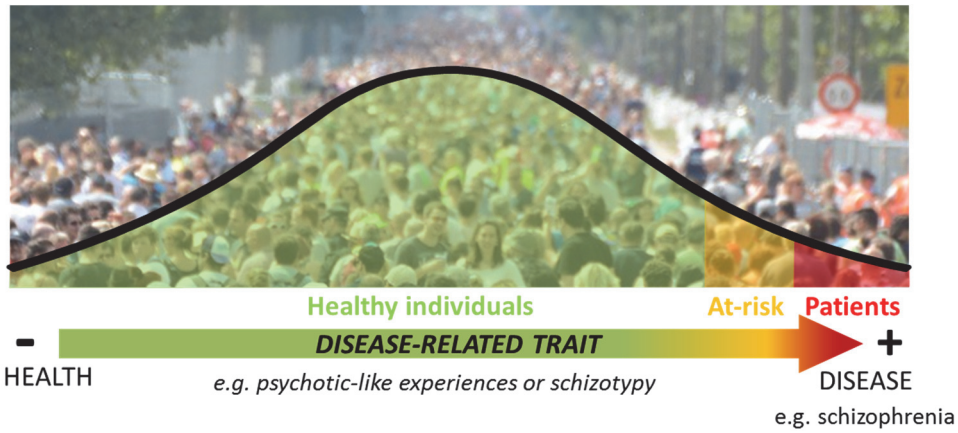

Figure 2 | The continuum hypothesis of psychosis.
From a dichotomic-categorical point of view, people from the general population can be classified as healthy or ill (e.g. schizophrenia). However, from a dimensional point of view –as when we assess quantitative traits associated with the disease (e.g. psychotic-like experiences or schizotypy)–, we can see a continuous distribution, with most individuals with low-medium scores (green), some individuals with high scores (at-risk) (orange) and patients with schizophrenia or other psychiatric disorders with the highest scores (red).

1.3.1. Schizotypy

Schizotypy is a measure of the common manifestation of the liability for psychosis-proneness from a "trait" dimension perspective. It describes a continuum of personality characteristics including magical ideation, superstition, cognitive disorganization, anhedonia and inhibition, among others, which can be found in healthy people, but are clearly increased in schizophrenia or other psychiatric disorders (Fanous *et al*, 2001).

As hypothesised by the psychosis continuum model, schizotypy shares a common multidimensional structure with schizophrenia, including the consistently replicated positive and negative dimensions (Gross *et al*, 2015; Kwapil *et al*, 2008). Similarly, the positive dimension include characteristics corresponding to an excess of a normal function (e.g. beliefs that somebody is following or controlling you), whereas the negative (or deficit-like) dimension include traits related with functional impairment features or disruptions to normal emotions and behaviours (e.g. diminished ability to feel or experience pleasure). Schizotypy has been found to predict the development of schizophrenia or schizophrenia-spectrum disorders, showing differential patterns of symptoms and impairment associated

with each dimension (Appels *et al*, 2004; Kwapil *et al*, 2008, 2013). Thus, schizotypy has been acknowledged and largely studied as an endophenotype of psychosis (Box 1).

1.3.2. Psychotic-like experiences

The vulnerability for psychosis can be measured also from a "symptom and trait" perspective, combining schizotypy traits as well as the expression of the trait in action, as psychotic-like experiences does. Psychotic-like experiences, also known in the literature as subclinical or attenuated psychotic experiences, are defined as strange and unusual experiences, similar to the ones reported by psychotic people, but attenuated. It has been estimated that about 5-6% of the healthy adult population report these experiences (McGrath *et al*, 2015; van Os *et al*, 2009). Although they are non-destabilising in the daily life, they may be a manifestation of genetic predisposition to develop schizophrenia (Kaymaz *et al*, 2012; Werbeloff *et al*, 2012), showing also shared risk factors as male sex, urbanicity or low educational level, among others, with the illness (van Os *et al*, 2000, 2009). In fact, undergoing such experiences in childhood represents an increased risk for psychosis in adult life (Poulton *et al*, 2000; Welham *et al*, 2009). Similarly as in schizophrenia, positive, negative and depressive dimension symptom scores can be measured in psychotic-like experiences. These experiences have been also recognised as valid endophenotypes of psychosis (Box 1).

Box 1 | The endophenotype concept in psychiatry

Endophenotypes or intermediate phenotypes are measurable components along the pathway between the genetic infrastructure and the presentation of a disorder (Gottesman and Gould, 2003). Endophenotypes are related with the disorder and genetically determined, but less complex and, thus, generally determined by a fewer number of genes. In other words, they are a product of the expression of certain genes involved in a more complex pathophysiological process that constitutes mental disease. The study of endophenotypes in general population samples is a promising approach for the examination of the processes involved in the development of psychopathology, but relatively free of the confounding consequences of clinical disorders such as diagnostic, medication or even hospitalization. Moreover, it has the advantage of diminishing the etiological and phenotypical complexity of the trait under study and facilitates the differentiation of dimension-specific subjacent mechanisms. Structural and morphometric brain alterations, neurocognitive deficits, schizotypal personality traits and psychotic-like symptoms are examples of intermediate phenotypes or endophenotypes of psychosis measurable throughout the psychosis continuum.

1.4. Risk factors for psychosis and related phenotypes

It is widely accepted that psychiatric disorders are multifactorial diseases and that the predisposition to develop psychopathology (including nonclinical related traits) is determined by a complex combination and/or interplay between genetic, environmental, personal and epigenetic factors. In the next sections the genetic and environmental risk factors for psychopathology, in which this thesis is focused, will be explained further.

1.4.1. Genetic factors

Evidence about the strong genetic component of psychosis has been largely provided in the literature, showing heritability estimates of about 80% in the case of schizophrenia (Cardno and Gottesman, 2000). The lifetime risk of developing psychosis is related with the degree of biological relatedness with an affected person, showing greater risks associated with higher levels of genes shared in an exponential way that clearly fits with a model of multiple genes of minor effect (Figure 3).

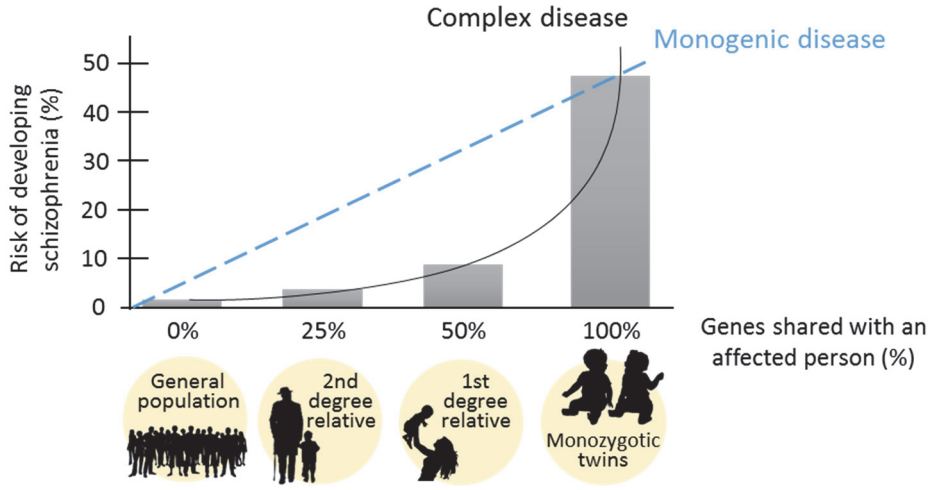

Figure 3 | Lifetime risk for developing schizophrenia according to percentage of genes shared with an affected individual.
Whereas the lifetime risk for the illness is about 1% in the general population, this risk increases exponentially depending on the percentage of genes shared with an affected individual. A lineal increase would represent a monogenic or Mendelian disease (blue line). However, this clearly fits with a model of multiple genes of minor effect or complex disease (black line). Moreover, the concordances described in monozygotic twins (risk of about 46%) suggest the involvement of environmental influences.

It is hypothesized that some allelic variations in multiple genes coding for proteins involved on neurobiological pathways (e.g. in dopaminergic, serotoninergic or glutamatergic neurotransmitter systems or pathways related with the neurodevelopment) may confer a lower efficiency on these systems. This would lead to specific pathophysiological disturbances that may impact the neural circuits and cause the development of phenotypes related with psychosis (clinical or nonclinical).

During the last decades, psychiatric genetics have tried to find the responsible genes for schizophrenia susceptibility using **candidate-gene research strategies**, which focuses on genes coding for proteins involved in pathways believed to be disrupted in schizophrenia, genes with a regulatory role on DNA expression (functional candidate genes), or genes located in genomic positions highly linked with schizophrenia (positional candidate genes). Some of the interesting classical genes that have arisen from these studies are the catechol-O-methyltransferase (*COMT*), the D-amino acid oxidase and its activator complex (*DAO* and *DAOA*), the dopaminergic receptors D1 and D4 (*DRD1* and *DRD4*), disrupted in schizophrenia 1 (*DISC1*), dysbindin (*DTNBP1*), the interleukin-1 cluster (*IL-1*), neuregulin-1 (*NRG1*) and the regulator of G-protein signalling 4 (*RGS4*) (Harrison and Owen, 2003; Owen *et al*, 2005). However, not all these findings have been replicated in independent cohorts, and the effect size of these putative schizophrenia-susceptibility genes is very low in all cases, never explaining more than a few percent of the disease cases.

The advances in our understanding of human genetic variation and the technology to measure such variation have enabled large-scale studies analysing up to a few million polymorphisms across the human genome in hundreds to thousands of samples, such as **genome-wide association studies** (GWASs). These studies have successfully identified many genetic variants (mainly single nucleotide polymorphisms, SNPs) contributing to the susceptibility for different complex diseases, such as schizophrenia (O'Donovan *et al*, 2008; Ripke *et al*, 2014). One example is the rs1344706 within the zinc-finger protein 804A (*ZNF804A*), which was the first genetic variant achieving genome-wide statistical significance (p-value $< 5 \times 10^{-8}$) in schizophrenia GWAS.

However, despite all efforts, the genetic variants discovered to date explain only a small fraction of the overall heritability of schizophrenia. For example, Ripke and colleagues showed that 8300 SNPs were contributing to the risk for schizophrenia, but they explained collectively only about 32% of the variance in liability (Ripke *et al*, 2013). One explanation of this "missing heritability" is that GWAS may not be well suited for detecting multiple variants with small effects, although this may be solved by exploring larger sample sizes or by diminishing the significance threshold or incorporating sub-thresholds (Hirschhorn and Daly, 2005). Other researchers have suggested that epistasis and gene-environment

interactions may account for at least a part of this missing heritability and should be considered (Woo *et al*, 2017).

In the past few years, new approaches have been developed with the aim to better reflect (and study) the intricacy of complex traits. For example, **polygenic-risk scores** allow the study of a huge number of polymorphisms, taking into account the relative risk associate to each one. It provides a score aggregating these risks representing a genetic risk profile (Halldorsdottir and Binder, 2017). These polygenic-risk scores also can be used to address more focused questions by exploring variants in genes involved in particular biological pathways (i.e. **pathway analyses**; Network and Pathway Analysis Subgroup of Psychiatric Genomics Consortium, 2015), and also enables the incorporation of gene-gene (e.g. Nicodemus *et al*, 2014) or gene-environment interactions (e.g. Trotta *et al*, 2016).

Another plausible explanation for the missing heritability is that genetic risk variants may not modulate the disorder itself but intermediate phenotypes related to psychosis (Box 1). There is convergent evidence showing shared genetic determinants between schizophrenia and intermediate phenotypes of the disease, such as schizotypy or psychotic-like experiences (Fanous *et al*, 2007; Stefanis *et al*, 2007). In this regard, the study of intermediate phenotypes or endophenotypes of the illness across the psychosis continuum seems to be a promising strategy in psychiatric genetics, still in the "post-GWAS" era. Some research groups have used this strategy, showing interesting results in nonclinical, subclinical and also clinical samples with genes such as the *RGS4* or the *ZNF804A* (e.g. Stefanis *et al*, 2008, 2013). The use of polygenic-risk scores and pathway analyses for the study of schizophrenia endophenotypes is also an interesting approach that some researchers have started to use, e.g. in relation to neurocognition (Hatzimanolis *et al*, 2015) or neural connectivity (Wang *et al*, 2017).

1.4.2. Environmental factors

Epidemiological studies have suggested that environmental factors also play a significant role in the aetiology of the disorder (van Os *et al*, 2010), including both biological and psychosocial adverse factors that occur during prenatal period, perinatal, adolescence or early adulthood. The ones that take place during prenatal or perinatal period –including those occurred during the intrauterine neurodevelopment, as viral infection, prenatal stress, malnutrition, infections and hypoxia– increase the vulnerability to develop psychosis for its influence in brain development. Whereas those that take place during adolescence or early adulthood might precipitate the development of the disease, as drug abuse (e.g. use of cannabis) or stressful social factors including childhood trauma, residence in urban areas, migration, etc. (van Os *et al*, 2010).

Exposure to **childhood trauma** is a robust environmental risk factor for psychosis that has demonstrated to be not merely a trigger of a genetic vulnerability, but a causal factor for psychosis (Read *et al*, 2008). It encompasses a range of adverse experiences suffered early in life, such as sexual, physical and emotional abuse, physical and emotional neglect, and other early-life events, such as the death of a parent. Childhood trauma has been strongly associated with schizophrenia, showing that individuals who had experienced childhood adversity were nearly three times more likely to exhibit psychotic symptoms (OR≈3; Varese *et al*, 2012). Patients with a child abuse history seem to be particularly likely to experience positive symptoms and to show the worst course and outcome of psychosis (Cutajar *et al*, 2010; Holtzman *et al*, 2013; Read *et al*, 2005). In the general population, associations between childhood trauma and intermediate phenotypes of psychosis such as schizotypy and psychotic-like experiences have also been reported (Arseneault *et al*, 2011; Velikonja *et al*, 2015). Particularly, Velikonja and colleagues reported in their review an association between all types of trauma and schizotypy, with ORs ranging between 2.01 and 4.15.

The exact mechanism by which childhood trauma increases the vulnerability for psychosis is not clear, although it seems that exposure to severe stress can alter the normal stress response mediated by the hypothalamus-pituitary-adrenal (HPA) axis (Figure 4) and have long-lasting effects on brain activity through a sensitization mechanism and/or aberrant neural circuit changes (Belda *et al*, 2015; De Bellis and Zisk, 2014). In this regard, alterations in HPA axis reactivity and related molecule levels have been found in patients with psychosis, as well as in subjects with schizotypal personality disorder and ultra-high risk subjects (Myin-Germeys and van Os, 2007), supporting that HPA axis function alteration may increase psychological vulnerability and predispose persons to develop psychotic symptoms.

However, despite all the evidences linking childhood trauma to psychosis-proneness, there are large individual differences in response to early stressful events (Holtzman *et al*, 2013). In fact, although some individuals develop psychosis, the majority of people who experience such stressful events seem to recover and do not develop psychopathology. The factors and mechanisms modulating and mediating an individual's risk and **resilience** (i.e. the individual successful adaptation and swift recovery after experiencing severe adversity during life) range from general environmental and sociocultural factors (e.g. high social support), to personal factors (e.g. secure attachment or positive emotions), involving also the molecular and cellular biology of an individual's neuronal circuitry (i.e. genes) (Rutten *et al*, 2013).

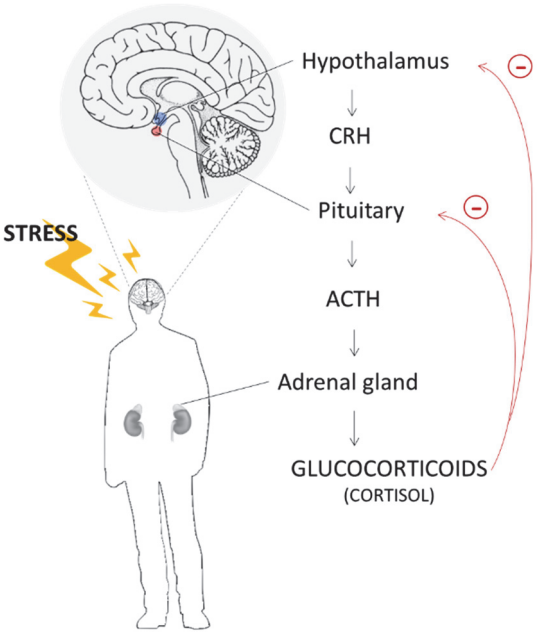

Figure 4 | The hypothalamus-pituitary-adrenal (HPA) axis.

The adaptive response to perceived stress is mediated by the HPA axis. In the presence of a stressor, corticotropin-releasing hormone (CRH) is secreted from the hypothalamus, which acts on the pituitary gland and results in the release of adrenocorticoid hormone (ACTH). This, stimulates the production and release of cortisol from the adrenal cortex, which binds to its receptor, the glucocorticoid receptor (GR), that once activated exerts a wide range of effects orchestrating the systemic stress response. Besides this, cortisol also inhibits the synthesis and release of CRH and ACTH in the hypothalamus and the pituitary, enabling a negative feedback regulation critical for the reduction of HPA axis activation and the restoration of homeostasis once the threat has subsided.

1.4.3. Gene-environment interaction

Ecogenetics is a discipline of genetics that studies genetic traits in relation to the response to environmental influences. It has demonstrated that genes modify both the exposure and sensitivity to environment and that environment impacts on gene expression and function (e.g. Kendler and Eaves, 1986; Van Os *et al*, 2008). Gene-environment interaction (GxE) may be an important variable explaining the observed differential risk to develop psychosis or related traits between individuals. In this sense, the different response against childhood adversity mentioned in the previous section would likely result from GxE. This means that the response (e.g. psychotic or psychotic-like outcome) of an individual to this environmental risk factor is moderated by genetic factors and thus, that it depends on the genetic variants that this individual carries, as represented in Figure 5 (Van Winkel *et al*, 2013).

Several theoretical models have been proposed to describe GxE. The most accepted and used framework is the **diathesis-stress model** or vulnerability-stress model (Zubin and Spring, 1977). This model stipulates that genetic vulnerability predisposes an individual to the development of a psychiatric disorder and, when a level of stressors exceeds this vulnerability threshold, symptoms emerge. In this sense, individuals carrying genetic risk variants will be more vulnerable to the effects of adverse environmental factors and thus more prone to develop psychopathology than those with the non-risk variants (Figure 5).

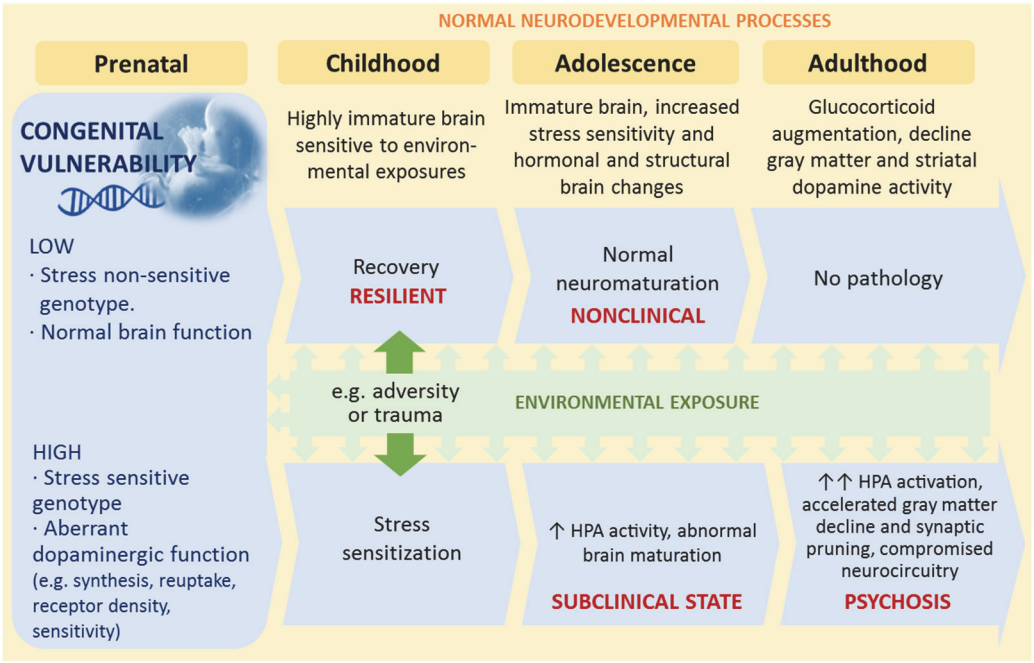

Figure 5 | GxE, stress and neurodevelopmental trajectories
The brain suffers several modifications during the normal neurodevelopmental processes. Congenital (genetic) vulnerability may determine brain structure and function. The presence of adverse environmental exposures (e.g. adversity or trauma) may lead to more changes in brain and neuronal activity which, influenced by the normal neurodevelopmental processes, will cause latent vulnerabilities (subclinical state) that will finally lead to the development of clinical symptomatology (psychosis). Adapted from Holtzman *et al*, (2013).

According to this model, polymorphic variants in genes involved in the moderation of the stress response driven by the HPA axis are thus plausible candidates mediating this GxE. For example, the FK506 binding protein 51 (*FKBP5*) gene has been studied in this regard, being observed to modulate the association between different types of childhood adversity and schizophrenia, major depression (Mihaljevic *et al*, 2016; Zimmermann *et al*, 2011), or also endophenotypes of psychosis such as amygdala reactivity, cognition and personality traits (Green *et al*, 2015; Shibuya *et al*, 2010; White *et al*, 2012). Genes involved in the neurodevelopment or in some neurotransmitter systems (e.g. *BDNF* or *COMT*) are also interesting to explore GxE in relation to childhood trauma (e.g. Alemany *et al*, 2011) or also other environmental adversities such as cannabis abuse (e.g. Caspi *et al*, 2005).

These studies are examples of the interest of exploring GxE to better understand the etiological factors underlying the psychosis-proneness phenotype. The inclusion of GxE in models exploring factors contributing to complex traits may increase the power to detect new genetic or environmental factors that influence the trait through an interaction and would not be detectable when the interaction is ignored. In this sense, the identification of

such interactions can help uncover disease-causing mechanisms, which could result in the development of new or better preventive measures.

2. Hypothesis and Objectives

Based on the background mentioned in the introduction showing i) that psychosis exists as a continuum of traits and characteristics that can be also observed in the general population in an attenuated level and ii) that environmental factors such as childhood trauma interact with genes in shaping the individual vulnerability to psychosis, the hypothesis and objectives of the present thesis are:

Hypothesis: At least a set of candidate genes for schizophrenia (G) will be underlying the vulnerability to develop psychotic-related phenotypes (P) in nonclinical subjects (G→P). Moreover, childhood trauma (E) will be associated with psychosis-proneness (P) in nonclinical subjects and genetic variability at genes involved in neurotransmission, neurodevelopment or stress-related systems will be modulating this association (GxE → P).

To explore this hypothesis, the following objectives were established:

Main objective: To explore the association between candidate genes for schizophrenia involved in the neurotransmission, neurodevelopment or stress-related systems and psychosis-proneness (section I) and to investigate the association between childhood trauma and psychosis-proneness, and the modulating effect of the mentioned genes in this association (section II) in a nonclinical sample.

Specific objectives of section I:

1) To analyse the association between genetic variability in the *COMT* gene, involved in the dopaminergic neurotransmission, and psychotic-like traits (i.e. schizotypy) and symptoms (i.e. psychotic-like experiences) in a sample of 808 nonclinical subjects.

2) To explore the association between genetic variability in the *RGS4* gene, a positional candidate gene for schizophrenia related with neurodevelopment and neurotransmission, and psychotic-like experiences in a sample of 808 nonclinical subjects.

3) To study the association between genetic variability in the genome-wide associated *ZNF804A* gene and schizotypy and psychotic-like experiences in a sample of 808 nonclinical subjects.

Specific objectives of section II:

1) To explore the association between childhood trauma and psychotic-like experiences in two independent nonclinical samples of 808 subjects and 621 female twins, and to investigate the modulating role of the genetic variability in the *BDNF* gene on this association.

2) To analyse the association between childhood trauma and schizotypy, psychotic-like experiences, depression and anxiety symptoms in a sample of 808 nonclinical subjects, and to explore the modulating role of genetic variability within the *FKBP5* gene on the mentioned associations in this nonclinical sample.

3) To review and discuss the evidences published about the modulating role of the *BDNF*, *COMT* and *FKBP5* genes on the association between childhood trauma and the continuum psychotic phenotype (i.e. psychosis and psychosis-proneness).

3. Publications

Supervisor's report on impact factor

The doctoral thesis "Candidate genes for psychosis and their interaction with environmental factors: impact on psychosis-proneness" is based on the original results obtained by Marta de Castro i Català. These results have been published or have been submitted to international peer reviewed journals. The impact factors of these journals demonstrate the quality of the research conducted, and are as follows:

1. ***COMT*-by-sex interaction effect on psychosis proneness**, published in *Biomed Research International*. This multidisciplinary journal publishes original research articles as well as review articles in several areas of life sciences. It is indexed in Journal Citation Reports (Science Edition) with a current impact factor of 2.134 and classified in the third quartile of the area of Medicine, Research and Experimental (ranking: 72/124).

2. **Association between *RGS4* variants and psychotic-like experiences in nonclinical individuals**, published in *European Archives of Psychiatry and Clinical Neurosciences*. This European journal is devoted to the publication of papers dealing with all aspects of psychiatry and related clinical neuroscience. It is indexed in Journal Citation Reports (Science Edition) with a current impact factor of 4.113 and classified in the first quartile of the area of Psychiatry (ranking: 30/142).

3. **The genome-wide associated candidate gene *ZNF804A* and psychosis-proneness: evidence of sex-modulated association**, submitted to *PLoS One* (PONE-D-17-19137). This international multidisciplinary journal features reports of original research from all disciplines within science and medicine. It is indexed in Journal Citation Reports (Science Edition) with a current impact factor of 3.057 and classified in the first quartile of the area of Multidisciplinary Sciences (ranking: 11/61).

4. **Childhood trauma, *BDNF Val66Met* and subclinical psychotic experiences. Attempt at replication in two independent samples**, published in *Journal of Psychiatric Research*. This international journal is dedicated to innovative and timely studies in psychiatry and cognate disciplines, including clinical and basic studies, as well as clinical laboratory techniques and advances in basic and

clinical research methodology. It is indexed in Journal Citation Reports (Science Edition) with a current impact factor of 4.465 and classified in the first quartile of the area of Psychiatry (ranking: 24/142).

5. **Interaction effect of *FKBP5* gene and childhood trauma on psychosis, depression and anxiety symptoms: study in a non-clinical sample**, submitted to *Psychoneuroendocrinology* (PNEC_2017_214; first revision submitted). This European journal is devoted to the publication of papers focused on understanding how a variety of psychobiological factors interact in the expression of the stress response as it relates to the development and/or maintenance of neuropsychiatric illnesses. It is indexed in Journal Citation Reports (Science Edition) with a current impact factor of 4.704 and classified in the first quartile of the area of Psychiatry (ranking: 23/142).

6. ***COMT*, *BDNF* and *FKBP5* genes as moderators of the association between childhood trauma, schizophrenia and psychosis proneness: a systematic review**, submitted to *Neuroscience & Biobehavioral Reviews* (NEUBIOREV_2017_296). This international journal publishes review articles dealing with all aspects of neuroscience, especially those related with the study of psychological processes and behaviour. It is indexed in Journal Citation Reports (Science Edition) with a current impact factor of 8.580 and classified in the first quartile of the area of Neurosciences (ranking: 16/256).

I hereby confirm the quality of the published and submitted articles.

Signed by Dr. Araceli Rosa

Barcelona, May 31st 2017

Section I: Impact of genetic variability on psychosis-proneness

3.1. *COMT*-by-Sex Interaction Effect on Psychosis Proneness

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Resum

Tant l'esquizotípia com les experiències psicòtiques es troben presents a la població general i comparteixen mecanismes etiopatogènics i factors de risc amb l'esquizofrènia, recolzant la idea de les psicosis com un fenotip continu que va des de trets atenuats no clínics fins al fenotip clínic propi de la malaltia. El gen de la *COMT* està implicat en la regulació de la dopamina al còrtex prefrontal i constitueix un gen candidat de susceptibilitat per l'esquizofrènia. Diversos estudis recents han mostrat diferències sexuals en l'impacte del genotip de la *COMT* en fenotips psiquiàtrics i cognitius, així com trets de personalitat. El present estudi va investigar l'associació entre el polimorfisme *Val158Met* (rs4680) de la *COMT* i les dimensions positiva i negativa de l'esquizotípia i de les experiències psicòtiques en una mostra no clínica de 808 individus.

Els nostres resultats van posar de manifest que el sexe (ser home o dona) modulava l'associació entre el genotip de la *COMT* i la dimensió negativa tant de l'esquizotípia com de les experiències psicòtiques. Els homes portadors de l'al · lel *Val* tendien a presentar majors puntuacions en la dimensió negativa d'ambdós trets. Els resultats del present estudi són consistents amb treballs recents que suggereixen una associació entre l'esquizotípia negativa i una disminució de la disponibilitat de dopamina prefrontal, i recolzen la idea que existeix una diferència biològica subjacent a les dimensions positiva i negativa. Addicionalment, aquests resultats recolzen evidències prèvies sobre els efectes específics de la *COMT* i que explicarien la predisposició diferencial tant per trastorns psiquiàtrics com per determinats trets de personalitat.

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Research Article

COMT-by-Sex Interaction Effect on Psychosis Proneness

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Schizotypy phenotypes in the general population share etiopathogenic mechanisms and risk factors with schizophrenia, supporting the notion of psychosis as a continuum ranging from nonclinical to clinical deviance. Catechol-O-methyltransferase (COMT) is a candidate susceptibility gene for schizophrenia that is involved in the regulation of dopamine in the prefrontal cortex. Several recent studies have reported a sex difference in the impact of COMT genotype on psychiatric and cognitive phenotypes and personality traits. The present study investigated the association of COMT Val158Met (rs4680) with psychometric positive and negative schizotypy and psychotic experiences in a sample of 808 nonclinical young adults. The main finding was that sex moderates the association of COMT genotype with the negative dimension of both schizotypy and psychotic experiences. Male subjects carrying the Val allele tended to score higher on the negative dimension of both trait and symptom-like measures. The results from the present study are consistent with recent work suggesting an association between negative schizotypy and diminished prefrontal dopamine availability. They support the idea that a biological differentiation underlies the positive and negative schizotypy dimensions. Additionally, these findings contribute to the growing literature on sex-specific effects of COMT on the predisposition to psychiatric disorders and personality traits.

1. Introduction

Functional, clinical, and genetic epidemiological studies show that many parameters of brain function and structure vary between men and women [1]. Similarly, most psychiatric disorders show sex differences in characteristics such as incidence, age at onset, clinical features, and outcome [2]. These differences are usually ascribed to the influence of sex hormones or the action of sex chromosome genes. However, there is evidence that autosomal genes may contribute to sex differences in the genetic predisposition to psychiatric phenotypes [3]. Despite this, the identity of the contributing genes is largely unknown. Several lines of evidence suggest that the catechol-O-methyltransferase (COMT) gene, which codes for an enzyme that plays an important role in the cortical dopamine metabolism, may be one such gene. It contains a functional polymorphism, a G>A substitution (rs4680), which produces a valine-to-methionine (Val/Met) substitution that influences COMT activity in the human prefrontal cortex. Thus, dopamine signalling is likely to be enhanced in Met carriers as compared to Val carriers. The deficit and excess of dopamine have been related to positive and negative schizophrenia symptoms [4]. It has been proposed that positive symptoms are due to a hyperactivity

of subcortical dopamine transmission, while negative symptoms may be caused by a deficit in dopamine transmission, particularly in the prefrontal cortex [5, 6]. However, the precise mechanisms are still not well understood.

Several studies have reported a sex difference in the impact of COMT genotype on psychiatric phenotypes and personality traits (reviewed by Harrison and Tunbridge [7, 8]), but few have explored the hypothesis of an interaction between sex and genotype [9, 10].

The association between schizophrenia and the high activity Val allele has been analysed in several studies that have culminated in different meta-analysis which showed inconsistent results [11, 12]. The influence of sex on the involvement of COMT genotype in schizophrenia vulnerability has been reported in several studies [13–16]. Candidate endophenotypes of schizophrenia, with which the disorder presumably shares a degree of overlapping genetic liability, include structural and morphometric brain alterations, neurocognitive deficits, and schizotypal personality traits or symptoms.

The underlying developmental vulnerability for schizophrenia and spectrum disorders is expressed across a dynamic continuum referred to as schizotypy or "psychosis proneness" that ranges from subclinical to clinical manifestations [17–19]. Nonpsychotic schizotypes experience similar, although attenuated, forms of the cognitive, emotional, and behavioural disturbances inherent in schizophrenia and are at heightened risk for developing schizophrenia-spectrum disorders [20]. Like schizophrenia, schizotypy is a multidimensional construct, positive and negative schizotypy dimensions being the most replicated factors [21]. According to family, twin and adoption studies [22] and prior genome-wide scans of schizophrenia and schizotypy, it seems that at least a subset of schizophrenia susceptibility genes also affect schizotypy in nonpsychotic relatives [23].

The nonclinical psychosis phenotype is observed and reliably measured at the level of schizotypy personality features (using trait-like measures) and subclinical psychotic experiences (PEs, i.e., unusual phenomena resembling clinical psychotic experiences) (using symptom-based measures). These phenotypes seem to share etiopathogenic mechanisms and risk factors with schizophrenia, thus supporting the notion of psychosis as a continuum ranging from nonclinical to clinical deviance [21, 24–28].

Evidence that the COMT Val158Met polymorphism may indeed have an impact on schizotypal endophenotypes was provided by Avramopoulos and colleagues [29]. They reported that the high activity Val allele is associated with self-reported schizotypy scores in a male population using the Perceptual Aberration Scale and the total score of the Schizotypal Personality Questionnaire. Further work showed that the Val allele was specifically related to the negative and disorganised dimensions of schizotypy [30, 31]. More recently, two independent studies analysing subjects carrying genetic liability for schizophrenia have found associations between the high activity allele and positive and negative schizotypy [32] and anhedonia (a central construct of negative schizotypy) [33]. To our knowledge, the association between COMT variability and PEs has not been analysed previously.

The present study aimed to explore (i) the association between this functional polymorphism in the COMT gene and dimensions of psychosis proneness using trait-like and symptom-based measures and (ii) sex-specific effects of the COMT on this phenotype in a sample of nonclinical young adults. We hypothesised that the Val allele of the Val158Met polymorphism, associated with diminished prefrontal dopamine availability, would be associated with higher scores on the negative dimension of both schizotypy and PEs.

2. Materials and Methods

2.1. Participants. The sample comprised 808 subjects from the general population, including 547 undergraduates enrolled in psychology courses at the Universitat Autònoma de Barcelona (UAB) and 261 students from technical training schools from Barcelona. Details of the two subsamples are given in Table 1. The final sample comprised 184 men (23%) and 624 women (77%). Males and females differed in terms of age (males: mean = 21.4, SD = 4.5; females: mean = 20.6, SD = 3.9, $P < 0.05$). Ethical approval was obtained from local research ethics committees. All participants volunteered to take part in the study and provided written informed consent. They were not preselected based upon any criteria.

2.2. Psychosis Proneness Assessment. All participants completed self-report measures assessing schizotypy and PEs. Schizotypy was assessed with the Spanish version [34] of the Wisconsin Schizotypy Scales (WSS), including the Perceptual Aberration [35], Magical Ideation [36], Revised Social Anhedonia [37], and Physical Anhedonia [38] Scales. The technical school volunteers completed the short version of the scales [39]. Exploratory and confirmatory factor analyses of the four scales reliably produce two factors, positive and negative schizotypy, that account for 80% of the variance [21]. Although the raw scores on the four scales are not comparable between the two samples, the factor structure underlying the short scales is comparable with the factor structure of the original scales (data not shown). Participants were assigned positive and negative schizotypy factor scores based upon factor loadings derived from a sample of 6137 college students [21].

PEs were assessed with the Spanish version of the Community Assessment of Psychic Experiences (CAPE) [34, 40], which evaluates three dimensions of symptoms: positive, negative, and depressive (the depressive subscale was not used in the present study). It has good validity and reliability and has been used in general population studies [41].

2.3. Genotyping. Genomic DNA was extracted from buccal mucosa on a cotton swab using the Real Extraction DNA kit (Durviz S.L.U., Valencia, Spain). DNA quality from all the samples was assessed by spectrophotometer readings (A260/280) using Nanodrop.

Table 1: Descriptive data for the undergraduate and technical training school students and comparison between the two subsamples.

	Undergraduates	Technical school	
Age (mean (SD))	20.60 (4.1)	21.20 (3.9)	$t = -2.01$ $P = \mathbf{0.045}$
Sex (men/women)	92/455	92/169	$\chi^2 = 34.13$ $P = \mathbf{0.000}$
COMT Val158Met (*n* (%))			
Val/Val	151 (28.8%)	72 (28.5%)	
Val/Met	253 (48.3%)	121 (47.8%)	$\chi^2 = 0.064$ $P = 0.969$
Met/Met	120 (22.9%)	60 (23.7%)	
Schizotypy (WSS) (mean (SD))			
Positive	−0.55 (0.8)	−0.26 (0.7)	$t = -5.33$ $P = \mathbf{0.000}$
Negative	−0.18 (0.9)	0.24 (0.9)	$t = -6.19$ $P = \mathbf{0.000}$
Psychotic experiences (CAPE) (mean (SD))			
Positive	7.24 (4.3)	9.72 (5.5)	$t = -6.96$ $P = \mathbf{0.000}$
Negative	8.92 (5.1)	9.62 (5.6)	$t = -1.69$ $P = 0.092$

WSS, Wisconsin Schizotypy Scales; CAPE, Community Assessment of Psychic Experiences. Differences were considered significant if P was below 0.05 (shown in bold).

Table 2: Descriptive data and ANCOVA statistics for the two WSS factors and CAPE subscales in relation to the COMT Val158Met genotype.

	Total sample mean (SD)	Val/Val mean (SD)	Val/Met mean (SD)	Met/Met mean (SD)	F (P)
Schizotypy (WSS)					
Positive	−0.46 (0.75)	−0.44 (0.85)	−0.48 (0.70)	−0.45 (0.74)	0.244 (0.783)
Negative	−0.04 (0.90)	−0.06 (0.90)	−0.01 (0.90)	−0.06 (0.90)	0.634 (0.531)
Psychotic experiences (CAPE)					
Positive	8.05 (4.85)	8.18 (4.84)	7.80 (4.78)	8.39 (5.01)	1.485 (0.227)
Negative	9.19 (5.26)	9.06 (4.96)	9.40 (5.45)	8.93 (5.24)	0.508 (0.602)

WSS, Wisconsin Schizotypy Scales; CAPE, Community Assessment of Psychic Experiences. Differences were considered significant if P was below 0.05.

The COMT Val158Met polymorphism (rs4680, G>A) was genotyped using TaqMan 5′ exonuclease assay (Applied Biosystems). The probe for genotyping the rs4680 was ordered through the TaqMan SNP genotyping Assays (ID: C_25746809_50) Applied Biosystems assay-on-demand service. The final volume was 5 μL, which contained 5 ng of genomic DNA, 2.5 μL of TaqMan Master Mix, and 0.25 μL of 20 genotyping assay. Polymerase chain reaction plates were read on an ABI PRISM 7900HT instrument and SDS v2.1 software (Applied Biosystems) was used for the genotype analysis of data.

2.4. Statistical Analyses. Hardy-Weinberg equilibrium for genotypic distribution was analysed using an online chi-squared Hardy-Weinberg equilibrium test calculator for biallelic markers (http://www.oege.org/software/hwe-mr-calc.shtml) [42].

All data were processed using SPSS 20.0 software. ANCOVA (analysis of covariance) was performed to test the effect of COMT Val158Met genotype (Val/Val, Val/Met, and Met/Met) on the positive and negative schizotypy and PEs scores. We also used ANCOVA to study the interaction between sex and COMT genotype on positive and negative schizotypy and PEs. The analyses were repeated comparing subjects who were carriers of at least one Val allele (Val carriers: Val/Val and Val/Met genotypes) versus Met/Met subjects.

3. Results

The total sample comprised 808 subjects. Differences were observed between the undergraduate and the technical school students in relation to age and sex. Also, the positive and negative schizotypy scores and the positive PEs scores were higher in the technical school sample than in the undergraduate student sample. See Table 1 for more details on the two groups. All the analyses were corrected by sex, age, and also considering whether individuals were from the university student sample or from the technical training school sample.

Of the whole sample, 805 individuals agreed on providing a DNA sample. COMT genotyping failed for 31 individuals. The two alleles Val and Met were present at overall frequencies of 52.8% and 47.2%, respectively. The three genotypes were Val/Val 28.7% (223/777), Val/Met 48.1% (374/777), and Met/Met 23.2% (180/777). The genotypes were in Hardy-Weinberg equilibrium. Both genotypic and allelic frequencies observed in our sample are in accordance with those found in other European populations and previous studies [30]. Mean scores and standard deviations of the WSS factors and CAPE subscales for the whole sample, as well as by genotype, are shown in Table 2.

The association of positive and negative schizotypy with the Val158Met genotype was not significant (positive schizotypy: $F = 0.244$, $P = 0.783$; negative schizotypy:

$F = 0.634$, $P = 0.531$; see Table 2). The lack of association held when analyses were conducted in the sample of males and females independently (data not shown). Two-way ANCOVA analyses (genotype∗sex) were conducted separately for positive and negative schizotypy. A significant interaction was found between COMT genotypes (Val/Val, Val/Met, and Met/Met genotypes) and sex with regard to negative schizotypy ($F_{(2,772)} = 3.924$, $P = 0.020$). No significant interaction was found for positive schizotypy ($F_{(2,772)} = 0.232$, $P = 0.793$).

The association between the two subscales of the CAPE and the Val158Met genotype was not significant (positive PEs: $F = 1.485$, $P = 0.227$; negative PEs: $F = 0.508$, $P = 0.602$; see Table 2). When analyses were conducted in males and females independently, a significant association was found in males with regard to negative PEs. Specifically, subjects with the Val/Met genotype showed higher scores than Val/Val and Met/Met subjects ($F = 3.591$, $P = 0.030$). Two-way ANCOVA analyses (genotype∗sex) were conducted separately for positive and negative PEs. A significant interaction was detected between the three COMT genotypes and sex with regard to negative PEs ($F_{(2,772)} = 4.229$, $P = 0.015$). No significant interaction was detected with positive PEs ($F_{(2,775)} = 0.152$, $P = 0.859$).

Further analyses grouping the COMT genotypes in Val carriers (Val/Val and Val/Met) and Met/Met revealed that these significant interactions resulted mostly from significant effects of the COMT Val allele in males. Males carrying Val alleles showed significantly higher scores for the negative dimension of both schizotypy (negative schizotypy: $F_{(1,772)} = 5.127$, $P = 0.024$; see Figure 1(a)) and PEs (negative PEs: $F_{(1,772)} = 8.282$, $P = 0.004$; see Figure 1(b)). Females, in contrast, did not show this pattern (Figure 1).

4. Discussion

In the current study we tested the hypothesis that the functional polymorphism Val158Met in the COMT gene would be associated with the negative dimension of the psychosis proneness phenotype in a nonclinical sample. Such phenotypes often referred to as "intermediate phenotypes" or endophenotypes with which schizophrenia presumably shares a degree of overlapping genetic liability are useful in studying the potential effect of candidate susceptibility genes for the disorder. Our main finding is that sex modulated the association between the COMT genotype and the negative dimension of both schizotypy and PEs.

These findings contribute to the growing literature on sex-specific effects of the role of COMT in the vulnerability to psychiatric or cognitive phenotypes and personality traits [10, 43–45]. In the present study, male subjects carrying the Val allele tended to score higher on the negative dimension of both trait and symptom-like measures. Our results are consistent with several studies that have investigated, at the population level, schizotypal traits as phenotypes in relation to the COMT in samples of young men [29–31]. In other nonclinical studies, although hypotheses regarding sex-specific associations were not specifically addressed, a trend towards a specific association in males was also detected [32, 46]. The association between COMT and CAPE scores has only been examined in studies of gene-environment interaction. In this regard, recent studies have shown that COMT constitutes a genetic risk factor for PEs in the context of combined exposure to childhood maltreatment and/or cannabis use [47–50]. However, the neurobiological bases of this interaction remain poorly understood.

The role of COMT in schizophrenia has been extensively studied and it seems that neither genetic variants nor the catalytic activity of the enzyme has great intrinsic influence on schizophrenia risk. However, multiple lines of evidence indicate that the high-activity COMT Val allele is associated with greater severity of negative and cognitive symptoms in schizophrenia, as well as specific endophenotypic impairments related to prefrontal deficits such as schizotypal traits [51]. In this regard, neuroimaging studies seem to point out the importance of low dopaminergic activity in the prefrontal cortex of schizophrenia patients. Specifically, the COMT Val carriers, with high enzyme activity, may have reduced dopamine levels in several brain regions and specifically in the prefrontal cortex, leading to a decrease in D1 receptors activation with subsequent impairment in cognitive tasks, such as working memory, as described in patients and healthy individuals [52]. The neurobiological mechanism underlying this association is highly debated and emerging findings from animal models seem to suggest that the significance of COMT for dopamine regulation is not limited to the prefrontal cortex [53].

The sex-specific association of the COMT gene with the negative dimension of psychosis proneness described here is in line with several studies in schizophrenia (reviewed by Godar and Bortolato [54]). In this regard, it seems that the effect of the Val158Met polymorphism on schizophrenia vulnerability is more directly related to male patients, possibly through an epistatic interaction with D1 receptors [15].

Higher COMT activity in the prefrontal cortex in men occurs despite a similar expression of the genes (i.e., mRNA and proteins) in both sexes. This dissociation between expression and activity may be explained by the ability of testosterone to increase COMT activity or the effect of estrogens. In this respect, it seems that there is a reciprocal COMT-estrogen modulation by which the COMT genotype influences the role that estrogens play in the brain, while estrogens affect COMT activity and its pathophysiological phenotype. An additional mechanism that may predict a lower COMT activity in females may be afforded by the function of catechol-estrogens and its role modulating the turnover of catecholamines. Further evidence on sexual dimorphic effects of this gene is provided by COMT knockout mouse studies. They provide evidence for an important sex- and region-specific contribution of COMT in the maintenance of steady-state levels of catecholamines in the brain and suggest a role for COMT in some aspects of emotional and social behaviour in mice [55].

The finding of an association between the COMT polymorphism and the negative dimension of the psychosis proneness phenotype provides evidence that the biological effects of the COMT gene may be relevant to the pathophysiology of schizophrenia. The balance of dopamine in

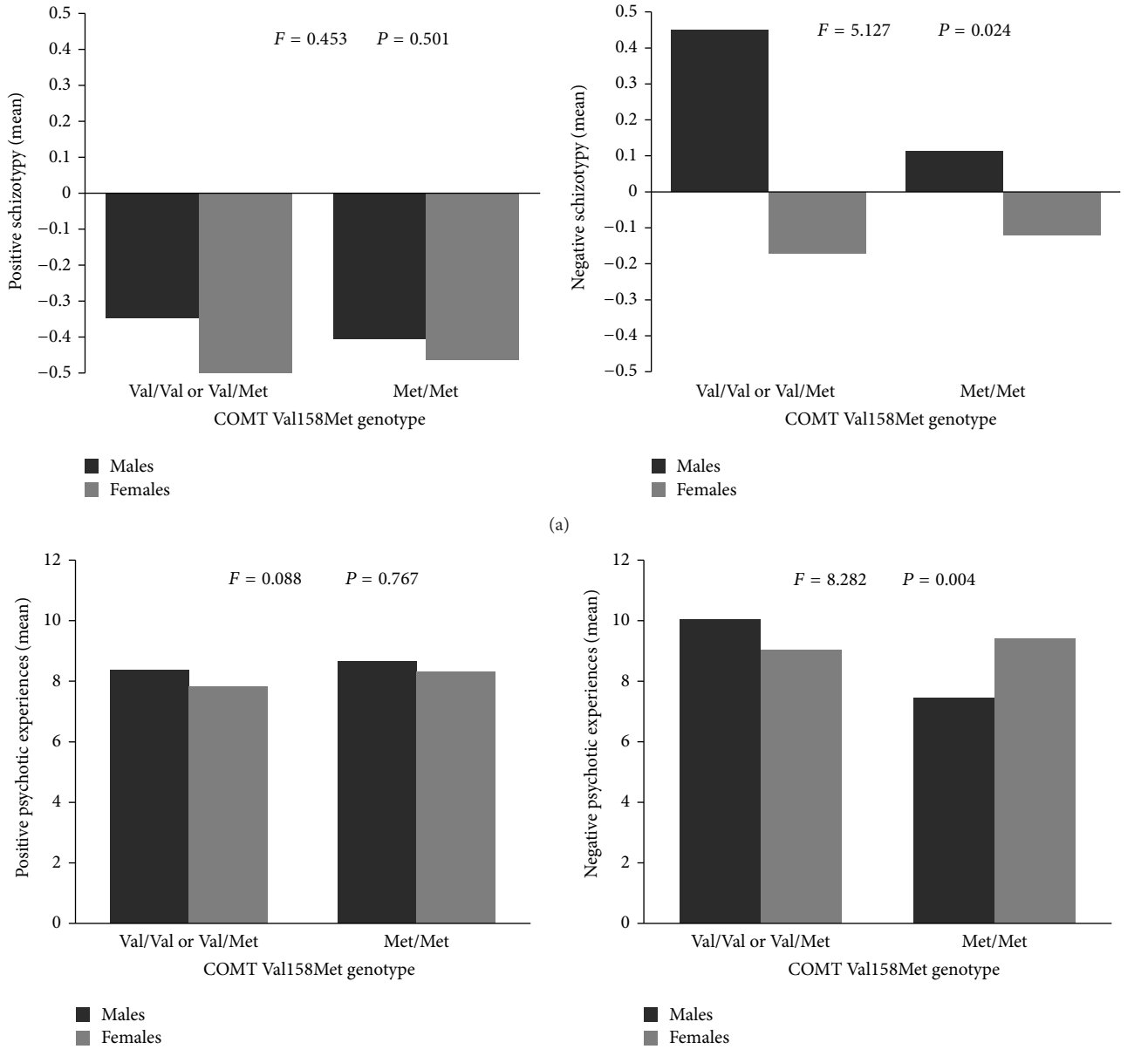

Figure 1: Mean scores of the psychosis proneness variables by sex and genotype (Val carriers versus Met/Met). (a) Positive and negative schizotypy and (b) positive and negative psychotic experiences.

prefrontal and striatal regions might be related to schizotypal characteristics evident in individuals who carry genetic liability for the condition. COMT gene is only a part of the complex neural dopaminergic system. Other dopamine genes and receptors and possibly other systems such as the serotoninergic system may all interact with the effects of this gene. The current evidence on the implication of other dopaminergic targets in gene-sex interactions is scant and mostly limited to dopaminergic receptors. Interestingly, several studies have shown that polymorphic variants in D1, D2, and D4 receptors are linked to different responses to antipsychotic medication in a gender-sensitive fashion.

The findings of this and previous studies seem to indicate that the role of gene∗sex interactions might affect brain substrates through different mechanisms with a particular impact on the catabolic action on dopamine. A comprehensive understanding of the genetic basis of schizotypy needs to consider the contributions of multiple genes and also environmental and biological factors. In this regard, the integration of preclinical research with neuroimaging and genetic studies will play a critical role enabling us to identify central neurobiological networks that underpin sex-specific, neurobehavioral endophenotypes of schizophrenia.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Supervisor's report on the contribution of the PhD candidate to the article.

Dr. Araceli Rosa de la Cruz, Senior Lecturer at the Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals of the Facultat de Biologia (Universitat de Barcelona) and supervisor of the present doctoral thesis by Marta de Castro i Català, hereby certifies that none of the co-authors of the article "*COMT*-by-Sex Interaction Effect on Psychosis Proneness" have used this publications for a doctoral thesis, and that the participation of the applicant in this article included the following tasks:

- Participation in the conception and design of the study.
- Participation in the sample collection.
- Laboratory tasks.
- Statistical analyses and interpretation of data.
- First drafting of the manuscript.
- Critical revision of the article for intellectual content.

Signed by Dr. Araceli Rosa

Barcelona, May 31st 2017

3.2. Association between *RGS4* variants and psychotic-like experiences in nonclinical individuals

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Resum

El fenotip de les psicosis s'expressa al llarg d'un *contínuum* que comprèn des de trets de personalitat i símptomes subclínics fins a la psicopatologia severa. L'estudi de les manifestacions subcliniques en individus no afectats minimitza els factors de confusió associats amb el fenotip clínic i facilita la diferenciació de mecanismes etiològics específics de cada dimensió. L'objectiu del present estudi va ser investigar l'associació entre la variació en el gen *RGS4*, un gen candidat per psicosi prèviament associat amb endofenotips de l'esquizofrènia i experiències psicòtiques atenuades. En total, 808 individus sans van completar el qüestionari CAPE (de l'anglès *Community Assessment of Psychic Experiences*) per mesurar experiències psicòtiques atenuades (dimensions positiva i negativa) i van proporcionar una mostra d'ADN. Es van genotipar dos polimorfismes d'un sol nucleòtid (SNP) del gen *RGS4* (rs951436 [SNP4] i rs2661319 [SNP18]). Es van utilitzar anàlisis de la covariància (ANCOVA) per explorar l'associació entre les dimensions positiva i negativa de les experiències psicòtiques atenuades i la variació del gen *RGS4*.

Els nostres resultats van mostrar una associació d'ambdues dimensions amb els dos polimorfismes estudiats, de manera que els individus amb l'al · lel *T* (SNP4) i l'al · lel *A* (SNP18) mostraven majors puntuacions tant per la dimensió positiva com la negativa. Les anàlisis haplotípiques recolzaven aquests resultats, mostrant puntuacions majors en aquells individus portadors de l'haplotip *TA* (SNP4-SNP18). Aquests resultats posen de manifest que les variants de l'*RGS4* podrien tenir un efecte en la vulnerabilitat per patir psicosi.

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ORIGINAL PAPER

Association between *RGS4* variants and psychotic-like experiences in nonclinical individuals

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Abstract The psychosis phenotype is expressed across a continuum known as schizotypy, which ranges from personality variation through subclinical symptoms to severe psychopathology. The study of subclinical manifestations in non-affected individuals minimizes confounding factors associated with the clinical phenotype and facilitates the differentiation of dimension-specific etiological mechanisms. The aim of the present study was to investigate the association between the variation in the regulator of G-protein signaling 4 (*RGS4*) gene, a putative candidate gene for psychosis previously associated with schizophrenia endophenotypes, and psychotic-like experiences (PLEs). In total, 808 healthy individuals completed the community assessment of psychic experiences (CAPE) to measure positive and negative PLEs and provided a DNA sample. Two *RGS4* single-nucleotide polymorphisms (SNPs) (rs951436 [SNP4] and rs2661319 [SNP18]) were genotyped. Analyses of covariance (ANCOVA) were used to explore the association of positive and negative PLEs with *RGS4* variation. Our results showed associations of positive and negative PLEs with the two polymorphisms studied: subjects with the T allele (SNP4) and the A allele (SNP18) had higher scores on both the positive and the negative dimensions. Haplotypic analyses supported these results, showing the highest scores in those with the TA haplotype (SNP4-SNP18). The *RGS4* variants might exert gene-specific modulating effects on psychosis proneness.

Keywords Psychotic-like experiences · Schizotypy · Psychosis proneness · *RGS4* gene

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Introduction

The regulator of G-protein signaling 4 (*RGS4*) gene codifies for a protein that modulates the intensity and duration of signal transduction via G proteins coupled to receptors on plasma membranes [1]. It accelerates the hydrolysis of GTP back to GDP, effectively ending the activation of a variety of signaling cascades. This protein is highly expressed in the prefrontal cortex (PFC) and has been found to be strategically positioned to regulate several aspects of neurotransmission, such as synaptic and volume transmission as well as internal calcium availability [2]. Thus, it seems that alterations in this protein could compromise many fundamental aspects of PFC physiology and cause modifications in several neurotransmitter systems.

The first evidence of the involvement of the *RGS4* in the pathogenesis of schizophrenia came from a microarray gene expression study showing that the expression of this gene was the most consistently reduced of the over 7800 genes explored [3]. In addition, this gene is located in the chromosome 1q23, a region highly linked with schizophrenia

[4, 5], as well as delusions and hallucinations [6]. Several association studies on schizophrenia have reported modest associations for certain *RGS4* single-nucleotide polymorphisms (SNPs) in independent samples of different geographic origin (e.g., [7–9]). However, following initial positive reports, negative results have also been found (e.g., [10, 11]). Given the inconsistencies observed among studies, three meta-analyses explored the variation of *RGS4* in relation to schizophrenia, but they found either no association or modest effects [12–14]. Notably, Talkowski and colleagues undertook an *RGS4* genotype-based meta-analysis using both published and unpublished schizophrenia family-based and case–control datasets [12]. They found significant over-transmission of two different *RGS4* haplotypes to patients in the family-based samples and an association between rs951436 [SNP4] and schizophrenia in their case–control exploration. Moreover, in the latest 'SZGene' meta-analysis, the *RGS4* rs2661319 polymorphism [SNP18] was associated with schizophrenia [15].

A plausible explanation for the inconsistent findings in the genetic association studies is that risk variants may not modulate the disorder itself, but intermediate phenotypes related to psychosis risk, such as schizotypal traits [16]. Research suggests that the psychosis phenotype is expressed across a continuum known as schizotypy, which ranges from personality variation through subclinical symptoms to severe psychopathology (e.g., [17]). Psychotic-like experiences (PLEs) are one such subclinical manifestation and refer to transient and/or attenuated forms of the full-blown symptoms exhibited by individuals with psychosis. Schizotypy traits and PLEs are presumed to be behavioral indicators of the underlying vulnerability for psychosis, as validated by their association with prodromal symptoms (e.g., [18]), cognitive impairment (e.g., [19]), daily-life malfunctioning (e.g., [20]) and prediction of schizophrenia spectrum disorders (e.g., [21]). Therefore, the study of subclinical manifestations in non-affected individuals minimizes confounding factors associated with the clinical phenotype and facilitates the differentiation of dimension-specific etiological mechanisms [22].

Interestingly, common genetic background that has emerged from genome-wide association studies (GWAS) of psychosis has also been associated with psychosis-related intermediate phenotypes such as cognitive performance, brain morphology, schizotypal traits and PLEs [23, 24]. In this regard, variation in *RGS4* has been also associated with structural brain alterations and functional activation and connectivity [25, 26], as well as schizotypal personality traits [16]. In this last study, Stefanis and colleagues found that common *RGS4* variants were associated with negative schizotypal personality traits in a large cohort of young healthy males. These findings concur with previous results, suggesting that at least a subset of schizophrenia susceptibility genes also affects subclinical psychosis [22].

The present study aimed to explore the association between the two most replicated *RGS4* polymorphisms (rs951436 [SNP4] and rs2661319 [SNP18]) and PLEs in nonclinical subjects. Given previous findings in psychosis, psychosis proneness and neuroimaging reports, we hypothesized that common risk alleles or haplotypes would be associated with positive and negative PLEs.

Methods

Sample

The sample consisted of 808 unselected individuals (23 % men, 77 % women; mean age = 20.79 years SD = 4.06) that volunteered to take part in the study and provided informed consent at assessments. The sample included 547 undergraduates from the Universitat Autònoma de Barcelona (UAB) and 261 students from technical training schools in Barcelona. Subjects were 93 % of European origin (subjects and both parents born in Europe), and only 7 % were of non-European origin (subjects with both parents born in non-European countries). Ethical approval was obtained from local research ethics committees. Further details of this sample can be found elsewhere [18, 27].

Psychometric assessment

PLEs were assessed using the Spanish version of the community assessment of psychic experiences (CAPE) [28], a self-report questionnaire that measures lifetime prevalence of PLEs, evaluating three dimensions of symptoms: positive, negative and depressive (note that the depressive dimension was not used in this study). It has good validity and reliability and has been used in general population studies [29, 18].

Genetic analyses

Participants provided buccal mucosa on a cotton swab from which we extracted genomic DNA using the Real Extraction DNA Kit (Durviz S.L.U., Valencia, Spain). The two single-nucleotide polymorphisms of the *RGS4* included in the study (rs951436 and rs2661319) were genotyped using TaqMan 5′ exonuclease assay (Applied Biosystems). The final volume of the PCR was 5 μL, which contained 5 ng of genomic DNA, 2.5 μL of TaqMan Master Mix and 0.125 μL of 40× genotyping assay (assays C_9619634_10 and C_16265745_10, respectively). The cycling parameters were as follows: 95 °C for 10 min followed by 40 cycles of denaturation at 92 °C for 15 s and annealing/extension at 60 °C

Table 1 Association analyses between *RGS4* variation (SNP4—rs951436 and SNP18—rs2661319) and positive and negative dimensions of psychotic-like experiences (PLEs)

	Allele	*n* (%)	Positive PLEs mean (SD)	Negative PLEs mean (SD)
SNP 4	GG	182 (23.98)	7.41 (4.39)	9.24 (4.91)
	GT	359 (47.30)	7.88 (4.89)	8.72 (5.04)
	TT	218 (28.72)	8.76 (5.01)	9.95 (5.88)
	F(*p*)		*5.89* (*0.003*)	*4.86* (*0.008*)
SNP 18	AA	197 (26.48)	8.64 (5.06)	9.72 (5.93)
	GA	350 (47.04	7.91 (4.93)	8.65 (4.75)
	GG	197 (26.48)	7.76 (4.62)	9.51 (5.09)
	F(*p*)		*3.99* (*0.019*)	*4.90* (*0.008*)

ANCOVA, *F*(*p* value) are in italic

for 1 min. Polymerase chain reaction plates were read on an ABI PRISM 7900HT instrument with SDS v2.1 software (Applied Biosystems). For accuracy of genotyping, 20 % of the samples (chosen randomly) were genotyped twice.

Statistical analyses

All data were analyzed using Stata v.13.1 (Stata/MP 13.1 for Windows, StataCorp LP, College Station, USA). Independent analyses of covariance (ANCOVA) were performed to explore the association of each SNP with PLEs dimensions. Age, sex and sample (whether they were undergraduate or technical school students) were added as covariates in all the analyses. When ANCOVAs yielded significant effects, pair-wise comparisons were computed to detail differences between genotypes.

Haplotypes were reconstructed with PHASE 2.1 [30] including the two polymorphisms ($D' > 0.9$). As each subject carries two haplotypes (one inherited from the father and the other from the mother), the combination of haploypes (diplotype) was calculated for each participant. ANCOVAs were performed using the positive and negative PLEs dimensions as dependent variables in separate models and the diplotype as the independent variable. Age, sex and sample were added as covariates. Additionally, pair-wise comparisons were computed to detail differences between diplotypes when significant associations were found.

Results

Descriptives

Of the 808 participants, one subject did not complete the CAPE positive dimension items correctly and four did not complete the CAPE negative dimension items. Three subjects did not agree on providing a buccal sample. The genotype frequencies were SNP4: GG 182 (23.98 %), GT 359 (47.30 %) and TT 218 (28.72 %) and SNP18: GG 197 (26.48 %), GA 350 (47.04 %) and AA 197 (26.48 %). Both polymorphisms were in Hardy–Weinberg equilibrium ($p > 0.1$).

Single-marker association analyses

In the association analyses between the three genotypes of each SNP and PLEs, our results showed significant associations of both SNPs with positive (SNP4 $F = 5.89$ $p = 0.003$ and SNP18 $F = 3.99$ $p = 0.02$) and negative PLEs (SNP4 $F = 4.86$ $p = 0.008$ and SNP18 $F = 4.90$ $p = 0.008$; Table 1). Post hoc analyses indicated that subjects with the TT genotype in SNP4 showed higher scores on the positive dimension than those with the GT and GG genotypes ($p = 0.009$ and $p = 0.008$, respectively). No differences were observed between GT and GG carriers. Regarding the negative dimension, individuals with the TT genotype showed higher scores than those with the GT genotype ($p = 0.006$). For the SNP18, subjects with the AA genotype scored higher on positive PLEs than GA subjects ($p = 0.02$). A trend was also observed toward higher scores on the positive dimension for AA as compared to GG subjects ($p = 0.062$). As for the negative dimension, AA subjects showed higher scores than GA subjects ($p = 0.012$).

Haplotype association analyses

Haplotypes were calculated including the two polymorphisms analyzed (SNP4 and SNP18), which were in high linkage disequilibrium (LD) ($D' = 0.94$). The haplotypic frequencies observed were: TA 48.2 % ($n = 779$), TG 3.8 % ($n = 61$), GG 46.5 % ($n = 751$) and GA 1.5 % ($n = 25$). The most frequent haplotypes were those including: (i) the risk alleles (T for SNP4 and A for SNP18) and (ii) protective alleles (G for SNP4 and G for SNP18), according to the single-marker analyses. Haplotypic combinations (diplotypes) were calculated for each subject. The most frequent diplotypes were TA/GG (43.94 %), TA/TA (24.51 %) and GG/GG (22.28 %); the other combinations showed frequencies lower than 5 % (i.e., TA/TG, TA/GA, GG/TG, GG/GA, TG/TG, TG/GA and GA/GA). To better examine the effect of each diplotype, subjects were divided into three groups: (1) carriers of at least one risk haplotype (i.e., TA/-, excluding those who were TA/GG), (2) carriers of at least one risk haplotype and one protective haplotype (TA/GG) and (3) carriers of at least one protective haplotype (GG/-, excluding those TA/GG). Subjects who did not carry a diplotype containing the TA or the GG haplotype (1.35 %) were excluded from the analyses.

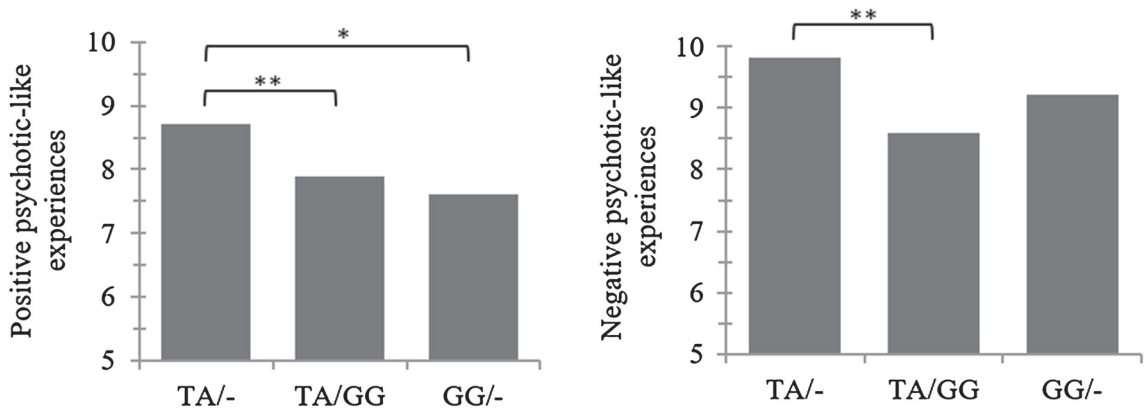

Fig. 1 Mean values for positive and negative psychotic-like experiences in relation to *RGS4* diplotypes (SNP4-SNP18) and pair-wise comparisons between diplotypes (* $p < 0.05$, ** $p < 0.01$)

The analyses showed significant associations between diplotypes and positive ($F = 5.35$ $p = 0.005$) and negative PLEs ($F = 5.36$ $p = 0.005$). Subjects carrying at least one TA haplotype (i.e., TA/-) scored higher than TA/GG and GG/- subjects on positive PLEs ($p = 0.01$ and $p = 0.016$, respectively). With respect to the negative dimension, subjects with the TA/- diplotype had higher scores than TA/GG subjects ($p = 0.006$) (see Fig. 1).

Discussion

Our results pointed out an association of positive and negative PLEs with two *RGS4* polymorphisms. Concretely, subjects carrying the T allele of SNP4 and/or the A allele of SNP18 showed the highest scores on PLEs, and this was confirmed in both the single-marker and the haplotypic analyses.

The *RGS4* gene has been widely studied in relation to schizophrenia in a series of replication studies and also in brain imaging endophenotypes, in which concordant but also discordant results were observed (e.g., [12, 19]). However, to our knowledge, this is the first study analyzing PLEs in relation to the variability of the *RGS4* in non-clinical subjects. PLEs can be regarded as the behavioral expression of liability for psychotic disorders, with high levels of PLEs having been associated with an increased risk of the development of clinical schizophrenia spectrum disorders, probably determined by the interaction of risk genetic factors with environmental exposures [31]. Thus, the study of such endophenotype may provide a better understanding of psychosis regarding its etiology, risk and expression [22].

The only published study on *RGS4* variability and the psychosis proneness phenotype is the one by Stefanis and colleagues, in which they explored the association between this gene and four schizotypal latent dimensions of the Schizotypal Personality Questionnaire (SPQ) (i.e., cognitive/perceptual, negative, disorganization and paranoid factors) [16]. It is worthwhile noting that both the SPQ cognitive/perceptual (or positive) dimension and the CAPE positive dimension as well as the negative dimension in both measures tap highly similar contents and are thus readily comparable. Stefanis et al.'s results showed an association between the negative, but not the positive, schizotypy factor and the same alleles and haplotypes reported in the current study. Negative schizotypy traits in the general population—similar to their illness counterpart, negative clinical symptoms—include several psychopathological constructs, probably reflecting the dysfunction of different neurobiological substrates. Although their underlying mechanisms are still unknown, several lines of evidence suggest that negative symptoms are associated with prefrontal deficits or damage: lower volumes, decreased metabolism and lower dopaminergic transmission [32]. The association of the *RGS4* gene with the negative dimension in its clinical and subclinical (i.e., schizotypy and PLEs) manifestations could indicate a contribution of the *RGS4* variants to the mentioned alterations in the prefrontal brain areas. This is supported by research on brain structure and function of first episode schizophrenia patients [25] and healthy volunteers [26]. Both studies showed lower volumes in the dorsolateral PFC in subjects carrying one or two copies of the T allele (SNP4) and also in first episode patients carrying one or two copies of the A allele (SNP18).

Regarding the association reported here between positive PLEs and *RGS4* variation, a previous study in schizophrenia spectrum disorders found that the 1q21-22 locus—where the *RGS4* gene is located—was associated with the reality distortion syndrome, characterized by delusions

and hallucinations (positive symptoms) [6]. The positive dimension is hypothesized to result from a common final pathway characterized by striatal hyperdopaminergia, based on findings that higher dopamine metabolite levels are related to greater symptoms and response to antipsychotic drug treatment [33]. The spatial expression pattern of RGS4 in the adult brain matches closely the dopaminergic and muscarinic G-protein-coupled receptor subtypes targeted by antipsychotic medications [34], suggesting a potential modulatory role of RGS4 in dopamine signaling, synaptic plasticity and motor behavior [35]. However, the exact biological mechanism by which the RGS4 is related to dopamine release is not still clear. Regarding its role in neurodevelopmental processes, RGS4 protein was found to be associated with neuronal differentiation in embryonic mice [36]. Moreover, zebrafish studies showed it to be essential for normal axon development during embryogenesis [37]. Alterations during neurodevelopment affect adult structure and function of the brain and may cause mental disabilities or other neurological problems such as psychosis. Although the *RGS4* SNPs studied here are not functional, SNP4 is located in a 5′ region of the gene that may be a putative promoter or regulator region. Regarding SNP18, it is located in an intronic region, and thus, it may determine splice variants. Thus, it cannot be excluded that these polymorphisms may have themselves an effect on the final protein structure and/or expression, affecting somehow the normal signaling pathways in which this protein is involved and leading to an abnormal signal transduction in neurons.

It is well accepted that, as in all complex diseases, there are no schizophrenia-predisposing genes with large effect sizes and thus that the effect of a single gene, such as the *RGS4*, cannot not be the only contributor to the final phenotype [38]. The RGS4 is an example of a molecule that may underlie increased vulnerability through different mechanisms by, probably, interacting with other proteins with subtle functions and the environment and may form a complex sum of subtle effects that produce an increased risk of psychotic phenomena.

Some limitations of this study should be mentioned. Firstly, only two RGS4 polymorphisms were studied, which do not represent the whole gene variation. In this regard, the observed associations may exist due to other risk variants at RGS4 that are in strong linkage disequilibrium with the two analyzed polymorphisms. Secondly, although RGS4 was a prominent candidate gene for schizophrenia before the GWAS era, as reported in a recent review analyzing candidate genes in light of the new mega-analyses in schizophrenia, the RGS4 gene is no longer considered to be significantly associated with the disorder [39]. However, the findings of the current study seem to indicate the possible involvement of RGS4 variants in modulating endophenotypes associated with schizophrenia rather than risk of the disease itself. These common variants might exert gene-specific modulating effects on psychosis proneness. Replication studies in independent community samples would be critical to further validate these findings.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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Supervisor's report on the contribution of the PhD candidate to the article.

Dr. Araceli Rosa de la Cruz, Senior Lecturer at the Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals of the Facultat de Biologia (Universitat de Barcelona) and supervisor of the present doctoral thesis by Marta de Castro i Català, hereby certifies that none of the co-authors of the article "Association between *RGS4* variants and psychotic-like experiences in nonclinical individuals" have used this publications for a doctoral thesis, and that the participation of the PhD applicant in this article included the following tasks:

- Participation in the conception and design of the study.
- Participation in the sample collection.
- Laboratory tasks.
- Statistical analyses and interpretation of data.
- First drafting of the manuscript.
- Critical revision of the article for intellectual content.

Signed by Dr. Araceli Rosa

Barcelona, 31st May 2017

3.3. The genome-wide associated candidate gene ZNF804A and psychosis-proneness: evidence of sex-modulated association

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Submitted to PLoS One

Resum

El gen que codifica per la proteïna ZNF804A és un prometedor gen candidat per esquizofrènia i l'ampli fenotip de les psicosis que ha sorgit dels estudis d'associació de tot el genoma (GWAS). Aquest gen està relacionat amb el neurodesenvolupament i s'ha trobat associat amb símptomes severs de la malaltia, alteracions estructurals del cervell, així com trets de personalitat esquizotípica de tipus positiu en mostres no clíniques. Addicionalment, alguns estudis han observat una associació entre el gen i la malaltia específicament en dones.

En el present estudi es va examinar l'associació entre dos polimorfismes del gen *ZNF804A* (rs1344706 i rs7597593) i la dimensió positiva de l'esquizotípia i les experiències psicòtiques atenuades en una mostra no clínica de 808 individus. A més, vam voler explorar si les diferències sexuals reportades en esquizofrènia es podien trobar també en el fenotip de vulnerabilitat a psicosi.

Els nostres resultats mostren una associació entre el marcador rs7597593 i l'esquizotípia. Aquesta associació era particularment forta en dones, de manera que aquelles portadores de l'al · lel C mostraven majors puntuacions en comparació amb les homozigotes TT. De manera similar, es va poder veure la tendència a una associació entre aquest polimorfisme i les experiències psicòtiques atenuades en dones, mostrant que les portadores de l'al · lel C eren les que presentaven puntuacions majors.

Els resultats d'aquest estudi recolzen la importància de l'estudi de la variabilitat del gen *ZNF804A* pel que fa al desenvolupament de psicopatologia en mostres no clíniques i la consideració del sexe com a moderador d'aquesta associació.

The genome-wide associated candidate gene *ZNF804A* and psychosis-proneness: evidence of sex-modulated association

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Abstract

The Zinc finger protein 804A (*ZNF804A*) is a promising candidate gene for schizophrenia and the broader psychosis phenotype that emerged from genome-wide association studies. It is related to neurodevelopment and associated to severe symptoms of schizophrenia and alterations in brain structure, as well as positive schizotypal personality traits in non-clinical samples. Moreover, a female-specific association has been observed between *ZNF804A* and schizophrenia.

The present study examined the association of two *ZNF804A* polymorphisms (rs1344706 and rs7597593) with the positive dimension of schizotypy and psychotic-like experiences in a sample of 808 non-clinical subjects. Additionally, we wanted to explore whether the sexual differences reported in schizophrenia are also present in psychosis-proneness.

Our results showed an association between rs7597593 and schizotypy. This association was driven by females, such those carrying the C allele had higher positive schizotypy scores compared to TT allele homozygotes. Similarly, this polymorphism was associated with psychotic-like experiences at a trend level in females, with C allele carriers having the highest symptom ratings.

The findings of the present study support the inclusion of *ZNF804* variability in studies of the vulnerability for the development of psychopathology in non-clinical samples and consideration of sex as a moderator of this association.

Keywords: ZNF804A gene, psychosis, psychotic-like experiences, schizotypy.

1. INTRODUCTION

Genome-wide association studies (GWAS) have identified thousands of genes and genetic variants contributing to the development of complex diseases, although the biological mechanisms by which most of these genes act remains unclear. Similarly, GWAS in schizophrenia have detected numerous candidate loci [e.g.: 1,2]. The first gene that has achieved genome-wide level of statistical significance in schizophrenia GWAS is the Zinc finger protein 804A (*ZNF804A*). Despite the substantial genetic evidence of two single-nucleotide polymorphisms (SNPs) within this gene (i.e. rs7597593 and rs1344708) [1,3], the function of the protein and the molecular mechanisms responsible for enhancing risk for psychosis remain unknown.

Further research showed that this gene was related to neurodevelopment and plasticity, influencing the expression of genes involved in cell adhesion and important processes such as neural migration, neurite outgrowth and synapse formation [4,5]. ZNF804A is expressed in the brain and contains a C2H2-type domain associated with the zinc-finger protein family with a role in transcription. More recent evidences suggested that ZNF804A localizes to synapsis and that it plays a role in neurite formation, maintenance of dendritic spines, and activity-dependent structural plasticity [6], which are found to be altered in brains of schizophrenia patients. Additionally, the expression of *ZNF804A* has been observed to peak in both the rat and human brain during the prenatal period when neuronal migration ends and neuronal differentiation and maturation begins [7,8]. Empirical work and predictive bioinformatic analyses have suggested that the two GWAS-associated polymorphisms (rs7597593 and rs1344708) may modify the affinity of the gene sequence for DNA- and/or RNA-binding proteins, which might in turn alter the expression levels of the gene [9]. In this sense, for example, the A allele of rs1344706 has been associated with lower ZNF804A expression during the second semester of fetal brain development [8].

In relation to the clinical phenotype of schizophrenia, this gene seems to influence the expression of several genes associated to the positive dimension of the illness [10,11], which includes psychotic symptoms such as hallucinations and delusions. Moreover, the A allele on rs1344705 was found related to elevated manic symptoms in psychotic patients [12], more severe symptoms in schizophrenia spectrum disorder patients [13], as well as poorer clinical outcome in first episode patients [14]. This allele has also been associated with altered brain macro- and micro-structure in healthy people, first episode patients, and patients with chronic schizophrenia or bipolar disorder [13,15–17]. The association of *ZNF804A* appears to be moderated by sex. Specifically, rs7597593 was strongly associated with schizophrenia in women, but not in men [18]. However, this sex differential associations

need more attention, because the other study exploring this did not find sex moderation [3].

Convergent evidence has shown shared genetic determinants between schizophrenia and intermediate phenotypes of the disease, such as functional brain alterations, neurocognitive deficits, schizotypal personality traits or psychotic-like experiences, which are observable across the psychosis continuum (i.e. non-clinical, subclinical and clinical samples) [19,20]. In this regard, several neurocognitive and neuroimaging studies pointed out that *ZNF804A* variability was associated with altered functional connectivity, relatively less impaired neuropsychological performances and reduced activation during measures of social cognition (e.g.: [13,21].

Only two previous studies have explored the effect of the *ZNF804A* gene on psychosis proneness in non-clinical samples. The first study, observed that subjects carrying the T allele of rs1344706 were showing higher schizotypy scores than those CC, specifically in the case of the disorganization schizotypy factor [22]. A more recent study, in a large cohort of 1507 healthy young male conscripts, found that the C alleles of both rs7597593 and rs1344706 were associated to schizotypy, specifically to self-rated paranoia and ideas of reference. The rs7597593 C allele was also associated with higher perceptual aberration and positive psychotic-like experiences [23]. Both studies found an effect for this gene in their non-clinical samples, although the results reported for the rs1344706 are controversial in terms of the allele conferring risk.

Considering the contradictory findings from the above mentioned studies, the aim of the present study was to study the association between two *ZNF804A* polymorphisms (rs7597593 and rs1344706) and schizotypy and psychotic-like experiences in a sample of 808 non-clinical subjects. We hypothesize that *ZNF804A* variability will be associated with the positive dimension of both traits. Moreover, given the differential sex results observed in the literature with schizophrenia, we wanted to explore for the first time whether these differences between males and females were also present in psychosis proneness.

2. MATERIALS AND METHODS

2.1. Participants

The sample consisted of 808 university and technical schools students from the area of Barcelona (mean age= 20.79 years, SD= 4.06). The final sample comprised 184 men (23%) and 624 women (77%). Males and females differed slightly in terms of age (males: mean = 21.4, SD = 4.5; females: mean = 20.6, SD = 3.9, $p < 0.05$). Ethical approval was obtained from local research ethics committees. All subjects volunteered to take part in the study and provided written informed consent at assessments, after being informed of the objectives of the study. They were not preselected based upon any criteria. Participants were 93% of European origin (subjects and both parents born in Europe) and only 7% were of

non-European origin (subjects with both parents born in non-European countries).

2.2. Psychosis Proneness Assessment

All participants completed self-report measures assessing positive schizotypy and positive psychotic-like experiences. Schizotypy was assessed with the Spanish version of the Wisconsin Schizotypy Scales (WSS), including the Perceptual Aberration, Magical Ideation, Revised Social Anhedonia, and Physical Anhedonia Scales [24–28]. The technical school volunteers completed the short version of the scales, which have comparable reliability and correlate highly with the original versions [29]. Exploratory and confirmatory factor analyses of the four scales reliably produce two factors, positive and negative schizotypy, that account for 80% of the variance. Magical Ideation and Perceptual Aberration Scales loaded on the positive schizotypy factor and Physical Anhedonia and Revised Social Anhedonia loaded on the negative schizotypy factor. The factor structure underlying the short scales is comparable with the factor structure of the original scales [30]. Participants were assigned positive schizotypy factor scores based upon factor loadings derived from a sample of 6137 college students [31].

Positive psychotic-like experiences were assessed with the Spanish version of the Community Assessment of Psychic Experiences (CAPE) [32,33]. The CAPE is a self-report questionnaire that measures lifetime prevalence of psychotic-like experiences on a frequency scale ranging from ‘never’ to ‘nearly always’, evaluating three dimensions of symptoms: positive, negative, and depressive. It has good validity and reliability and has been used in general population studies [34]. Note that only the positive dimension of both schizotypy and psychotic-like experiences were used to explore our hypothesis.

2.3. Genotyping

Genomic DNA was extracted from buccal mucosa on a cotton swab using the Real Extraction DNA kit (Durviz S.L.U., Valencia, Spain). The two single nucleotide polymorphisms of the ZNF804A included in the study (rs7597593 and rs1344706) were genotyped using TaqMan 5’ exonuclease assay (Applied Biosystems).

The final volume was 5 µL, which contained 5 ng of genomic DNA, 2.5 µL of TaqMan Master Mix, and 0.125 µL of 40x genotyping assay (assays C_223561_10 and C_2834835_10, respectively). The cycling parameters were as follows: 95ºC for 10 min followed by 40 cycles of denaturation at 92ºC for 15s and annealing/extension at 60ºC for 1 min. Polymerase chain reaction plates were read on an ABI PRISM 7900HT instrument and SDS v2.1 software (Applied Biosystems) was used for the genotype analysis of data. Both polymorphisms were in Hardy-Weinberg equilibrium. For accuracy of genotyping, 20 % of the samples (chosen randomly) were genotyped twice.

2.4. Statistical Analyses

All data were processed using Stata v.13.1 (Stata/MP 13.1 for Windows, StataCorp LP, College Station, USA). Independent analyses of covariance (ANCOVA) were performed to explore the association of each SNP with positive schizotypy and positive psychotic-like experiences, adding age, sex and sample (whether they were undergraduate or technical school students) as covariates. Differences between males and females were also analysed. When ANCOVAs yielded significant effects, pairwise comparisons and analyses comparing carriers of one allele vs homozygotes of the other allele were computed to detail differences between genotypes.

3. RESULTS

The initial sample contained 808 subjects. However, three did not complete the WSS, one did not complete the CAPE, three did not consent to provide a DNA sample, and genotyping failed for 56 individuals (6.9%) for rs7597593 and for 33 individuals (4.3%) for rs1344706. Descriptive statistics for the WSS (standardized factor scores) and CAPE (raw scores), as well as genotype frequencies of the two SNPs are presented in Table 1. No differences were observed in the genotypic frequencies between males and females (see Table 1).

The association analyses between rs7597593 and positive schizotypy showed a significant association in the whole sample (Table 2). Post-hoc pairwise comparisons revealed that TT subjects were reporting lower scores than CT (t=-2.60 p=0.03) and further analyses grouping subjects in carriers of the C allele (i.e. CC and CT genotypes) and TT homozygotes showed that those TT were reporting lower scores compared to C carriers (t=1.94 p=0.03). Sex-stratified analyses showed that this effect was driven by females but not males (see Table 2). In this regard, post-hoc pairwise comparisons indicated that females with the TT genotype showed lower scores than those with the CT genotype (t=-2.82 p=0.02, Figure 1a) and further analyses revealed that females TT were reporting lower scores compared to C carriers (t=2.54 p=0.006).

The analyses between rs7597593 and positive psychotic-like experiences did not show a significant association in the whole sample (F=2.34 p=0.1, Table 2). Sex-stratified analyses between this SNP and psychotic-like experiences revealed that rs7597593 was border-line associated in females, but not in males (Table 2). In this regard, post-hoc pairwise comparisons indicated that females with the TT genotype were showing lower positive psychotic-like experiences scores than CT (t=-2.46 p=0.04, Figure 1b). Further analyses showed that females with the TT genotype were reporting lower scores compared to those carrying the C allele (t=2.37 p=0.009).

Regarding rs1344706, no association was observed with schizotypy or psychotic-like experiences, neither in the whole sample nor by sex (Table 2).

4. DISCUSSION

In the present study, we aimed to provide further evidence for the implication of *ZNF804A* gene variation (i.e. rs7597593 and rs1344708) on psychosis proneness and explore, for the first time, whether sex was playing a role on this association as suggested by a previous study in schizophrenia. Our main result is that non-clinical females T homozygotes for rs7597593 were reporting lower positive schizotypy and psychotic-like experiences scores compared to C carriers, whereas this was not detected in males.

Given the strong association detected between genetic variants in the *ZNF804A* in schizophrenia GWAS [1,3,35], considerable efforts have been focused on exploring the genetic variation within this gene and its influence on schizophrenia and the broader psychosis phenotype, as well as its biological mechanisms and neuronal functions (see [9,36]). For example, the GWAS associated alleles (i.e. rs1344708 A allele and rs7597593 T allele), apart from being replicated in independent case-control studies (e.g. [37]), have been found related to higher and severe clinical symptoms [12,13], worst outcome in first episode patients [14] and brain alterations (e.g. [13,15]).

Convergent evidence from family studies have shown a phenotypic relationship between levels of schizotypy or psychotic-like experiences and schizophrenia that can be attributed to shared genetic effects, which points towards a significant overlap between the underlying genetic factors inducing both psychosis prone-

Table 1 Descriptive data for the positive dimensions of schizotypy and psychotic-like experiences (mean ± standard deviation and range) and details on the genotypic frequencies for the two analysed SNPs (rs7597593 and rs1344706). Data are given for the whole sample, as well as by sex (comparisons between males and females are given in italics).

		Total	**Males**	**Females**
Positive Schizotypy (WSS)		-0.46 ±0.75 (-1.72 – 3.23)	-0.35 ±0.79 (-1.45 – 2.24)	-0.49 ±0.74 (-1.72 – 3.23)
			t=2.18 p=0.03	
Positive psychotic-like experiences (CAPE)		8.04 ±4.88 (0 – 29)	8.51 ±5.02 (0 – 29)	7.90 ±4.83 (0 – 29)
			t=1.48 p=0.14	
***ZNF804A* polymorphisms**				
rs7597593	*CC*	294 (39.10%)	69 (38.98%)	225 (39.13%)
	CT	348 (46.28%)	78 (44.07%)	270 (46.96%)
	TT	110 (14.63%)	30 (16.95%)	80 (13.91%)
			χ^2= *1.1 p=0.6*	
rs1344706	AA	304 (39.23%)	64 (35.56%)	240 (40.34%)
	CA	347 (44.77%)	90 (50.00%)	257 (43.19%)
	CC	124 (16.00%)	26 (14.44%)	98 (16.47%)
			χ^2= *2.6 p=0.3*	

ness and schizophrenia [38]. In this sense, despite all the new studies with *ZNF804A*, the variability within this gene has been understudied in relation to psychosis proneness. However, only two previous studies have explored the implication of *ZNF804A* on psychosis-related (or attenuated) traits in non-clinical samples.

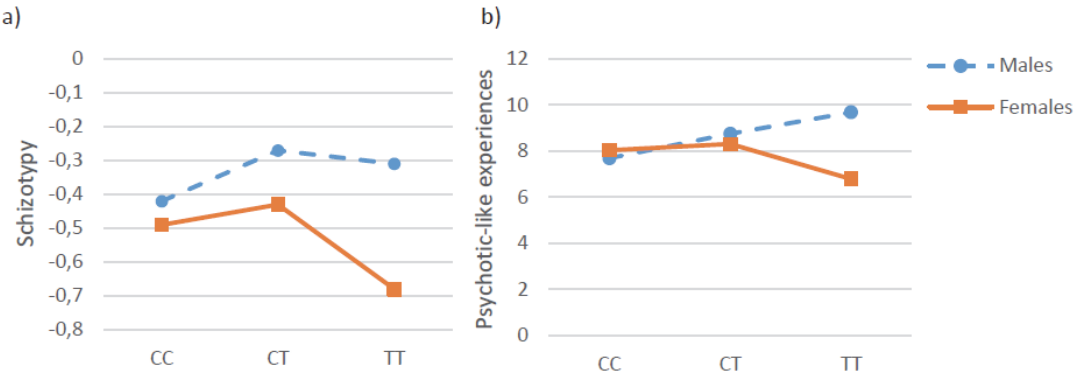
In the first study, Yasuda and colleagues showed that subjects carrying the T allele of rs1344706 were reporting higher schizotypy scores (particularly for the disorganized factor) in a healthy Japanese sample [22]. In contrast, in the other study exploring this, Stefanis and colleagues observed higher schizotypy scores (specifically paranoia and ideas of reference) in rs1344706 C carriers. One possible explanation for this discordant result in terms of risk allele, could be the different population origin of both samples (i.e. Japanese and European). To date, converging data suggest that *ZNF804A* is undoubtedly a risk gene for the psychotic phenotype in populations of European ancestry, but this is not clear for Asian populations. An study comparing LD patterns of the genomic region covering *ZNF804A* between Asians and Europeans showed sharp differences supporting the genetic heterogeneity, probably because differential population histories [39].

As regards to the other polymorphism studied here (rs7597593), our results are in line with those of Stefanis and colleagues reporting that subjects with the C allele showed higher scores

Table 2 Association analyses between *ZNF804A* variation (rs7597593 and rs1344706) and the positive dimensions of schizotypy and psychotic-like experiences (mean ± standard deviation). Data is given for the whole sample, as well as by sex (F and p-values are shown in italics).

		Total	**Males**	**Females**
rs7597593				
Schizotypy	CC	-0.47 ± 0.77	-0.42 ± 0.83	-0.49 ± 0.75
	CT	-0.39 ± 0.76	-0.27 ± 0.79	-0.43 ± 0.75
	TT	-0.58 ± 0.76	-0.31 ± 0.75	-0.68 ± 0.76
		F=3.68 p=0.03	*F=0.49 p=0.62*	*F=3.98 p=0.02*
Psychotic-like experiences	CC	7.94 ± 4.88	7.68 ± 3.76	8.02 ± 5.18
	CT	8.4 ± 5.14	8.74 ± 5.73	8.3 ± 4.96
	TT	7.56 ± 4.54	9.67 ± 5.6	6.78 ± 3.82
		F=2.34 p=0.1	*F=1.96 p=0.14*	*F=3.05 p=0.05*
rs1344706				
Schizotypy	AA	-0.44 ± 0.76	-0.31 ± 0.76	-0.47 ± 0.76
	CA	-0.48 ± 0.74	-0.39 ± 0.79	-0.52 ± 0.72
	CC	-0.44 ± 0.78	-0.29 ± 0.89	-0.48 ± 0.75
		F=0.20 p=0.82	*F=0.51 p=0.60*	*F=0.05 p=0.95*
Psychotic-like experiences	AA	8.17 ± 5.15	8.95 ± 5.58	7.97 ± 5.02
	CA	7.99 ± 4.76	8.57 ± 5.05	7.83 ± 4.65
	CC	7.87 ± 4.83	7.84 ± 3.41	7.89 ± 5.15
		F=0.15 p=0.86	*F=0.96 p=0.39*	*F=0.05 p=0.95*

Figure 1 Mean scores for the positive dimension of schizotypy (a) and psychotic-like experiences (b) in relation to rs7597593 genotype in males and females.



in schizotypy, perceptual aberration and positive psychotic-like experiences.

Regarding the differences between males and females in relation to *ZNF804A* genetic variability, to the best of our knowledge, this is the first study exploring this in psychosis proneness. In our sample, we detected a female-specific association between rs7597593 and both schizotypy and psychotic-like experiences. This result expand upon Stefanis and colleagues by including females, although, contrary to them, no significant results were found in the males of our sample.

In schizophrenia, the published studies exploring this sex-specific associations have shown discordant results. Zhang et al detected a strong association between the rs7597593 T allele and schizophrenia in females of European ancestry in their sex-stratified analyses, and a trend towards interaction between sex and this SNP in their schizophrenia case-control [18]. However, no significant interaction was detected in an Irish study by Riley et al (i.e. rs17508595, rs13393273, rs7597593 and rs1344706) (Riley *et al*, 2010) or in a recent Chinese case-control (i.e. rs1344706) (Wang *et al*, 2016). In this regard, as Zhang and colleagues pointed out that both the Irish and Chinese samples were predominately male, which might have influenced the results given the analyses of female subjects could have had less statistical power to detect the association. In our study, the predominantly female sample has allowed us to detect the *ZNF804A* by-sex effect in psychosis proneness, although the lower number of males included is a limitation that might have influenced our results.

The results of the present study support the idea of a shared underlying aetiology for schizophrenia and related attenuated phenotypes present at the different levels of severity [41,42], although different alleles have been associated in clinical and non-clinical studies. In this regard, it is difficult to determine the biological mechanisms underlying these

associations, because the function of this gene is still unknown. Zinc finger domains are relatively small protein motifs containing multiple finger-like protrusions that make tandem contacts with their target molecule (e.g. DNA, RNA, protein, lipid substrates), having a function in gene transcription, translation, mRNA trafficking, cytos-keletal organization, protein folding and chromatin remodelling, among others. Their binding properties depend on the amino acid sequence of the finger domains, on the linker between fingers, the number of fingers, as well as on higher-order structures. Although the role of the ZNF804 protein is not clear, variation within the *ZNF804A* may affect its binding to the target and compromise the pathways to which they are involved, including its own mRNA expression [43,44], as seem to suggest recent neuroimaging studies [45,46]. The *ZNF804A* influences the expression of three genes involved directly in dopaminergic transmission (i.e. *DRD2* and *COMT*) and cAMP signalling (i.e. *PDE4B*), two pathways thought to underlie many of the symptoms of psychosis (Girgenti *et al*, 2012). Determining the genes regulated by *ZNF804A* may help to understand the function of this gene and how it may be involved in psychopathology. In this sense, *ZNF804A* was associated with positive schizotypy and psychotic-like experiences in the present study. Positive symptomatology has been related to hyperactivity of subcortical dopamine transmission, in which *DRD2* and/or *COMT* could be involved [47,48]. Interestingly, for both *DRD2* and *COMT,* gender-specific effects on psychosis have also been reported (e.g. [49,50]), as Zhang [18] and our study in psychosis proneness have found with *ZNF804A*. It is tempting to speculate, thus, that *ZNF804A* could have an effect on positive symptoms through a dopaminergic-related pathway that may be influenced by sexual hormones [51]. Further functional experiments together with bioinformatics analysis might help with our understanding of these results.

The findings of the present study support the involvement of *ZNF804* variability on the vulnerability for the development of psychopathology at a non-clinical level and the implication of sex in this association. However, elucidating the mechanisms by which this gene affects mental health will be relevant in the near future.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: A. Rosa, N. Barrantes-Vidal and T.R. Kwapil. Performed the experiments and assessments: M. de Castro-Catala, A. Mora-Solano, A. Racioppi, P. Cristóbal-Narváez and T. Sheinbaum. Analysed the data: M. de Castro-Catala, A. Mora-Solano, T.R. Kwapil and A. Rosa. Wrote the paper: M. de Castro-Catala, A. Mora-Solano and A. Rosa. Critically revised the manuscript: T.R. Kwapil, P. Cristóbal-Narváez, T. Sheinbaum, A. Racioppi and N. Barrantes-Vidal.

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Supervisor's report on the contribution of the PhD candidate to the article.

Dr. Araceli Rosa de la Cruz, Senior Lecturer at the Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals of the Facultat de Biologia (Universitat de Barcelona) and supervisor of the present doctoral thesis by Marta de Castro i Català, hereby certifies that none of the co-authors of the article "The genome-wide associated candidate gene *ZNF804A* and psychosis-proneness: evidence of sex-modulated association" have used this publications for a doctoral thesis, and that the participation of the PhD applicant in this article included the following tasks:

- Participation in the conception and design of the study.
- Participation in the sample collection.
- Laboratory tasks.
- Statistical analyses and interpretation of data.
- First drafting of the manuscript.
- Critical revision of the article for intellectual content.

Signed by Dr. Araceli Rosa

Barcelona, May 31st 2017

Section II: Interaction effect between genetic variability and environment on psychosis-proneness

3.4. Childhood trauma, *BDNF* Val66Met and subclinical psychotic experiences. Attempt at replication in two independent samples

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Resum

L'exposició al trauma durant la infància és un important factor de risc ambiental per les psicosis. No obstant, no tots els individus exposats desenvoluparan símptomes psicòtics en el futur. Alguns estudis han posat de manifest que el polimorfisme *Val66Met* (rs6265) en el gen *BDNF* podria modular els efectes inductors de psicosi del trauma durant la infància en mostres clíniques i no clíniques. El nostre estudi pretenia investigar l'efecte de la interacció entre el trauma durant la infància i el polimorfisme *Val66Met* del *BDNF* sobre les experiències psicòtiques atenuades. Amb aquest objectiu es van explorar dues mostres no clíniques independents: (i) una mostra d'estudiants universitaris i de formació professional (n=808, mostra 1) i (ii) una mostra de bessones (n=621, mostra 2).

Els resultats van mostrar una forta associació entre el trauma durant la infància i l'existència d'experiències psicòtiques tant positives com negatives. Addicionalment, en totes dues mostres es va detectar que existia un efecte moderador del polimorfisme *Val66Met* sobre aquesta associació. No obstant, els resultats observats eren discordants en termes de l'al · lel associat a un major risc. Mentre que a la mostra 1 els portadors de l'al · lel *Val*, en especial els homes, eren més vulnerables als efectes del trauma durant la infància pel que fa a les experiències psicòtiques, a la mostra 2 les portadores de l'al · lel *Met* presentaven majors puntuacions que les *Val*, davant l'exposició al trauma durant la infància. A més, a la mostra 2, es va detectar un efecte d'interacció sobre la dimensió negativa. El nostre estudi replica parcialment un estudi previ i mostra que alguns individus són més vulnerables a desenvolupar experiències psicòtiques, havent patit adversitat infantil, a causa d'una combinació complexa de múltiples factors.

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Childhood trauma, *BDNF* Val66Met and subclinical psychotic experiences. Attempt at replication in two independent samples

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ABSTRACT

Childhood trauma exposure is a robust environmental risk factor for psychosis. However, not all exposed individuals develop psychotic symptoms later in life. The Brain-derived neurotrophic factor (*BDNF*) Val66Met polymorphism (rs6265) has been suggested to moderate the psychosis-inducing effects of childhood trauma in clinical and nonclinical samples. Our study aimed to explore the interaction effect between childhood trauma and the *BDNF* Val66Met polymorphism on subclinical psychotic experiences (PEs). This was explored in two nonclinical independent samples: an undergraduate and technical-training school student sample (n = 808, sample 1) and a female twin sample (n = 621, sample 2). Results showed that childhood trauma was strongly associated with positive and negative PEs in nonclinical individuals. A *BDNF* Val66Met x childhood trauma effect on positive PEs was observed in both samples. These results were discordant in terms of risk allele: while in sample 1 Val allele carriers, especially males, were more vulnerable to the effects of childhood trauma regarding PEs, in sample 2 Met carriers presented higher PEs scores when exposed to childhood trauma, compared with Val carriers. Moreover, in sample 2, a significant interaction was also found in relation to negative PEs. Our study partially replicates previous findings and suggests that some individuals are more prone to develop PEs following childhood trauma because of a complex combination of multiple factors. Further studies including genetic, environmental and epigenetic factors may provide insights in this field.

1. Introduction

The maturation of most brain areas starts early in the embryonic stages and continues into childhood and adolescence (Lenroot and Giedd, 2006), requiring a complex interplay of genetic and environmental factors. Disruption of these factors can alter normal

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development, causing modifications in neuronal structure, function or connectivity (Lewis and Levitt, 2002). Specifically, prenatal environmental exposures (e.g. maternal nutrition deficiency, stress or infections during pregnancy) and postnatal environmental factors (e.g. early life adversity, growing up in an urban environment, minority group position and drug abuse) have been associated with psychotic outcomes (van Os et al., 2010). In this regard, early psychological stress, such as childhood trauma, has been related to the expression of psychotic symptoms in clinical and non-clinical samples, as shown in a recent meta-analysis (Varese et al., 2012).

Childhood trauma encompasses a range of adverse experiences suffered early in life, such as sexual, physical and emotional abuse, physical and emotional neglect, and other early-life events, such as the death of a parent. In this regard, patients from psychiatric units with a child abuse history are particularly likely to experience positive symptoms and tend to have the worst course and outcome of psychosis (Holtzman et al., 2013; Read et al., 2005). In the general population, associations between childhood trauma and schizotypy and psychotic experiences (PEs) have also been reported (Sheinbaum et al., 2014; Velikonja et al., 2014), including an increased expression of these traits and experiences in daily life for those exposed to childhood trauma (Cristóbal-Narváez et al., 2016a). However, there are large individual differences in response to early stressful events (Holtzman et al., 2013), which suggest that genetic factors may be moderating this response (van Winkel et al., 2013). Some studies have reported several genes that may moderate the impact of childhood adversity on mental health, such as the Brain-derived neurotrophic factor gene (*BDNF*) (Alemany et al., 2011), the Serotonin transporter gene (*SERT*) (Karg et al., 2011) or the FK506 binding protein gene (*FKBP5*) (Alemany et al., 2016; Collip et al., 2013; Cristóbal-Narváez et al., 2016b).

BDNF is a neurotrophin that plays an important role in several neurodevelopmental processes (e.g.: neuronal differentiation), synaptic and cognitive plasticity and, in addition, seems to influence some neurotransmitter systems (Buckley et al., 2011; Carvalho et al., 2008). Also, as a neurotrophin, a role of BDNF has been established in cell survival in response to stress (Sofroniew et al., 2001). BDNF secretion is affected by the common functional polymorphism (rs6265, C > T) that results in valine (Val) to methionine (Met) substitution at codon 66 (Val66Met). The Val allele of this polymorphism has been associated with risk for psychosis (e.g. schizophrenia or bipolar disorder) in both case-control (Lohoff et al., 2005; Neves-Pereira et al., 2005) and family-based studies (Geller et al., 2004; Neves-Pereira et al., 2002; Rosa et al., 2006; Sklar et al., 2002).

Gene-environment interaction (G x E) studies have investigated the role of *BDNF* as a moderator of the association between childhood trauma and different psychiatric phenotypes. Only two previous studies have analysed the childhood trauma x *BDNF* Val66Met interaction in relation to subclinical PEs in general population samples. The first study explored the interactions between *BDNF* and abuse, and *BDNF* and neglect (Alemany et al., 2011). They found that individuals carrying the Met allele who had been exposed to childhood abuse reported more positive PEs than those with the Val/Val genotype. The second study by Ramsay and colleagues attempted to replicate those findings, but was unable to find any significant interaction (Ramsay et al., 2013). Despite these contradictory results, there is independent evidence indicating that this polymorphism moderates the effects of stress exposure on cognition, brain structure and anxiety (Aas et al., 2013; Chen et al., 2006), in line with Alemany et al.'s findings.

Given these previous results, the aim of our study was to explore the modulating role of *BDNF* Val66Met on the association between childhood trauma and PEs, using two independent samples of healthy individuals. Consistent with functional studies and previous G x E research with the Val66Met polymorphism (i.e.: Aas et al., 2013; Alemany et al., 2011; Chen et al., 2006), we hypothesised that individuals carrying the Met allele (Met/Met and Val/Met) who have been exposed to childhood trauma are at higher risk of presenting with PEs in adulthood as compared with Val/Val subjects.

2. Material and methods

2.1. Sample 1 – general population undergraduate and technical school students

Sample 1 comprised 808 subjects, including 547 university students from the Universitat Autònoma de Barcelona (UAB) and 261 students from 7 technical training schools in Barcelona (77% were women; n = 622). The mean age was 20.79 years (SD = 4.06; range = 17–54). Ninety-three percent of the subjects were of European origin (subject and both parents born in Europe). Further details of this sample can be found elsewhere (Barrantes-Vidal et al., 2013a, 2013b; de Castro-Catala et al., 2016).

To assess childhood trauma, all subjects were administered the Spanish version of the short form of the Childhood Trauma Questionnaire (CTQ) (Bernstein and Fink, 1998; Hernandez et al., 2013). This questionnaire consists of 28 questions enquiring about five types of childhood trauma: emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect. The score for each item ranges from 1 (never true) to 5 (very often true) according to the frequency to which subjects have experienced the statement during their childhood and adolescence. Following the guidelines for classification of CTQ scores proposed by Bernstein and Fink (1998), the prevalence of each type of childhood trauma was calculated. A total childhood trauma score was computed by summing all the CTQ items. Also, the five types of childhood trauma were grouped into childhood abuse (sum of emotional, physical and sexual abuse) and childhood neglect (sum of emotional and physical neglect), in order to explore the differences between these two types of trauma. Childhood trauma data was available for 807 subjects.

PEs were assessed using the Spanish version of the Community Assessment of Psychic Experiences (CAPE) (Ros-Morente et al., 2011; Stefanis et al., 2002), a validated instrument for assessing subclinical symptoms in general population samples (Konings et al., 2006). The CAPE is a self-report questionnaire that measures the lifetime prevalence of PEs on a frequency scale ranging from 0 (never) to 3 (nearly always). It consists of 42 items that evaluate three dimensions of symptoms: positive, negative, and depressive. A total sum score per dimension (positive and negative) of the frequency items was used for analyses.

2.2. Sample 2 – general population twins

Participants from sample 2 came from the *East Flanders Prospective Twin Survey* (Derom et al., 2013, 2002), a population-based survey that has prospectively recorded all multiple births in the province of East Flanders. As part of the survey, subjects were interviewed five times (T0 - T4) at approximately 3- to 4-monthly intervals. The five measurement points included the baseline measurement (T0), carried out at individuals' home, and four follow-up measurements (T1 - T4), collected using questionnaires. At baseline, sample 2 comprised 621 female subjects, including 174 monozygotic twin pairs, 112 dizygotic twin pairs, 2 twin pairs of unknown zygosity, and 45 of their non-twin sisters. Zygosity of each multiple birth was determined through examination of the placental membranes and vascular anastomoses, blood groups and DNA fingerprints. The mean age was 27.8 years (SD = 7.9;

range = 18–61). Participants were of white ethnic group and of Belgian origin; 62% had a higher education, 36% had followed higher secondary school and 2% had finished primary school only.

Childhood trauma was assessed at baseline using a self-report questionnaire based on the Dutch version of the 70-item CTQ original questionnaire (Arntz and Wessel, 1996; Bernstein et al., 1994). A shorter CTQ version was used in which the most explicit items related to sexual and physical abuse were omitted, as requested by the Twin Registry. Thus, subjects completed a 21-item questionnaire measuring positive events such as a happy childhood or youth, interparental or marital harmony and love, feeling safe and respect of privacy as well as negative events such as physical abuse, emotional neglect, material problems in parental household and stressful life events. The frequency of each item was rated on a scale from 1 (never) to 5 (always). Positive events were recoded to reflect adverse experiences or trauma. Cronbach's alpha for this 21-item questionnaire was 0.93. As in sample 1, a total childhood trauma score was calculated by summing the 21 items of the questionnaire. In this sample, childhood abuse and neglect could not be calculated. From the total sample, 612 subjects completed the childhood trauma assessment.

All subjects completed the Dutch version of the CAPE (http://cape42.homestead.com) to assess PEs. They completed this questionnaire three times, at T0, T2 and T4. The CAPE total scores per dimension at each interview time were calculated as in sample 1.

The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the Universitat Autònoma de Barcelona (UAB) (sample 1) and the Maastricht University Medical Centre (sample 2). All subjects volunteered to take part in the study after being informed of the objectives of our research and provided informed consent at assessments.

2.3. Genotyping

From sample 1, 805 participants agreed on providing buccal mucosa on cotton swabs, from which genomic DNA was extracted using the Real Extraction DNA kit (Durviz S.L.U., Valencia, Spain). In this sample, the *BDNF* Val66Met polymorphism was genotyped using the TaqMan assay (Applied Biosystems). The genotyping reaction was performed according to the manufacturer's protocol on ABI PRISM 7900HT. The final volume was 5ul, which contained 5 ng of genomic DNA, 2.5ul of Taqman Master Mix and 0.125μl of 40x genotyping assay (C_11592758_10). The genotype analysis of data was done with SDS v2.3 software (Applied Biosystems). For accuracy of genotyping, 20% of the samples (chosen randomly) were genotyped twice. Genotyping failed for 6 individuals from this sample.

In sample 2, genomic DNA was extracted from placental tissue, blood samples or buccal cell samples on sterile swabs. According to the appropriate protocol for each sample type, genomic DNA was extracted using QUIAamp DNA Mini Kits (Qiagen, Venlo, the Netherlands). The *BDNF* Val66Met was determined by KBioscience (Hertz, UK) using their proprietary allelic discrimination assay. For every monozygotic twin in the sample with genotypic data, the same genotypic data were included for the co-twin, assuming identical genotypes for both twins. All dizygotic twins were genotyped. From the whole sample, genetic data were obtained for 473 subjects.

No genotyping discrepancies were expected neither between DNA obtained from different sample types (de Vries et al., 1996), nor between different genotyping technologies. Hardy-Weinberg equilibrium (HWE) was verified for each sample using an on-line Chi-squared HWE test calculator for biallelic markers (Rodriguez et al., 2009).

Due to the low frequency of Met/Met genotype, *BDNF* genotype was converted into a binary variable for the analyses: Met carriers (genotypes Met/Met and Val/Met) and Val/Val genotype.

2.4. Statistical analyses

Linear regression analyses were used to study the childhood trauma - *BDNF* Val66Met interaction in sample 1. CAPE positive and CAPE negative symptoms were tested as dependent variables using separate models, controlling for age and sex. Abuse x *BDNF* Val66Met and neglect x *BDNF* Val66Met effects were also examined in this sample. In the female twin sample (sample 2), given the different structure of the data, multilevel regression analysis was used (XTMIXED command). Two additional levels were added: (i) adjusting for clustering within twin pairs (as scores in the analysed variables are likely to be more similar between twin pairs) and (ii) adjusting for clustering within subjects (as the CAPE was measured three times and CAPE scores are more likely to be similar within subjects). Age was added as covariate in the multilevel analyses. All data were analysed using Stata version 13.1 (Stata/MP 13.1 for Windows, StataCorp LP, College Station, USA).

3. Results

3.1. Descriptive of the variables

CAPE positive and negative data were available for 807 and 804 subjects from sample 1, respectively. In sample 2, 620 participants

Table 1
Descriptives of the two samples included in the study: *BDNF* Val66Met genotype frequencies (Val/Val and Met carriers, including Met/Met and Val/Met genotypes) and mean scores (SD, range) for total childhood trauma, abuse and neglect, and for the positive and negative dimensions of subclinical psychotic experiences.

	Sample 1 n = 808	Sample 2 n = 621	Comparison
Genotype frequencies (n (%))			
BDNF Val/Val	501 (63%)	302 (64%)	$\chi^2 = 0.167$ p = 0.683
BDNF Met carriers	298 (37%)	171 (36%)	
Subclinical psychotic experiences[a] (mean (SD, range))			
Positive	8.04 (4.88, 0–29)	3.60 (3.54, 0–28)	**t = 19.13 p < 0.001**
Negative	9.15 (5.26, 0–35)	7.08 (5.07, 0–27)	**t = 7.49 p < 0.001**
Childhood trauma[b] (mean (SD, range))			
Total childhood trauma	32.95 (8.76, 25–84)	34.89 (11.98, 19–95)	–
Abuse	18.15 (5.03, 15–50)	–	–
Neglect	14.79 (4.92, 10–41)	–	–

Significant results are shown in bold.
[a] Subclinical psychotic experiences were assessed using the Community Assessment of Psychic Experiences (CAPE).
[b] Childhood trauma was assessed using the Childhood Trauma Questionnaire (CTQ) (sample 1) and a CTQ-based questionnaire (sample 2). Note that no comparisons have been done between the total childhood trauma scores, given the two different questionnaires used.

Table 2
Main effects of total childhood trauma, abuse and neglect, and the *BDNF* Val66Met polymorphism (Val/Val *vs.* Met carriers) on positive and negative subclinical psychotic experiences in the two samples included in the present study.

	Positive subclinical psychotic experiences		Negative subclinical psychotic experiences	
	Sample 1	Sample 2	Sample 1	Sample 2
Total childhood trauma	**B = 0.11 s.e. = 0.02 p < 0.001 95% IC 0.08–0.15**	**B = 0.11 s.e. = 0.01 p < 0.001 95% IC 0.09–0.14**	**B = 0.15 s.e. = 0.02 p = < 0.001 95% IC 0.11–0.19**	**B = 0.15 s.e. = 0.02 p < 0.001 95% IC 0.12–0.18**
Abuse	**B = 0.20 s.e. = 0.03 p < 0.001 95% IC 0.13–0.26**	–	**B = 0.18 s.e. = 0.04 p < 0.001 95% IC 0.11–0.25**	–
Neglect	**B = 0.15 s.e. = 0.03 p < 0.001 95% IC 0.08–0.21**	–	**B = 0.28 s.e. = 0.04 p < 0.001 95% IC 0.24–0.35**	–
BDNF Val66Met	B = 0.12 s.e. = 0.35 p = 0.726 95% IC −0.55–0.80	B = 0.04 s.e. = 0.34 p = 0.911 95% IC −0.62–0.70	B = −0.56 s.e. = 0.38 p = 0.145 95% IC −1.31–0.19	B = 0.15 s.e. = 0.48 p = 0.755 95% IC −0.80–1.1

Significant results are shown in bold.

had complete CAPE positive data and 619 had complete CAPE negative data at T0, 438 participants had available CAPE positive and negative data at T2, and 480 participants had complete CAPE positive and negative data at T4. The prevalence of each type of childhood trauma (see in brackets the threshold considered as presence of each type of trauma, according to Bernstein and Fink (1998)) in sample 1 was: (i) emotional abuse [>8] 23.17%, (ii) physical abuse [>7] 7.93%, (iii) sexual abuse [>5] 9.67%, (iv) emotional neglect [>9] 36.18% and (v) physical neglect [>7] 12.39%. This was not calculated in sample 2, given the alternative questionnaire used to assess childhood trauma. Descriptives of the variables included in the analyses are shown in Table 1. Genotype frequencies (Val/Val and Met carriers) were similar in both samples ($p > 0.05$, Table 1). No differences were observed in genotypic frequencies between males and females in sample 1 (Val/Val: males 62% and females 63%, $p > 0.05$). Males and females from sample 1 showed similar positive and negative PEs scores ($p > 0.05$, data not shown). Positive and negative PEs scores were higher in sample 1 than in sample 2 ($p < 0.05$, Table 1). This was also observed when considering only females ($p < 0.0001$ for both positive and negative dimensions). Complete data (PEs, childhood trauma and *BDNF* Val66Met genotype) were available for 799 and 460 participants from sample 1 and 2, respectively.

3.2. Impact of childhood trauma and BDNF genotype on psychotic experiences

There was a significant main effect of total childhood trauma in the model of positive and negative PEs in both sample 1 (positive PEs: B = 0.11, s.e. = 0.02, 95% CI: 0.08–0.15, $p < 0.001$ and negative PEs: B = 0.15, s.e. = 0.02, 95% CI: 0.11–0.19, $p < 0.001$) and sample 2 (positive PEs: B = 0.11, s.e. = 0.01, 95% CI: 0.09–0.14, $p < 0.001$ and negative PEs: B = 0.15, s.e. = 0.02, 95% CI: 0.116–0.182, $p < 0.001$). In sample 1 both abuse and neglect were found to predict positive and negative PEs (see Table 2). No significant main effect of *BDNF* Val66Met genotype was found for positive or negative PEs in either sample (Table 2).

3.3. Impact of childhood trauma x BDNF Val66Met in predicting psychotic experiences

In sample 1, a significant interaction was found between total childhood trauma and *BDNF* Val66Met on positive PEs (B = −0.08, s.e. = 0.04, 95% CI: - 0.16 to −0.01, p = 0.036). Specifically, Val/Val subjects reported more positive PEs than Met carriers when exposed to childhood trauma. When we divided the childhood trauma scores into abuse and neglect, this interaction effect on positive PEs was also found with neglect (B = −0.15, s.e. = 0.07, 95% CI: -0.28 to −0.01, p = 0.033), but not abuse, although a trend in the same direction was observed (B = −0.13, s.e. = 0.07, 95% CI: −0.26 to −0.003, p = 0.056). Regarding negative PEs, no significant interaction was found with any of the childhood trauma variables studied (data not shown).

In Sample 2, in which only total childhood trauma was estimated, there was a significant interaction effect on positive PEs (B = 0.05, s.e. = 0.03, 95% CI: 0.001–0.1, p = 0.045); carriers of the Met allele with childhood trauma exposure showed more positive PEs than Val/Val carriers (Fig. 1G). This interaction was also significant for negative PEs (B = 0.13, s.e. = 0.04, 95% CI: 0.06–0.21, p = 0.001). Similarly, Met carriers exposed to childhood trauma reported more negative PEs than those Val/Val (Fig. 1H).

When we observed that the results across both samples were in the opposite direction, we explored whether the G x E found in sample 1 was influenced by sex. This was considered relevant given that this sample was composed of both male and female

participants (whereas sample 2 was all female). Sex-stratified analyses on positive PEs revealed that the interaction effects observed in sample 1 with total childhood trauma, abuse and neglect were significant only for males (Table 3, Fig. 1A–C), but not females (see Table 3). Regarding negative PEs, sex-stratified analyses revealed significant interactions between the *BDNF* Val66Met and total childhood trauma, as well as abuse and neglect, on negative PEs in males (Table 3, Fig. 1D, E, 1F) but not females (see Table 3).

4. Discussion

The present study investigated the possible interplay between *BDNF* Val66Met genotype and childhood trauma on subclinical PEs using two independent samples of nonclinical young adults. Firstly, our results show a consistent association between childhood trauma and PEs in healthy individuals. Secondly, a *BDNF* Val66Met x childhood trauma effect on PEs was observed in both samples, although discordant in terms of risk allele. Moreover, the G x E effect found in sample 1 seems to be sexually dimorphic.

4.1. Childhood trauma and subclinical psychotic experiences

In the present study, we observed an increased risk for PEs related to childhood traumatic experiences. This was detected in both samples, despite the differences in PEs' scores between samples, which probably is a consequence of the mean age of each sample (i.e.: sample 1 is approximately 7 years younger than sample 2). A pattern of diminished PEs prevalence over the life course after a peak during adolescence has been observed in other studies (Kelleher et al., 2012; Verdoux et al., 1998), suggesting that these symptoms may be part of normal development during childhood and adulthood development, but become abnormal (indicating pathology) with age. This was considered in the present study, correcting all analyses by age.

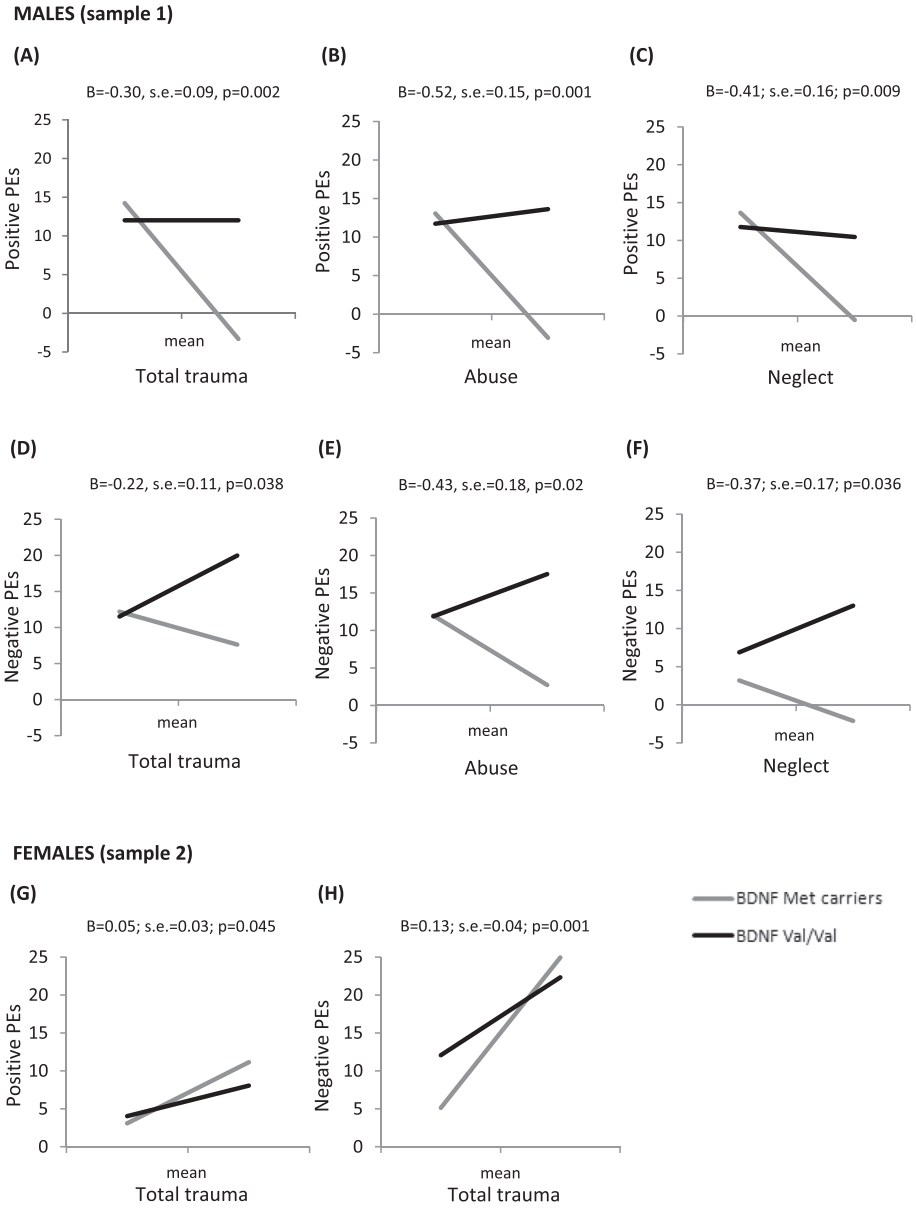

Fig. 1. Graphic representation of the significant interaction effects by sex (males and females) between childhood trauma (total, abuse and neglect) and the *BDNF* Val66Met polymorphism (Val/Val *vs.* Met carriers) on positive and negative subclinical psychotic experiences (PEs) in the two samples studied (i.e.: sample 1 and sample 2).

Table 3
Interaction effects by sex (males and females) between childhood trauma (total, abuse and neglect) and the *BDNF* Val66Met polymorphism (Val/Val vs. Met carriers) on positive and negative subclinical psychotic experiences in the two samples studied (sample 1 and sample 2).

	Sex	Positive psychotic experiences		Negative psychotic experiences	
		Sample 1	Sample 2	Sample 1	Sample 2
Childhood abuse x BDNF	Males	**B = −0.52 s.e. = 0.15 p = 0.001 95% CI: 0.82 − −0.21**	–	**B = −0.43 s.e. = 0.18 p = 0.02 95% CI: 0.78 − −0.07**	–
	Females	B = −0.03 s.e. = 0.07 p = 0.728 95% CI: −0.17–0.12	–	B = 0.05 s.e. = 0.08 p = 0.576 95% CI: −0.11–0.2	–
Childhood neglect x BDNF	Males	**B = −0.41 s.e. = 0.16 p = 0.009 95% CI: 0.72 − −0.11**	–	**B = −0.37 s.e. = 0.17 p = 0.036 95% CI: 0.71 − −0.03**	–
	Females	B = −0.08 s.e. = 0.08 p = 0.267 95% CI: −0.23–0.07	–	B = −0.0001 s.e. = 0.08 p = 0.999 95% CI: −0.16–0.16	–
Total childhood trauma x BDNF	Males	**B = −0.30 s.e. = 0.09 p = 0.002 95% CI: 0.48 − −0.11**	No males in this sample	**B = −0.22 s.e. = 0.11 p = 0.038 95% CI: 0.43 − −0.01**	No males in this sample
	Females	B = −0.23 s.e. = 0.04 p = 0.469 95% CI: −0.11–0.05	**B = 0.05 s.e. = 0.03 p = 0.045 95% CI: 0.001–0.11**	B = 0.02 s.e. = 0.05 p = 0.710 95% CI: −0.07–0.11	**B = 0.13 s.e. = 0.04 p = 0.001 95% CI: 0.06–0.21**

Note that childhood trauma was assessed with the CTQ in sample 1 and an adapted version of the CTQ in sample 2.
Significant results are shown in bold.

Our results relate traumatic experiences to a greater risk for PEs in two independent nonclinical samples are consistent with other studies analysing this in healthy individuals (van Nierop et al., 2014). Similar results have been found in a meta-analysis including nonclinical and clinical studies analysing childhood adversities in relation to psychotic symptoms and psychosis. The odds ratios (ORs) associated were situated around 3 for both phenotypes (Varese et al., 2012). Evidence in the same line has been provided in a review by Velikonja et al. (2014) focusing on schizotypy, the underlying vulnerability for psychosis-spectrum psychopathology that is expressed across a wide range of personality traits, PEs, subclinical and clinical psychosis phenomenology, presumably reflecting the expression of common causal factors (Barrantes-Vidal et al., 2015; van Os et al., 2009). In the Velikonja et al. review, they found an association between all types of trauma and schizotypy, with ORs ranging between 2.01 and 4.15.

Exposure to stress causes the activation of the hypothalamic-pituitary-adrenal (HPA) axis, which activates several pathways that regulate gene expression for metabolism, immune function, cognition, and brain development, preparing the body to respond to stress. Exposure to severe stress (e.g. childhood trauma) can alter this normal stress response (De Bellis and Zisk, 2014) and may precipitate a cascade of events that leads to aberrant neural circuit changes, including an abnormal increase in dopamine signalling or neurotrophic factors (e.g. BDNF) (Carbone and Handa, 2013; van Winkel et al., 2008). Alterations in HPA axis reactivity and related molecule levels have been found in patients with psychosis, as well as in subjects with schizotypal personality disorder, and ultra-high risk subjects (Myin-Germeys and van Os, 2007), suggesting that HPA axis function alteration may increase psychological vulnerability, predisposing persons to develop psychotic symptoms.

4.2. Childhood trauma x BDNF Val66Met and subclinical psychotic experiences

In the present study, we examined whether *BDNF* Val66Met moderated the association between childhood trauma and PEs in two independent samples of healthy subjects. According to our results, in sample 1, Val/Val subjects who had been exposed to childhood trauma or childhood neglect reported more positive PEs. This association was specific to males, as this was not observed in females. In sample 2, Met carriers with exposure to childhood trauma were observed to have more positive and negative PEs. It is challenging to interpret this results because, to our knowledge, there are only two previous studies analysing this specific G x E in healthy subjects, but none of them have examined sex differences. The first study showed a significant effect of Met allele x childhood abuse on positive PEs (Alemany et al., 2011), which seems to converge with the results of this study in sample 2, although this sample was composed only by female participants. The second study, by Ramsay et al. (2013), did not find any significant interaction. Overall, the results of our sample 1 support the importance of examining sex differences when analysing the *BDNF* x trauma effect on PE dimensions. There are also studies exploring this G x E interaction in clinical samples. Aas and colleagues, for example, explored the *BDNF* x childhood abuse effect on cognition and brain abnormalities in a sample of schizophrenic and bipolar patients. In this study, Met carriers with childhood trauma exposure showed poorer cognitive functioning, reduced hippocampal volumes and larger ventricles (Aas et al., 2013). However, these findings have not been replicated in other samples (Hernaus et al., 2014).

The BDNF is a neurotrophin required for proper neurodevelopment and is also involved in essential functions in the mature brain (e.g.: synaptic plasticity) (see Autry and Monteggia (2012) for an extensive review on BDNF). The *BDNF* Val66Met

polymorphism is reported to affect intracellular processing and secretion of the mature protein influencing neurogenesis and plasticity. In this sense, the BDNF-Met protein shows a defective secretion and is associated with lower distribution of BDNF protein in neurons (Chen et al., 2006, 2004; Egan et al., 2003). Despite these findings, the underlying neurobiology mediating the effect of this particular polymorphism on brain functioning and its interaction with other genetic factors are still not well understood. However, it seems evident that inappropriate or inadequate neurotrophic support during brain development could underlie structural and functional disorganization of neural and synaptic networks (Lu and Martinowich, 2008), leading to an impaired brain with, probably, a decreased ability to make the normal and necessary adaptive changes according to the inputs received.

Along these lines, the *BDNF* Met allele has been related to reduced hippocampal and prefrontal cortex volumes (Pezawas et al., 2004), two brain areas highly involved in cognition, consolidation of information, memory, and behaviour. Consistent with this, *BDNF* Met carriers have shown memory dysfunctions (Chen et al., 2004), impairment in learning and memory (Chen et al., 2006; Soliman et al., 2010), and cognition (Altmann et al., 2016; Lu et al., 2012). Such impairments are a core feature of schizophrenia (Heinrichs and Zakzanis, 1998; Medalia and Thysen, 2008) and can also be observed in an attenuated form in non-clinical and at-risk populations (Piskulic et al., 2016). Case-control and family studies have shown contradictory results in relation to the allele of risk of this polymorphism. Although a recent meta-analysis pointed towards Met as the risk allele for schizophrenia (Kheirollahi et al., 2016), some studies have found associations with the Val allele (Neves-Pereira et al., 2005; Rosa et al., 2006). These apparently contradictory findings regarding the allele of risk are also observable across studies conducted in relation to other psychiatric phenotypes, such as bipolar disorder and depression (Lohoff et al., 2005; Oswald et al., 2004). The reason for these inconsistencies is unclear. The different pattern of results may suggest that cumulative and/or interactive effects of other genes of minor effect with the variability on *BDNF* (e.g. the genes Catechol-O-methyltransferase (*COMT*) or *SERT*; Gutiérrez et al., 2015; Han et al., 2008) or epigenetic factors (Boulle et al., 2012) are operating in the expression of psychopathology.

Another factor to consider may be the subject's sex. There is evidence of sex differences in healthy brain structure, function, and neurotransmission (Cosgrove et al., 2007). These differences can also be observed in relation to risk for psychopathology (Aleman et al., 2003; Cahill, 2006), showing males to have a higher susceptibility for schizophrenia, with earlier age of onset and an overall poorer clinical prognosis (Godar and Bortolato, 2014). Moreover, several studies have shown a different genetic effect on psychosis risk depending on the subject's sex, for example with the *COMT* gene (e.g.: de Castro-Catala et al., 2015; Harrison and Tunbridge, 2008; Tunbridge and Harrison, 2011) or the Zinc finger protein 804A gene (Zhang et al., 2011). In line with this, we found a *BDNF* x childhood trauma effect on psychosis proneness in males, but not in females (sample 1). All these studies point out the existence of a gender-specific mechanism underlying brain development and functioning, that may be led by sexual hormones (i.e.: estrogens and androgens) (see review by Godar and Bortolato, 2014). In addition, these biological differences may interact with gender-dimorphic sociocultural factors influencing the impact of childhood trauma on the brain-mind development and gene expression patterns. Actually, there is some evidence suggesting that sex differences might be relevant not only in terms of differential rates of childhood adversities (e.g., Tolin and Foa, 2006) and PEs (e.g., Maric et al., 2003), but also in the association between childhood trauma and psychosis phenotypes (Fisher et al., 2009; Garcia et al., 2016). Since sample 2 was composed of females only, this sex-specific effect could not be explored.

The findings of the present study, although partially replicate previous findings, do not provide conclusive results. They should be considered in light of some limitations, such as the two different questionnaires used to assess childhood trauma in each sample. In sample 2 some explicit questions on physical and sexual abuse were omitted, which could have resulted in an underestimation of experienced trauma. It is possible that some individuals scored zero for trauma, although they had experienced abuse. However, since the correlation between different types of trauma tends to be high (Bellis et al., 2014), the effect of the omission of these items is likely limited. Furthermore, the present study may be underpowered for the sex-stratified interaction analyses. The retrospective assessment of childhood trauma which may bias incidence rates, the proportion of males and females studied, and the sample size are also limitations of the present study. In this regard, future studies should be done in this field considering larger samples with comparable sex groups and genetic, environmental and/or epigenetic factors to better understand the modulating effect of this gene on psychosis and psychosis proneness.

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Contributors

N. Barrantes-Vidal, T. Kwapil, R. van Winkel and A. Rosa managed the design, analysis and interpretation of the data. T. Sheinbaum, P. Cristóbal-Narváez, M. de Castro-Catala and E. Peña participated in the collection and design of databases of sample 1. N. Jacobs, C. Derom, E. Thiery and J. van Os participated in the collection and design of the sample 2 study. M. de Castro-Catala and E. Peña conducted the lab work of sample 1. M.de Castro-Catala and M. van Nierop undertook statistical analysis with input from T. Kwapil, R. van Winkel and A. Rosa. M de Castro-Catala and M. van Nierop wrote the initial manuscript, which was further edited by all the authors. All authors contributed to and approved the final manuscript.

Conflict of interest

The authors have no conflicts of interest to disclose.

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Supervisor's report on the contribution of the PhD candidate to the article.

Dr. Araceli Rosa de la Cruz, Senior Lecturer at the Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals of the Facultat de Biologia (Universitat de Barcelona) and supervisor of the present doctoral thesis by Marta de Castro i Català, hereby certifies that none of the co-authors of the article "Childhood trauma, *BDNF* Val66Met and subclinical psychotic experiences. Attempt at replication in two independent samples" have used this publications for a doctoral thesis, and that the participation of the PhD applicant in this article included the following tasks:

- Participation in the conception and design of the study.
- Participation in the collection of sample 1.
- Laboratory tasks of sample 1.
- Stay at the Maastricht University.
- Statistical analyses and interpretation of data.
- First drafting of the manuscript.
- Critical revision of the article for intellectual content.

Signed by Dr. Araceli Rosa

Barcelona, May 31st 2017

3.5. Interaction effect of *FKBP5* gene and childhood trauma on psychosis, depression and anxiety symptoms: study in a non-clinical sample

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Resum

Patir adversitat durant la infància ha estat associat a un risc incrementat per desenvolupar tant psicopatologia clínica com no-clínica durant l'edat adulta. Els gens relacionats amb la resposta a l'estrés, com ara l'*FKBP5*, són possibles gens moderadors d'aquesta associació. Aquest estudi tenia l'objectiu d'explorar la implicació de la variabilitat genètica de l'*FKBP5* en l'associació entre diferents tipus de trauma i símptomes subclínics de psicosis, depressió i ansietat en una nostra no clínica. Es van obtenir mesures d'esquizotípia, experiències psicòtiques atenuades, símptomes de depressió i ansietat i de trauma durant la infància de 808 adults joves. Dos blocs haplotípics dins el gen *FKBP5* van ser detectats: bloc 1 (rs3800373 - rs9296158 - rs1360780) i bloc 2 (rs9470080 - rs4713916). Els individus van ser classificats en dos grups en funció de si eren portadors o no de l'haplotip de risc descrit prèviament a la literatura (bloc 1: CAT i bloc 2: TA). Es van dur a terme anàlisis de regressió linear per (i) estudiar l'associació entre trauma i els blocs haplotípics i (ii) explorar l'efecte de la seva interacció en les diferents formes de psicopatologia mencionades.

Totes les escales de trauma, excepte la d'abús sexual, es van trobar associades amb l'esquizotípia, les experiències psicòtiques atenuades, i els símptomes de depressió i ansietat. Cap d'aquests símptomes es va trobar associat amb la variabilitat genètica de l'*FKBP5*. El nostre resultat principal va ser una interacció entre l'haplotip del bloc 2 i l'abús en les variables que mesuren símptomes depressius. Específicament, els individus que havien estat exposats a abús emocional i físic que no eren portadors de l'haplotip TA (bloc 2) mostraven un major risc per presentar símptomes depressius. Aquests resultats suggereixen que l'exposició a abús durant la infància podria incrementar el risc per patir símptomes depressius a un nivell subclínic, depenent de la variabilitat genètica en l'*FKBP5*. No obstant, calen més estudis per aclarir quin és el paper d'aquest gen en la salut mental.

Interaction between *FKBP5* gene and childhood trauma on psychosis, depression and anxiety symptoms in a non-clinical sample

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ABSTRACT

Background: Childhood trauma has been associated with a heightened risk for presenting clinical and non-clinical psychopathology in adulthood. Genes related with the stress response, such as the FK506 binding protein 51 (*FKBP5*), are plausible candidates moderating the effects of childhood trauma on the emergence of such symptoms later on. The present study aimed to explore the moderating role of *FKBP5* genetic variability on the association of different types of childhood trauma with subclinical psychosis, depression and anxiety in a non-clinical sample.

Methods: Schizotypy, psychotic-like experiences, depression and anxiety symptoms and childhood trauma were assessed in 808 young adults. Two *FKBP5* haplotypic blocks were detected: block 1 (rs3800373 - rs9296158 - rs1360780) and block 2 (rs9470080 - rs4713916). Subjects were classified in two groups according to whether they carried or not the risk haplotype previously described in the literature (block 1: CAT and block 2: TA). Linear regression analyses were used to study (i) the main effects of childhood trauma and *FKBP5* haplotype blocks and (ii) their interaction effects on the mentioned forms of psychopathology.

Results: All childhood trauma scales, except sexual abuse, were associated with schizotypy, psychotic-like experiences, depression and anxiety symptoms. None of the analysed symptoms was associated with *FKBP5* genetic variability. Our main finding was a block 2-haplotype x abuse interaction on the variables tapping depressive symptoms. Specifically, subjects who were exposed to emotional and physical abuse and were non-TA carriers (haplotype block 2) were shown to be at higher risk for depressive symptoms.

Conclusions: Our data suggest that exposure to childhood abuse may increase the risk for depressive symptoms below the diagnostic threshold depending on *FKBP5* genetic variability. Further research is needed to better elucidate the role of *FKBP5* on mental health.

Key-words: schizotypy, psychotic-like experiences, depression, anxiety, childhood trauma, *FKBP5* gene, gene-environment interaction.

1. INTRODUCTION

Stressful life events, especially those taking place during sensitive early development periods, predict risk for a number of psychiatric disorders (Varese et al., 2012). Childhood trauma, as a form of psychological stress, is one of the most replicated adverse experiences associated with the development of clinical and non-clinical psychotic symptoms (Sheinbaum and Barrantes-Vidal, 2015; Spauwen et al., 2006; Velikonja et al., 2015), depression and anxiety (Hovens et al., 2010; Mandelli et al., 2015) in adulthood. Some of these studies have suggested that different types of childhood trauma (e.g.: abuse and neglect) may have differential effects on the brain and may thus be associated with the emergence of different symptoms (Heins et al., 2011; Mandelli et al., 2015). However, others pointed out that other adversity-related variables (e.g.: age of exposure or intention to harm) may be more relevant than the specific type of trauma in predicting psychopathology (Van Nierop et al., 2014; Varese et al., 2012). It is believed that exposure to such childhood adversities causes a sensitization that leads to increased behavioural, neurochemical, or psychological responses to subsequent exposures to (even less severe) stressful events.

However, most individuals exposed to childhood trauma do not develop psychotic, depressive or anxious symptoms, which points out that these adverse experiences are neither a necessary nor a sufficient cause for psychopathology. In this regard, individual genetic vulnerability, among other factors, has been suggested to play an essential role in the differential emergence of such symptoms following adversity (Van Winkel et al., 2013). Thus, current evidence suggests that a complex interplay between environmental and biological factors is likely to underlie the development of clinical and non-clinical levels of psychopathology.

Hypothalamus-pituitary-adrenal (HPA) axis dysregulation is one of the plausible biological systems underlying sensitization, contributing to a final common pathway of dopamine sensitization in mesolimbic regions (Belda et al., 2015; Van Winkel et al., 2008). This neuroendocrine axis mediates the principal adaptive response to perceived stress via glucocorticoid (cortisol) release by the adrenal cortex. Cortisol binds to its receptor, the glucocorticoid receptor (GR), that once activated exerts a wide range of effects orchestrating the

systemic stress response. Besides the stress response effects, cortisol is also critical for restoring homeostasis to the HPA axis. This is achieved through a negative feedback loop on several levels of the axis, anterior pituitary and hypothalamus, involving the activation of GR and the subsequent changes in expression of those genes participating in HPA axis regulation. An impaired negative feedback regulation via GR has been proposed to be a potential risk factor for stress-related psychopathology (Binder, 2009).

In the frame of a genetic susceptibility model, polymorphic variants in genes involved in the moderation of the stress response driven by the HPA axis are, thus, plausible candidates mediating this gene-environment interaction (G x E). One such gene is the FK506 binding protein 51 (*FKBP5*), located on chromosome 6p21.31. *FKBP5* codes for a co-chaperone that binds to a complex of proteins including other co-chaperones and GR. FKBP5 binding promotes a receptor complex conformation that has lower affinity for cortisol and inhibits GR activity. Cortisol binding in the complex promotes the exchange of FKBP5 for FK506 binding protein 52 (FKBP4) and activates GR signalling which, among other functions, promotes the synthesis of FKBP5 and regulates its own activity via a negative feedback loop (Zannas and Binder, 2014). Notably, a functional haplotype spanning the whole gene has been related to a differential FKBP5 mRNA and protein induction by GR activation and also to variation in GR sensitivity (Binder, 2009; Binder et al., 2004; Zannas and Binder, 2014). This haplotype is tagged by rs3800373, rs9296158 or rs1360780 single nucleotide polymorphisms (SNPs), and further explorations identified rs1360780 as the variant most likely conferring this functionality (Zannas and Binder, 2014).

Several studies have focused on exploring the interaction effect of *FKBP5* genetic variability and childhood trauma on psychotic, depression or anxiety symptoms in non-clinical, subclinical and clinical samples, showing a G x E effect of several *FKBP5* SNPs and different early adversities. In the case of depression, increased symptoms were observed in general population subjects homozygous for the minor allele of rs1360780 who were exposed to physical abuse and/or severe levels of sexual and emotional abuse (Appel et al., 2011). Another study showed increased depressive symptoms in subjects carriers of minor alleles for rs1360780, rs9470080 and rs9394309 who were separated from their parents during childhood (Lahti et al., 2016). More recently, the minor alleles of rs3800373, rs926158, rs1360780, rs9470080 and rs4713916 were associated with higher risk of developing anxiety and depressive disorders in children who experienced a high number of mild to moderate (but not severe) life events (Scheuer et al., 2016). In the same line, two independent studies found an effect of FKBP5 SNPs on anxiety (Isaksson et al., 2016) and depression symptoms (VanZomeren-Dohm et al., 2015), but specifically in adolescent females who were exposed to violence or peer victimization. Although there are abundant studies analysing this G x E in relation to anxiety and depression,

this has been scarcely explored on psychosis or psychotic-like symptoms. In this regard, the first study analysing this G x E on psychosis showed a significant *FKBP5* x childhood trauma effect on psychosis across three levels of psychosis severity (Collip et al., 2013). Specifically, non-clinical subjects carrying rs9296158 and rs1360780 minor alleles and/or rs1043805 major allele were more likely to exhibit positive psychotic-like experiences when exposed to childhood trauma. The rs992105 and rs4713916 minor alleles were related to positive schizotypy in patients' relatives who had suffered childhood trauma. In patients, significant interaction results were observed with those carrying the minor allele of rs9296158 on positive psychotic symptoms. Another study explored *FKBP5* rs1360780 x childhood abuse interaction in a non-clinical sample and showed a significant effect in the same direction (minor allele carriers had higher scores) on positive psychotic-like experiences (Alemany et al., 2016).The aforementioned studies have shown that polymorphisms in the 3' region of the *FKBP5* (such as rs3800373, rs9296158 or rs1360780) are related to the stress response. However, less is known about the potential genetic influences of the 5' region of this gene in this context. In terms of functionality, according to the ENCODE annotation of DNA elements (Encode Consortium, 2012), the 5' region of this gene contains a significant number of functional elements, including promoters and other regulatory regions, with potential effects on the expression of different *FKBP5* isoforms. In this regard, four of the abovementioned G x E studies identified two polymorphisms in the 5' region of the *FKBP5* (i.e.: rs9470080 or rs4713916) as relevant in G x E analysis on psychosis (Collip et al., 2013), depression (Lahti et al., 2016; Scheuer et al., 2016) and anxiety (Isaksson et al., 2016; Scheuer et al., 2016).

Based on these previous findings, the aim of our study was to explore the effect of the interaction between *FKBP5* gene and childhood trauma on psychotic, depression and anxiety symptoms in a non-clinical sample. Our approach aimed to analyse two *FKBP5* haplotypic blocks. Thus, in the 3'region we studied diplotypes (i.e. haplotypic combinations) including rs3800373, rs9296158 and rs1360780, described previously as functional (Zannas and Binder, 2014). In the 5´region, diplotypes involving rs9470080 and rs4713916, tagging the first exons and introns of the gene and the promoter were analysed.

According to previous studies, we hypothesised that subjects carrying specific diplotypes would be at higher risk of presenting psychotic, depression and anxiety symptoms when exposed to childhood trauma. Additionally, we wanted to ascertain whether different subtypes of childhood trauma have a different effect on such G x E interactions.

2. MATERIAL AND METHODS

2.1. Subjects

The sample included 808 unselected non-clinical students enrolled at university (67.7%) or

technical training (32.3%) courses in the Barcelona area. This sample was collected to investigate the role of genetic and environmental factors and their interaction effect in predicting psychosis proneness (Barrantes-Vidal et al., 2013; de Castro-Catala et al., 2017, 2015). All subjects volunteered to take part in the study and provided written informed consent at assessments, after being informed of the objectives of the study. Ethical approval was obtained from local research ethics committees.

2.2. Measures

Participants completed a battery of self-reported measures assessing schizotypy, psychotic-like experiences, depression and anxiety symptoms, as well as childhood trauma.

Schizotypy was assessed with the Spanish version of the Wisconsin Schizotypy Scales (WSS; Ros-Morente et al., 2010), including the Perceptual Aberration, Magical Ideation, Revised Social Anhedonia, and Physical Anhedonia Scales. Exploratory and confirmatory factor analyses of the four scales reliably produce two factors, positive and negative schizotypy, accounting for 80% of the variance (Kwapil et al., 2008). Although the technical school participants completed the short version of the scales (Winterstein et al., 2011), which raw scores are not comparable to those obtained with the original scales, the factor structure underlying both the short and the original scales is comparable (Gross et al., 2015). Participants were assigned positive and negative schizotypy factor scores based upon factor loadings derived from a sample of 6137 college students (Kwapil et al., 2008).

Psychotic-like experiences were measured using the Spanish version of the Community Assessment of Psychic Experiences (CAPE; Ros-Morente et al., 2011), a validated self-reported instrument for assessing such experiences in general population samples (Konings et al., 2006). The CAPE consists of 42 items measuring the lifetime prevalence of positive and negative psychotic-like experiences as well as depressive symptoms on a frequency scale ranging from “never” to “nearly always” (scoring 0 to 3). A total sum score per dimension on the frequency items was used in the analyses.

Depression and anxiety symptoms were assessed using the depression (13 items) and anxiety (10 items) subscales of the Symptom Checklist-90-Revised (SCL-90-R) (de las Cuevas et al., 1991; Derogatis, 1994), a validated self-report questionnaire. Each item is scored on a 5-point scale from 0 (“not at all”) to 4 (“extremely”). The sum scores for each subscale were used for analyses.

Childhood trauma was assessed using the Spanish version of the short form of the Childhood Trauma Questionnaire (CTQ) (Bernstein and Fink, 1998; Hernandez et al., 2013). The CTQ consists of 28 questions enquiring about five types of childhood trauma: emotional abuse, physical abuse, sexual abuse, emotional neglect and physical neglect. The score for each item ranges from “never true” to

"very often true" (scoring 1 to 5). For better comparison with previous studies, the variables childhood abuse (sum of emotional, physical and sexual abuse), neglect (sum of emotional and physical neglect) and a total childhood trauma score (sum of the five types of childhood trauma) were computed as in Alemany et al. (2016, 2011).

2.3. Genetic data

Participants provided a biological sample consisting of buccal mucosa on cotton swabs. Genomic DNA was extracted using the REALpure genomic DNA extraction kit (Durviz S.L.U., Valencia, Spain). Five single-nucleotide polymorphisms (SNPs) within the *FKBP5* gene were genotyped using TaqMan 5' exonuclease assay (Applied Biosystems): rs3800373 (SNP1), rs9296158 (SNP2), rs1360780 (SNP3), rs9470080 (SNP4), and rs4713916 (SNP5). See assay codes in Figure 1. Polymerase chain reaction plates were read on an ABI PRISM 7900HT instrument with SDS v2.1 software (Applied Biosystems). For accuracy of genotyping, twenty percent of the sample, randomly selected, was genotyped twice. Compliance with the Hardy-Weinberg Equilibrium was assessed for each SNP.

Linkage disequilibrium (LD) between the five FKBP5 polymorphisms in the sample under study was examined by pair-wise comparisons of r2 and D' using Haploview version 4.2 (Barrett et al., 2005). LD pattern was similar to that observed in the reference Iberian population (IBS) from 1000 genomes project (data not shown). Two haplotype blocks were defined including SNP 1 to SNP 3 (haplotype 1; r^2 = 0.79, D' = 0.89) and SNP 4 to SNP 5 (haplotype 2; r^2 = 0.70, D' = 0.97) (Figure 1). As each subject carries two haplotypes (one inherited from the father and the other from the mother), in each block, the combination of haplotypes (diplotypes) was estimated for each participant using a Bayesian approach implemented in PHASE software (Stephens and Donnelly, 2003). Only subjects with a diplotypic estimation probability higher than 80% were included in the analyses.

2.4. Statistical analysis

Linear regression analyses were conducted using STATA v.13.1 (Stata/MP 13.1 for Windows, StataCorp LP, College Station, USA). First, we explored the main effects of childhood trauma and *FKBP5* diplotypes on schizotypy, psychotic-like experiences, and depression and anxiety symptoms. Second, the interaction between *FKBP5* diplotypes and the childhood trauma variables was added in the regression. Analyses were performed using each dimension or symptom measure as dependent variables in separate models. Childhood trauma variables, genetic data and their interaction were added as independent variables. Age, sex and cohort membership (whether they were undergraduate or technical school students) were added as covariates in all the analyses. P-values corrected for multiple testing were calculated performing permutation testing of 10000 simulations using *lmPerm* package (Wheeler, 2010) in R 3.2.1 software (R Development Core Team, 2011).

Plots were generated using the *ggplot2* package (Wickham, 2009) in R 3.2.1 software.

3. RESULTS

3.1. Descriptive data

Participants were aged 17 to 54 years (mean age = 20.79, SD = 4.06). They were mainly women (77 %) and of European origin (93 % subjects with both parents born in Europe). Males and females slightly differed in terms of age (males: mean = 21.4, SD = 4.5; females: mean = 20.6, SD = 3.9, $p < 0.05$). Descriptive statistics for schizotypy, psychotic-like experiences, depressive and anxiety symptoms, as well as correlation between each dimension or symptom measure, are shown in Table 1. For the CTQ questionnaire, only one subject had no valid data. Descriptive statistics for the childhood trauma variables, as well as the correlations between them, can be found in Table 2. Details on the groups according to levels of trauma are shown in Table 2. Further details on total childhood trauma, abuse and neglect data in this sample can be found elsewhere (de Castro-Catala et al., 2016).

Details on genotype data are shown in Figure 1. No differences were observed for genotype frequencies between males and females ($p > 0.05$, data not shown). In block 1, fourteen subjects (1.73%) showed diplotypic probability estimates < 80% and, therefore, were excluded for analyses. The most frequent diplotypes within this block were AGC/AGC (41.69 %), AGC/CAT (38.04 %) and CAT/CAT (8.94 %), the other combinations showed frequencies lower than 5%. Subjects were classified in two groups according to whether they carried or not at least one hypothetical risk haplotype (i.e. CAT): CAT carriers (n = 400, 50.38 %) and non-CAT carriers (n = 394, 49.62 %).

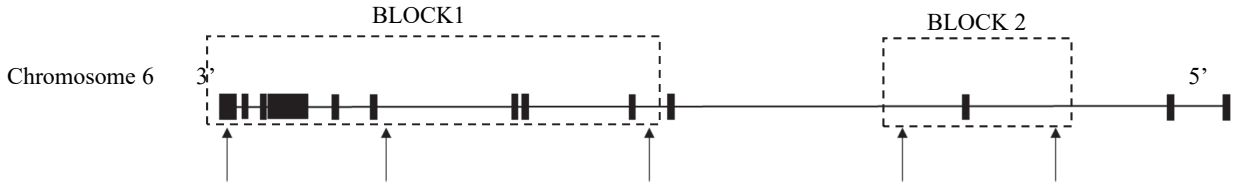
Table 1 Descriptive data of the CAPE scales (positive, negative and depressive), the WSS scales (positive and negative), and the SCL-90-R scales (depression and anxiety) in the present sample and correlation between these variables (all p-values < 0.01). Note that WSS positive and negative scores are Z scores derived from factor analyses.

	mean	s.d.	range	n	Correlation between variables						
CAPE positive	8.04	4.88	0 – 29	807	1.00						
CAPE negative	9.15	5.26	0 – 35	804	0.44	1.00					
CAPE depressive	5.95	2.99	1 – 22	806	0.45	0.56	1.00				
WSS positive	- 0.46	0.75	- 1.71 – 3.23	805	0.67	0.37	0.40	1.00			
WSS negative	- 0.04	0.90	- 1.75 – 4.27	805	0.15	0.34	0.18	0.16	1.00		
SCL-90-R depression	11.60	8.60	0 – 49	801	0.44	0.58	0.77	0.42	0.23	1.00	
SCL-90-R anxiety	6.28	5.58	0 – 33	805	0.46	0.36	0.59	0.43	0.16	0.64	1.00
					CAPE positive	CAPE negative	CAPE depressive	WSS positive	WSS negative	SCL-90-R depression	SCL-90-R anxiety

Table 2 Descriptive data of the childhood trauma variables included in the present study and correlations among trauma variables (all p-values ≤ 0.0012).

	mean	s.d.	range	n	Correlation between variables							
Abuse	18.15	5.03	15 – 50	807	1.00							
Emotional abuse	7.24	3.36	5 – 24	807	0.91	1.00						
Physical abuse	5.56	1.62	5 – 21	807	0.72	0.54	1.00					
Sexual abuse	5.36	1.60	5 – 25	807	0.50	0.21	0.11	1.00				
Neglect	14.79	4.92	10 – 41	807	0.55	0.57	0.38	0.15	1.00			
Emotional neglect	8.90	3.74	5 – 24	807	0.52	0.55	0.33	0.15	0.96	1.00		
Physical neglect	5.90	1.70	5 – 18	807	0.44	0.42	0.39	0.11	0.78	0.57	1.00	
Total trauma	32.94	8.76	25 – 84	807	0.88	0.84	0.63	0.37	0.88	0.84	0.69	1.00
					Abuse	Emotional abuse	Physical abuse	Sexual abuse	**Neglect**	Emotional neglect	Physical neglect	**Total trauma**

Figure 1 Details on the five *FKBP5* single nucleotide polymorphisms (SNPs) and the haplotype blocks (block 1 and 2) included in the present study.



SNP	**SNP1**	**SNP2**	**SNP3**	**SNP4**	**SNP5**
rs	rs3800373	rs9296158	rs1360780	rs9470080	rs4713916
Location [1]	6:35574699	6:35599305	6:35639794	6:35678658	6:35702206
Position	3' UTR	Intron 6	Intron 9	Intron 10	Intron 11
Alleles [2]	A/C	G/A	C/T	C/T	G/A
MAF	0.33	0.36	0.33	0.36	0.22
AB assay code	C_27489960_10	C_1256775_10	C_8852038_10	C_92160_10	C_1979246_10
Genotype frequencies	AA: 357 (45.89%) AC: 342 (46.96%) CC: 79 (10.15%)	GG: 356 (45.01%) GA: 345 (43.62%) AA: 90 (11.38%)	CC: 369 (46.13%) CT: 340 (42.50%) TT: 91 (11.38%)	CC: 314 (42.38%) CT: 328 (44.26%) TT: 99 (13.36%)	GG: 397 (49.44%) GA: 341 (42.47%) AA: 65 (8.09%)
Hardy-Weinberg equilibrium	$\chi^2 = 0.05$ p = 0.826	$\chi^2 = 0.64$ p = 0.214	$\chi^2 = 0.89$ p = 0.347	$\chi^2 = 0.82$ p = 0.364	$\chi^2 = 0.48$ p = 0.489

[1] assembly GRCh38.p2. [2] Major/Minor allele; MAF: Minor allele frequency from the 1000 genomes project, AB: Applied Biosystems

In block 2, thirty subjects (3.71%) showed diplotypic probability estimates < 80% and were excluded. In this block, the most frequent diplotypes were CG/CG (40.36 %), CG/TA (37.92 %), TA/TA (8.10 %), CG/TG (6.56 %) and TG/TA (5.27 %); other combinations showed frequencies < 5%.Subjects were classified in TA (risk haplotype) carriers (n = 401, 51.54 %) and non-TA carriers (n = 377, 48.46 %).

3.2. Main effects of childhood trauma and *FKBP5* on the studied phenotypes

All childhood trauma subscales included in the present study, except sexual abuse in some cases, showed significant main effects on CAPE subscales (Table A.1), schizotypy (Table A.2), and depressive and anxiety symptoms (Table A.3). Note that the main effect of abuse, neglect and total trauma on positive and negative psychotic-like experiences in this sample was reported in a previous study (de Castro-Catala et al., 2016), but these results are also shown here for the sake of completeness. None of the two FKBP5 haplotype blocks showed a significant main effect on CAPE subscales (Table A.1), schizotypy (Table A.2) or the affective measures (Table A.3).

3.3. Interaction between childhood trauma x *FKBP5* haplotype block 1 on the studied phenotypes

The interaction of haplotype block 1 with childhood trauma variables did not show a significant effect on CAPE subscales, positive and negative schizotypy, or depressive symptoms. Regarding anxiety, no interaction effect was observed for abuse, neglect or total trauma. However, further exploration of the trauma subscales revealed a significant interaction between the diplotypic groups analysed and both emotional (β = -0.18, s.e. = 0.11, p = 0.039) and physical abuse (β = - 0.33, s.e. = 0.24, p = 0.012). Specifically, subjects carrying the CAT haplotype showed lower anxiety scores than non-carriers when exposed to emotional or physical abuse (data not shown). Further details are shown in Tables A.1-A.3.

3.4. Interaction between childhood trauma x *FKBP5* haplotype block 2 on the studied phenotypes

The interaction analyses of haplotype block 2 and childhood trauma did not show any significant effect for abuse (Figure 2b), neglect or any of the trauma variables on positive or negative schizotypy.

Regarding psychotic-like experiences, no significant effect was detected for any of the trauma scales (Table A.1).

In the case of negative psychotic-like experiences, abuse and total trauma were found to have an interaction effect with the diplotypic groups (abuse: β = - 0.31, s.e. = 0.07, p = 0.025; total trauma: β = - 0.32, s.e. = 0.04, p = 0.023), with TA carriers reporting lower scores than non-TA carriers, when exposed to abuse or total trauma (Figure 2a). Further analyses revealed

that this effect was driven by emotional abuse (β = - 0.21, s.e. = 0.11, p = 0.022).

In the case of the CAPE depressive scale, an interaction with abuse was detected (β = - 0.29, s.e. = 0.04, p = 0.029), by which individuals carrying the TA haplotype were reporting lower scores when exposed to abuse (Figure 2a). This, reflected the effect in the same direction of emotional abuse (β = - 0.18, s.e. = 0.06, p = 0.039).

On depressive symptoms as measured with SCL-90-R, only abuse showed an interaction effect (β = - 0.33, s.e. = 0.12, p = 0.013), which was driven by emotional (β = - 0.19, s.e. = 0.18, p = 0.028) and physical abuse (β = - 0.34, s.e. = 0.38, p = 0.009). In this sense, subjects carrying the TA haplotype were reporting lower depressive symptoms than non-carriers, when exposed to abuse (Figure 2c). With respect to anxiety symptoms, abuse showed an interaction effect with the group of diplotypes considered (β = - 0.29, s.e. = 0.08, p = 0.030), showing that carriers of the TA haplotype were presenting lower anxiety symptom scores than those non-carriers when exposed to abuse (Figure 2c). In this case, emotional (β = - 0.20, s.e. = 0.11, p = 0.023) and physical abuse (β = - 0.31, s.e. = 0.25, p = 0.018) were the scales having an effect. Further details are given in Tables A.1-A.3.

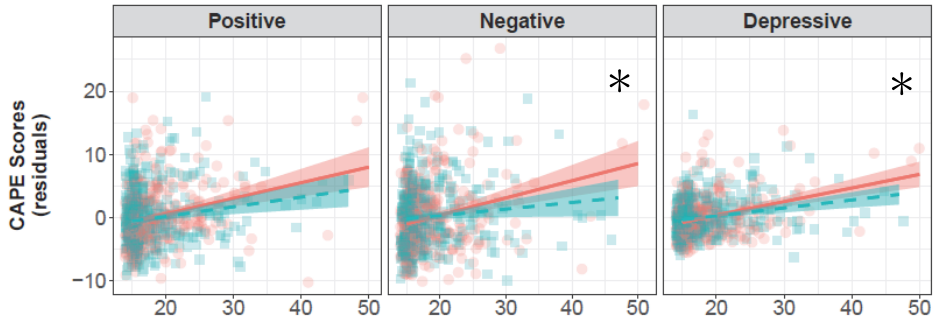
4. DISCUSSION

The interest of studying the *FKBP5* gene in mental health relies on its implication in the HPA axis stress response by regulating GR affinity and signalling (Zannas and Binder, 2014). Stress-related psychopathology has been related to an impaired negative feedback regulation via GR (Binder, 2009). Additionally, in animal models, genetic predisposition for altered stress reactivity has been found to interact with early-life stress by modifying coticosterone release during a critical brain development period, exerting lasting effects on the HPA axis (McIlwrick et al., 2016). In this line, the interaction between childhood trauma and *FKBP5* polymorphisms has been related to altered glucocorticoid (cortisol) levels (Buchmann et al., 2014) and a heightened threat-related amygdala reactivity (White et al., 2012). Other studies have reported a G x E effect in other psychosis-related phenotypes such as impaired attention (Green et al., 2015) or higher dissociative symptoms (Yaylaci et al., 2016). Also, widespread structural changes in subcortical and cortical emotion-processing brain areas have been related to FKBP5 and childhood abuse (Grabe et al., 2016).

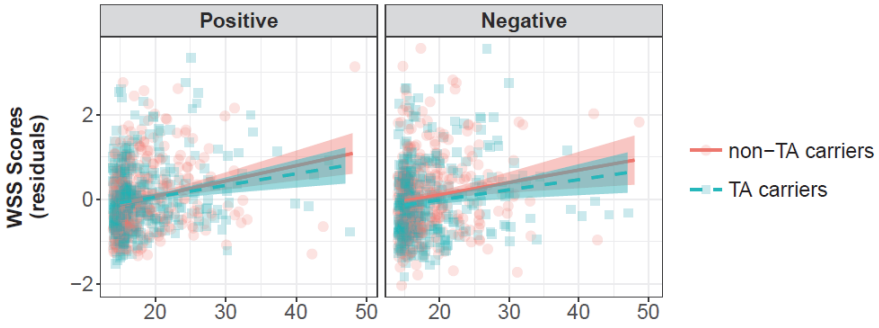
The present study examined the interaction effect of *FKBP5* gene variability and childhood trauma on schizotypy, psychotic-like experiences and depression and anxiety symptoms in non-clinical young adults. It provides further insights on the implication of *FKBP5* genetic variability – including a haplotype in the 3´region previously described as a functional (Zannas and Binder, 2014) and a haplotype in the less studied 5´region – on the association between childhood trauma and different types of psychopathology.

Figure 2 Graphic representation of the interaction effects between the *FKBP5* haplotype block 2 groups (TA carriers and non-TA carriers) and abuse on (a) CAPE positive, negative and depressive scores, (b) WSS positive and negative scores and (c) SCL-90-R depressive and anxiety scores. Significant interactions are indicated with *. Note that residuals corrected by age, sex and cohort membership were used in the plots.

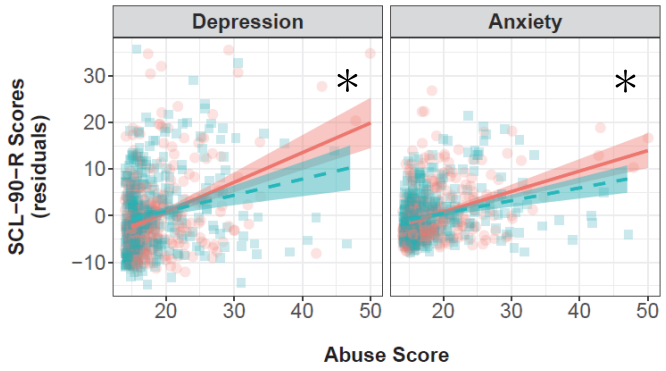
(a)



(b)



(c)



First, our results support the strong association between different types of trauma with personality deviances and psychopathology. Many lines of evidence have shown that people who have suffered trauma at early ages are more vulnerable to develop psychopathology (Mandelli et al., 2015; Varese et al., 2012; Velikonja et al., 2015). In this line, all types of trauma but sexual abuse were associated with psychotic, depressive and anxiety symptoms in our sample. Some researchers have suggested that specific types of trauma might be related to specific symptoms (Heins et al., 2011; Schenkel et al., 2005) and one meta-analysis found sexual abuse to be less related to depressive symptoms (Infurna et al., 2016). However, the fact that we could not detect an effect for sexual abuse in all variables should be considered with caution, since participants reported very low levels of this specific type of abuse with the consequences it implies regarding statistical power in our sample. Non-clinical samples often have low (but not negligible) levels of sexual abuse (Bendall et al., 2008) and, moreover, subjects in general tend to underreport this type of abuse (Read et al., 2005), which makes the exploration of its effects on mental health substantially difficult. This should be considered in future studies exploring sexual abuse in non-clinical samples.

Secondly, our results support that *FKBP5* genetic variability plays a role in the development of psychopathology in subjects who have been exposed to childhood trauma. As far as we know, few studies ascertaining these specific G x E effects on psychopathology have included, as ours, haplotypic combinations in their analyses. The study of haplotypes is of great interest because it increases the power to find genetic associations (Crawford and Nickerson, 2005). Our analyses based on block 1 haplotypes and the psychosis-related traits studied (schizotypy and psychotic-like experiences) could not detect any effect. These results differ from previous studies that detected a G x E effect for the SNPs within this block in relation to psychiatric symptoms (Comasco et al., 2015), psychotic-like experiences (Alemany et al., 2016; Collip et al., 2013) or also in the real-world expression of psychosis proneness and social stress reactivity using Experience Sampling Methodology (Cristóbal-Narváez et al., 2016). In our non-clinical sample, we observed an interaction effect of these diplotypic groups with emotional and physical abuse on anxiety symptoms, by which subjects carrying the CAT haplotype were at lower risk of presenting those symptoms. A similar interaction effect has been recently reported with these SNPs (i.e. SNP1, 2 and 3) and risk of developing anxiety and/or depressive disorder, although opposite in terms of risk alleles in children who suffered mild to moderate life events, but not in those that suffered severe life events (Scheuer et al., 2016). This same effect was observed in another study exploring anxiety in female adolescents and young adults exposed to violence (Isaksson et al., 2016). Also, SNP3 was found to play a role on depression in the same direction in interaction with childhood separation (Lahti et al., 2016),

childhood physical abuse and severe emotional and sexual abuse (Appel et al., 2011), and current peer victimization in girls (VanZomeren-Dohm et al., 2015).

As regards to haplotype block 2, located in the less explored 5' region of the gene, our interaction analyses showed that, upon childhood abuse, subjects carrying the TA haplotype were at lower risk of developing negative psychotic symptoms, as well as depression and anxiety symptoms. These associations were mostly driven by the emotional and physical abuse scales, and not observed for sexual abuse. It is worth noting that the effects we detected here are mainly on depression and anxiety symptoms. The CAPE negative dimension has been found to be highly saturated with depression (Barrantes-Vidal et al., 2013) and we observed that it is highly correlated with depression symptoms in our sample as measured both with the CAPE and SCL-90-R. Therefore, unlike the negative dimension of schizotypy (as measured with the WSS), we believe that the associations detected with the CAPE negative dimension are more related to depression rather than to subclinical negative symptoms. To the best of our knowledge, the two polymorphisms comprised in haplotype block 2 have been analysed in three G x E studies in relation to depression and/or anxiety (although none of these studies included a childhood trauma assessed by CTQ). In these studies, minor allele carriers for these polymorphisms were shown to be at higher risk for developing anxiety and/or depression when they were exposed to mild to moderate (but not severe) life events (Scheuer et al., 2016), separation from parents during childhood (Lahti et al., 2016) or violence (Isaksson et al., 2016). In this last study, Isaksson and colleagues could not detect an effect on depression, and the associations with anxiety were observed only in female participants, but not males.

As regards to schizotypy and psychotic-like experiences, there are no studies to date analysing the variability in this 5' region and its interaction effect on schizotypy and only one explored SNP5 x childhood trauma on psychotic-like experiences. In this study, Collip and colleagues found an effect in their sample of healthy siblings, showing the minor allele as the risk allele for developing such symptoms. However, this was not detected in their general population, healthy controls and patients samples (Collip et al., 2013). The results observed in our sample regarding haplotype block 2, although consistent with the depression and anxiety measures we have studied, are inconsistent in terms of allele of risk with previous published studies. Such an inconsistency between previous results and our study (based on more than 800 subjects accurately assessed for the relevant phenotypes) suggests that understanding the role of FKBP5 in this context requires further studies.

The present study has several limitations that should be considered. First, the modest levels of childhood trauma detected in the sample could have made more difficult the detection of G x E interaction effects. Studies comprising participants reporting low, medium and high

levels of childhood trauma may be better positioned to detect *FKBP5* x childhood trauma effects than those with unselected samples. Second, the retrospective self-assessment of childhood trauma could have biased real incidence rates. However, such bias has been shown to be low and does not invalidate childhood trauma retrospective measures (Fisher et al., 2011; Hardt and Rutter, 2004).

In conclusion, our data suggest that exposure to childhood abuse (especially emotional and physical abuse) may increase the risk for depressive symptoms below the diagnostic threshold depending on *FKBP5* genetic variability, although further research is needed to better clarify the role of *FKBP5* on mental health.

Conflict of interest: None declared.

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APPENDICES

Table A.1 Main effects and interaction effects between childhood trauma variables (CTQ) and *FKBP5* genetic variability on CAPE positive, negative and depressive scores. Note that each section correspond to a separate model (i.e.: childhood trauma, *FKBP5* genetic variability and interaction).

	CAPE POSITIVE				CAPE NEGATIVE				CAPE DEPRESSIVE			
	β	s.e.	95% IC	p-value	β	s.e.	95% IC	p-value	β	s.e.	95% IC	p-value
CHILDHOOD TRAUMA												
Abuse[1]	*0.20*	*0.03*	*0.13 – 0.26*	*<0.0001***	*0.17*	*0.04*	*0.11 – 0.25*	*<0.0001***	0.28	0.02	0.13 – 0.21	<0.0001**
Emotional abuse	0.20	0.05	0.20 – 0.38	<0.0001**	0.18	0.05	0.18 – 0.39	<0.0001**	0.30	0.03	0.20 – 0.32	<0.0001**
Physical abuse	0.15	0.10	0.25 – 0.65	<0.0001**	0.12	0.12	0.16 – 0.61	0.001**	0.17	0.06	0.18 – 0.43	<0.0001**
Sexual abuse	0.07	0.10	0.005 – 0.41	0.045	0.03	0.12	- 0.13 – 0.32	0.406	0.09	0.06	0.04 – 0.29	0.012**
Neglect[1]	*0.15*	*0.03*	*0.08 – 0.21*	*<0.0001***	*0.26*	*0.04*	*0.20 – 0.35*	*<0.0001***	0.25	0.02	0.11 – 0.19	<0.0001**
Emotional neglect	0.14	0.04	0.09 – 0.27	<0.0001**	0.28	0.05	0.31 – 0.49	<0.0001**	0.26	0.03	0.16 – 0.26	<0.0001**
Physical neglect	0.13	0.10	0.18 – 0.57	<0.001**	0.12	0.11	0.16 – 0.59	0.001**	0.13	0.06	0.12 – 0.36	<0.001**
Total trauma[1]	*0.20*	*0.02*	*0.08 – 0.15*	*<0.0001***	*0.24*	*0.02*	*0.11 – 0.19*	*<0.0001***	0.30	0.01	0.08 – 0.12	<0.0001**
***FKBP5* GENETIC VARIABILITY**												
Block 1: CAT carriers *vs* non-carriers	0.01	0.33	- 0.53 – 0.78	0.704	0.02	0.38	- 0.51 – 0.97	0.536	0.01	0.21	- 0.34 – 0.49	0.721
Block 2: TA carriers *vs* non-carriers	- 0.03	0.34	- 0.91 – 0.41	0.457	0.01	0.38	- 0.59 – 0.89	0.691	0.006	0.21	- 0.38 – 0.46	0.866
INTERACTION												
BLOCK 1 **Abuse**	0.03	0.07	- 0.11 – 0.14	0.796	- 0.08	0.07	- 0.19 – 0.10	0.559	- 0.21	0.04	- 0.14 – 0.01	0.104
Emotional abuse	- 0.03	0.10	- 0.23 – 0.16	0.716	- 0.09	0.11	- 0.32 – 0.11	0.340	- 0.15	0.06	- 0.22 – 0.02	0.094
Physical abuse	0.19	0.21	- 0.10 – 0.71	0.140	- 0.001	0.23	- 0.46 – 0.45	0.995	- 0.17	0.13	- 0.41 – 0.09	0.210
Sexual abuse	- 0.04	0.22	- 0.51 – 0.37	0.769	- 0.08	0.25	- 0.64 – 0.35	0.558	- 0.12	0.14	- 0.40 – 0.15	0.382
BLOCK 1 **Neglect**	- 0.06	0.07	- 0.17 – 0.10	0.612	- 0.11	0.07	- 0.21 – 0.08	0.353	- 0.11	0.04	- 0.12 – 0.04	0.330
Emotional neglect	- 0.04	0.09	- 0.21 – 0.14	0.695	- 0.09	0.10	- 0.28 – 0.10	0.360	- 0.04	0.05	- 0.13 – 0.08	0.677
Physical neglect	- 0.06	0.20	- 0.47 – 0.30	0.661	- 0.06	0.22	- 0.52 – 0.34	0.673	- 0.21	0.12	- 0.44 – 0.04	0.107
BLOCK 1 **Total trauma**	- 0.04	0.04	- 0.08 – 0.06	0.768	- 0.15	0.04	- 0.13 – 0.04	0.293	- 0.24	0.02	- 0.08 – 0.01	0.084
BLOCK 2 **Abuse**	- 0.19	0.07	- 0.23 – 0.03	0.148	- 0.31	0.07	- 0.32 – - 0.02	0.025*	- 0.29	0.04	- 0.17 – - 0.01	0.029*
Emotional abuse	- 0.12	0.10	- 0.33 – 0.06	0.160	- 0.21	0.11	- 0.47 – - 0.04	0.022*	- 0.18	0.06	- 0.25 – - 0.01	0.039*
Physical abuse	- 0.07	0.21	- 0.53 – 0.29	0.561	- 0.23	0.23	- 0.86 – 0.07	0.093	- 0.17	0.13	- 0.43 – 0.09	0.204
Sexual abuse	- 0.09	0.23	- 0.60 – 0.30	0.515	- 0.04	0.26	- 0.59 – 0.42	0.752	- 0.12	0.15	- 0.41 – 0.16	0.396
BLOCK 2 **Neglect**	- 0.18	0.07	- 0.25 – 0.02	0.107	- 0.15	0.07	- 0.24 – 0.05	0.187	- 0.03	0.04	-0.09 – 0.07	0.806
Emotional neglect	- 0.14	0.09	- 0.30 – 0.05	0.150	- 0.12	0.10	- 0.31 – 0.07	0.202	0.03	0.06	- 0.09 – 0.12	0.789
Physical neglect	- 0.19	0.20	- 0.69 – 0.09	0.133	- 0.17	0.22	- 0.73 – 0.15	0.197	- 0.20	0.13	-0.44 – 0.06	0.131
BLOCK 2 **Total trauma**	- 0.26	0.04	- 0.15 – 0.003	0.058	- 0.32	0.04	- 0.18 – - 0.01	0.023*	-0.22	0.02	-0.09 – 0.01	0.104

[1] Main effects of abuse, neglect and total trauma on CAPE positive and negative in this sample were reported in de Castro-Catala et al., 2016, but have been also shown in this table (in italics) for a better comprehension of the present results). s.e., standard error; 95% IC, 95% confidence interval. P-values after 10000 iterations are indicated as follows: *p<0.1, **p<0.05.

Table A.2 Main effects and interaction effects between childhood trauma variables (CTQ) and *FKBP5* genetic variability on positive and negative schizotypy (WSS). Note that each section corresponds to a separate model (i.e.: childhood trauma, *FKBP5* genetic variability and interaction).

		WSS POSITIVE				WSS NEGATIVE			
		β	s.e.	95% IC	p-value	β	s.e.	95% IC	p-value
CHILDHOOD TRAUMA									
Abuse		0.21	0.005	0.02 – 0.04	<0.0001**	0.14	0.006	0.01 – 0.04	<0.0001**
Emotional abuse		0.20	0.008	0.03 – 0.06	<0.0001**	0.16	0.009	0.03 – 0.06	<0.0001**
Physical abuse		0.12	0.02	0.02 – 0.09	0.001**	0.10	0.02	0.02 – 0.10	0.003**
Sexual abuse		0.09	0.02	0.01 – 0.08	0.006**	0.02	0.02	- 0.03 – 0.05	0.632
Neglect		0.17	0.005	0.02 – 0.04	<0.0001**	0.26	0.006	0.04 – 0.06	<0.0001**
Emotional neglect		0.17	0.007	0.02 – 0.05	<0.0001**	0.28	0.008	0.05 – 0.08	<0.0001**
Physical neglect		0.12	0.02	0.02 – 0.08	0.001**	0.12	0.02	0.03 – 0.10	<0.001**
Total trauma		0.21	0.003	0.01 – 0.02	<0.0001**	0.23	0.003	0.02 – 0.03	<0.0001**
***FKBP5* GENETIC VARIABILITY**									
Block 1: CAT carriers *vs* non-carriers		- 0.004	0.05	- 0.11 – 0.10	0.911	- 0.06	0.06	- 0.23 – 0.01	0.063
Block 2: TA carriers *vs* non-carriers		- 0.005	0.05	- 0.11 – 0.10	0.890	- 0.07	0.06	- 0.24 – 0.004	0.057
INTERACTION									
BLOCK 1	**Abuse**	0.02	0.01	- 0.02 – 0.02	0.880	0.01	0.01	- 0.02 – 0.03	0.941
	Emotional abuse	- 0.01	0.02	- 0.03 – 0.03	0.876	0.005	0.02	- 0.04 – 0.04	0.954
	Physical abuse	0.03	0.03	- 0.06 – 0.07	0.843	- 0.18	0.04	- 0.13 – 0.02	0.177
	Sexual abuse	0.08	0.04	- 0.05 – 0.09	0.554	0.08	0.04	- 0.05 – 0.11	0.531
	Neglect	0.01	0.01	- 0.02 – 0.02	0.901	-0.12	0.01	- 0.04 – 0.01	0.265
	Emotional neglect	0.04	0.01	- 0.02 – 0.03	0.706	- 0.10	0.02	- 0.05 – 0.01	0.277
	Physical neglect	- 0.05	0.03	- 0.07 – 0.05	0.756	- 0.08	0.04	- 0.09 – 0.05	0.551
	Total trauma	0.0001	0.006	- 0.01 – 0.01	1	- 0.10	0.005	- 0.02 – 0.01	0.470
BLOCK 2	**Abuse**	- 0.11	0.01	- 0.03 – 0.01	0.416	- 0.06	0.01	- 0.03 – 0.02	0.670
	Emotional abuse	- 0.06	0.02	- 0.04 – 0.02	0.535	0.04	0.02	- 0.03 – 0.04	0.691
	Physical abuse	- 0.01	0.04	- 0.07 – 0.07	0.933	- 0.22	0.04	- 0.15 – 0.01	0.107
	Sexual abuse	- 0.06	0.04	- 0.09 – 0.06	0.673	- 0.07	0.04	- 0.10 – 0.06	0.612
	Neglect	- 0.03	0.01	- 0.02 – 0.02	0.776	0.03	0.01	- 0.02 – 0.03	0.775
	Emotional neglect	0.001	0.01	- 0.03 – 0.03	0.988	0.01	0.02	- 0.03 – 0.03	0.885
	Physical neglect	- 0.12	0.03	- 0.09 – 0.03	0.352	0.03	0.04	- 0.06 – 0.08	0.803
	Total trauma	- 0.11	0.006	- 0.02 – 0.01	0.428	- 0.05	0.007	- 0.02 – 0.01	0.696

s.e., standard error; 95% IC, 95% confidence interval. P-values after 10000 iterations are indicated as follows: *p<0.1, **p<0.05.

Table A.3 Main effects and interaction effects between childhood trauma variables (CTQ) and *FKBP5* genetic variability on depression and anxiety symptoms (SCL-90-R). Note that each section corresponds to a separate model (i.e.: childhood trauma, *FKBP5* genetic variability and interaction).

		SCL-90-R DEPRESSION				SCL-90-R ANXIETY			
		β	s.e.	95% IC	p-value	β	s.e.	95% IC	p-value
CHILDHOOD TRAUMA									
Abuse		0.27	0.06	0.35 – 0.58	<0.0001**	0.32	0.04	0.28 – 0.43	<0.0001**
Emotional abuse		0.29	0.09	0.58 – 0.91	<0.0001**	0.33	0.06	0.44 – 0.66	<0.0001**
Physical abuse		0.17	0.18	0.54 – 1.26	<0.0001**	0.21	0.12	0.50 – 0.97	<0.0001**
Sexual abuse		0.07	0.19	0.02 – 0.75	0.037*	0.08	0.12	0.05 – 0.53	0.017**
Neglect		0.27	0.06	0.36 – 0.59	<0.0001**	0.26	0.04	0.22 – 0.38	<0.0001**
Emotional neglect		0.29	0.08	0.51 – 0.81	<0.0001**	0.27	0.05	0.30 – 0.50	<0.0001**
Physical neglect		0.16	0.18	0.46 – 1.15	<0.0001**	0.18	0.11	0.36 – 0.81	<0.0001**
Total trauma		0.31	0.03	0.24 – 0.37	<0.0001**	0.33	0.02	0.17 – 0.25	<0.0001**
***FKBP5* GENETIC VARIABILITY**									
Block 1: CAT carriers *vs* non-carriers		0.02	0.61	- 0.85 – 1.53	0.575	0.003	0.40	- 0.74 – 0.81	0.932
Block 2: TA carriers *vs* non-carriers		0.03	0.61	- 0.65 – 1.75	0.371	- 0.01	0.40	- 0.89 – 0.69	0.806
INTERACTION									
DIPLOTYPE 1	**Abuse**	- 0.16	0.12	- 0.36 – 0.09	0.235	- 0.19	0.07	- 0.25 – 0.04	0.153
	Emotional abuse	- 0.11	0.17	- 0.56 – 0.12	0.208	- 0.18	0.11	- 0.45 – - 0.01	0.039*
	Physical abuse	- 0.18	0.37	- 1.24 – 0.21	0.163	- 0.33	0.24	- 1.08 – - 0.14	0.012**
	Sexual abuse	- 0.05	0.41	- 0.96 – 0.64	0.693	0.21	0.27	- 0.11 – 0.93	0.121
	Neglect	- 0.10	0.12	- 0.34 – 0.13	0.380	- 0.03	0.08	- 0.17 – 0.13	0.816
	Emotional neglect	- 0.05	0.15	- 0.39 – 0.22	0.587	0.01	0.10	- 0.19 – 0.21	0.899
	Physical neglect	- 0.14	0.35	- 1.06 – 0.32	0.296	- 0.09	0.23	- 0.60 – 0.30	0.510
	Total trauma	- 0.19	0.07	- 0.22 – 0.03	0.153	- 0.16	0.04	- 0.14 – 0.03	0.226
DIPLOTYPE 2	**Abuse**	- 0.33	0.12	- 0.53 – - 0.06	0.013**	- 0.29	0.08	- 0.32 – - 0.02	0.030*
	Emotional abuse	- 0.19	0.18	- 0.73 – - 0.04	0.028*	- 0.20	0.11	- 0.48 – - 0.04	0.023*
	Physical abuse	- 0.34	0.38	- 1.74 – - 0.25	0.009**	- 0.31	0.25	- 1.07 – - 0.10	0.018**
	Sexual abuse	- 0.006	0.42	- 0.84 – 0.80	0.968	0.13	0.27	- 0.29 – 0.78	0.362
	Neglect	0.009	0.12	- 0.23 – 0.24	0.936	0.03	0.08	- 0.13 – 0.18	0.774
	Emotional neglect	0.04	0.16	- 0.24 – 0.38	0.646	0.04	0.10	- 0.16 – 0.25	0.672
	Physical neglect	- 0.13	0.36	- 1.06 – 0.36	0.330	- 0.03	0.24	- 0.52 – 0.41	0.817
	Total trauma	- 0.22	0.07	- 0.24 – 0.02	0.105	- 0.18	0.04	- 0.14 – 0.03	0.191

s.e., standard error; 95% IC, 95% confidence interval. P-values after 10000 iterations are indicated as follows: *p<0.1, **p<0.05.

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Supervisor's report on the contribution of the PhD candidate to the article.

Dr. Araceli Rosa de la Cruz, Senior Lecturer at the Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals of the Facultat de Biologia (Universitat de Barcelona) and supervisor of the present doctoral thesis by Marta de Castro i Català, hereby certifies that none of the co-authors of the article "Interaction effect of *FKBP5* gene and childhood trauma on psychosis, depression and anxiety symptoms: study in a non-clinical sample" have used this publications for a doctoral thesis, and that the participation of the PhD applicant in this article included the following tasks:

- Participation in the conception and design of the study.
- Participation in the sample collection.
- Laboratory tasks.
- Stay at the Institute of Psychiatric Phenomics and Genomics (IPPG), Medical Center of the University of Munich.
- Statistical analyses and interpretation of data.
- First drafting of the manuscript.
- Critical revision of the article for intellectual content.

Signed by Dr. Araceli Rosa

Barcelona, May 31st 2017

3.6. *COMT*, *BDNF* and *FKBP5* genes as moderators of the association between childhood trauma, schizophrenia and psychosis proneness: a systematic review of gene-environment studies

de Castro-Catala M, Fañanás L, Barrantes-Vidal N, Rosa A.

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Resum

Els estudis d'interacció gen-ambient (GxE) han posat de manifest que alguns gens candidats per psicosi o vulnerabilitat per psicosi podrien ser moderadors de l'impacte de l'adversitat infantil en determinades patologies mentals. Aquests gens estarien implicats en la neurotransmissió dopaminèrgica, com el que codifica per la *COMT,* que regula la degradació de dopamina, o bé en neuroplasticitat o gestió de la resposta a l'estrès com els gens del *BDNF* i de l'*FKBP5.* L'objectiu d'aquest article va ser revisar els estudis GxE que han analitzat aquests gens en relació a l'associació entre trauma durant la infància i la vulnerabilitat a psicosi. Vuit articles incloent 13 estudis basats en mostres independents van complir els criteris d'inclusió, dels quals tres estudiaven la *COMT*, tres el *BDNF* i set l'*FKBP5.* D'acord amb els treballs revisats, en general, existeixen evidències d'interacció GxE pels tres gens explorats. Els estudis revisats suggereixen que hi ha diversos aspectes etiològics compartits entre l'esquizofrènia i els fenotips atenuats relacionats, presents en els diferents nivells de severitat, i recolzen la hipòtesi del contínum de les psicosis en aquest model causal d'interacció GxE. De la mateixa manera, els resultats apunten a l'existència d'una complexa xarxa subjacent a la vulnerabilitat a patir psicosi que inclou múltiples factors que interaccionen entre si.

COMT, *BDNF* and *FKBP5* genes as moderators of the association between childhood trauma, schizophrenia and psychosis proneness: a systematic review of gene-environment studies

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Abstract

Childhood trauma is associated with an increased risk for psychotic disorders and related subclinical traits. However, this risk is likely modified by genetic factors. The genes *catechol-O-methyltransferase* (*COMT*), *brain-derived neurotrophic factor* (*BDNF*) and *FK506 binding protein 51* (*FKBP5*) have been related with psychosis proneness. Studies suggested that, rather than have a major role, they may be moderating the association between childhood trauma and psychosis. This paper reviews the available evidences examining the modulating role of polymorphisms in *COMT*, *BDNF* and *FKBP5* genes on the association between childhood trauma and psychosis proneness. Eight papers encompassing 14 studies based on independent samples fulfilled our inclusion criteria. Overall, the presence of a gene-environment (GxE) interaction is confirmed for the three genes explored, but pointing towards a more complex network of multiple factors interacting underlying psychosis proneness. The studies reviewed suggest there are some shared aetiological aspects between schizophrenia and related attenuated phenotypes present at the different levels of severity, and support the hypothesis of the psychosis continuum in this GxE interaction causal model.

Keywords: *COMT*; *BDNF*; *FKBP5*; gene–environment interaction; childhood trauma; psychosis proneness; psychosis

1. INTRODUCTION

Schizophrenia is considered one of the most severe forms of mental disorder and one of the top ten causes of long-term disability worldwide. This severe psychopathological condition has traditionally been thought of as dichotomous, with subjects classified as healthy or ill. However, from an epidemiological point of view, evidence shows that psychotic-related traits have a distribution in the general population (Johns & van Os, 2001; Stefanis et al., 2002), suggesting the existence of a continuous range between health and pathology. According to this dimensional or continuum model, schizophrenia represents the extreme and most severe manifestation of a range of cognitive, affective and behavioural features that can be found, to a less intense degree, in many subjects within the general population (van Os & Kapur, 2009). Such common manifestations of psychosis proneness can be measured from a trait dimension perspective (i.e. schizotypy) or from a symptom-and-trait perspective, by combining schizotypy traits with the expression of the trait in action (i.e. psychotic-like symptoms or experiences).

Different authors have suggested that both schizotypy and psychotic-like experiences constitute an underlying vulnerability to schizophrenia (e.g. Barrantes-Vidal et al., 2013). In fact, although not all the individuals who present them will develop schizophrenia in the future (in fact, most never will), they have been observed to have an increased vulnerability to the disease, suggesting that at least a subset of schizophrenia susceptibility genes also affects schizotypy and psychotic-like experiences in non-psychotic subjects (Fanous et al., 2007; Stefanis et al., 2007). According to the most recent genome-wide association studies (GWAS), schizophrenia appears as a complex polygenic disorder involving hundreds of genes, some involved in dopaminergic functions, synaptic plasticity and response to stress (Ripke et al., 2014). Thus, taking all this evidence into account, we can hypothesize that the variability of a subset of these genes will contribute to the propensity for both schizophrenia and related attenuated phenotypes.

Different pre- and postnatal environmental exposures have been shown to disrupt normal brain development and underlie the development of psychotic outcomes (Fernandes and Osório, 2015; Mandelli et al., 2015; van Os et al., 2010). Particularly, childhood trauma, as a form of early psychological stress, has been related to psychotic symptoms at the level of both psychotic disorders and the extended psychosis phenotype of subclinical psychotic experiences in the general population (Read et al., 2005; Varese et al., 2012; Velikonja et al., 2015). These early experiences are believed to increase the risk for psychosis though an abnormal stress response via the hypothalamic-pituitary-adrenal (HPA) axis. Exposure to stress causes activation of the HPA axis, which triggers several pathways involved on the regulation of gene expression for metabolism, immune function, cognition, and brain

development, thereby preparing the body to respond to stress. However, severe stress exposure can alter this normal stress response (De Bellis and Zisk, 2014) and may precipitate a cascade of events that leads to aberrant neural circuit changes, including an abnormal increase in dopamine signalling or neurotrophic factors (Carbone and Handa, 2013; Van Winkel et al., 2008). Alterations in HPA axis reactivity and the levels of related molecules have been found in patients with depression, anxiety, psychosis, as well as in subjects with schizotypal personality disorder, and ultra-high risk subjects (Elzinga et al., 2010; Myin-Germeys and van Os, 2007). This supports the idea that altered HPA axis function increases psychological vulnerability, predisposing people to develop psychotic symptoms.

In line with this, although childhood trauma has robustly been established as a causal factor for psychosis, there are large individual differences in response to early stressful events. This suggests that childhood trauma is not a sufficient cause of psychosis and that underlying genetic vulnerability could play an essential role in bringing about psychotic symptoms following adversity (Van Winkel et al., 2013). In this regard, gene-environment (G x E) interaction studies have reported several candidate genes for psychosis or related traits (see Box 1) as feasible moderators of the impact of childhood adversity on mental health. Those included genes involved in neuronal neurotransmitter degradation such as catechol-O-methyltransferase (*COMT*), but also some that are not specifically involved in psychosis but more generally in neuroplasticity or in the stress-response system as brain-derived neurotrophic factor (*BDNF*) or the FK506 binding protein 51 (*FKBP5*), respectively (Alemany et al., 2016, 2011; Collip et al., 2013; Cristóbal-Narváez et al., 2016; Karg et al., 2011). It is hypothesized that some allelic variations in these genes may confer a lower

Box 1 Evidences of the impact of *COMT*, *BDNF* and *FKBP5* on mental health

COMT | Dopaminergic abnormalities have consistently been observed in schizophrenia and its prodrome (Howes, McCutcheon, Owen, & Murray, 2017). The Val allele of the Val158Met polymorphism within this gene has been associated with different schizophrenia-related endophenotypes such as schizotypy (e.g. Stefanis et al., 2004) or impaired cognition (e.g. Oken et al., 2006). Val allele carriers, who present high enzyme activity, have reduced dopamine levels in the prefrontal cortex and other brain regions and may show an impairment in dopamine-related traits, e.g. movement, memory, mood, cognition, attention or learning (Laatikainen, Sharp, Harrison, & Tunbridge, 2013).

BDNF | It is essential for several neurodevelopmental processes, as proliferation, differentiation and survival of neuronal cells, and synaptic plasticity and connectivity in the adult brain (Autry & Monteggia, 2012). It may influence glutamatergic neurotransmitter systems (Carvalho et al., 2008). Also, it may play a role in cell survival in response to stress (Sofroniew, Howe, & Mobley, 2001). The Met allele in the Val66Met polymorphism is associated with a lower distribution of BDNF in neurons and with increased risk for schizophrenia or related phenotypes, although others reported the Val allele with increased risk for schizophrenia (e.g. Chen et al., 2004; Kheirollahi et al., 2016; Lu et al., 2012; Rosa et al., 2006).

FKBP5 | A role for this gene has been established as modulator of the HPA axis response to stress. A functional haplotype spanning the whole gene was related to a differential *FKBP5* mRNA and protein induction by glucocorticoid receptors (GR) activation and also to variation in GR sensitivity (Binder, 2009; Binder et al., 2004; Zannas & Binder, 2014). This haplotype was tagged by the polymorphisms rs3800373, rs9296158 and rs1360780, and further exploration showed that the variant most likely to confer this functionality was rs1360780 (Zannas & Binder, 2014). Specifically, it seems that the transcription start is enhanced in rs1360780 T-allele carriers (Klengel et al., 2013) which may lead to a prolonged response to stress.

efficiency on the systems in which the corresponding proteins are involved, as reflected in Figure 1. This would lead to specific pathophysiological disturbances of molecular pathways that may impact the neural circuits associated with the characteristic symptoms of psychosis and schizophrenia, but also with the attenuated phenotypes observed in the general population.

The studies analysing these genes, point towards an implication of *COMT*, *BDNF* and *FKBP5* on psychosis proneness, but suggest that, rather than having a major role, they are involved through a complex framework including environmental fac-tors as childhood trauma. Considering this and the lack of a published revision summarizing these findings, the purpose of this work was to provide an update on the studies that have examined the modulating role of variability in the genes *COMT*, *BDNF* and *FKBP5* on the association between childhood trauma and schizophrenia at the clinical, subclinical and non-clinical levels.

2. METHODS

2.1 Selection criteria

The design of the systematic review was based on recommendations from Khan and colleagues (2003). Two independent inves-tigators (M.C.C and A.R.) conducted a systematic literature search of the PubMed and PsychINFO databases. The search term was: "(BDNF OR COMT OR FKBP5) AND (childhood trauma OR childhood maltreat-ment OR childhood abuse OR childhood neglect) AND (psychosis OR psych* OR schizoid* OR schizotyp* OR psychosis proneness)". Given PsychINFO includes a wide range of literature, including publica-tions in non -scientific journals, in this case, the terms were searched exclusively in abstracts. As shown in Figure 2, studies were selected using the following inclusion criteria: (1) written in English, (2) original research published in scientific journals (i.e. exclusion of reviews), (3) analysed G x E interaction, but not triple interactions (e.g. *COMT* x trauma x cannabis), (4) explored an outcome of psychosis or psychosis proneness clinically or psychometrically evaluated (exclusion of neuroimaging, cognition studies or exploring biological outcomes such as cortisol or mRNA levels). The final set consisted of 8 papers to be reviewed, encompassing 14 studies —some reported the results of two or more independent samples or for more than one of the selected candidate genes. They were included in the systematic review by consensus of all authors.

3. RESULTS

All the studies reviewed here are listed by the gene they studied, in chronological order of publication in Table 1. For each study, we have specified the sample characterization and study design, the clinical outcome or intermediate phenotype (P) explored, the childhood trauma measure (environment, E), the gene (G) and polymorphisms studied, and the main results detected regarding the associations between both childhood trauma and the phenotype (E → P) and the gene variation and the phenotype (G→P), and also the G x E interaction effect on

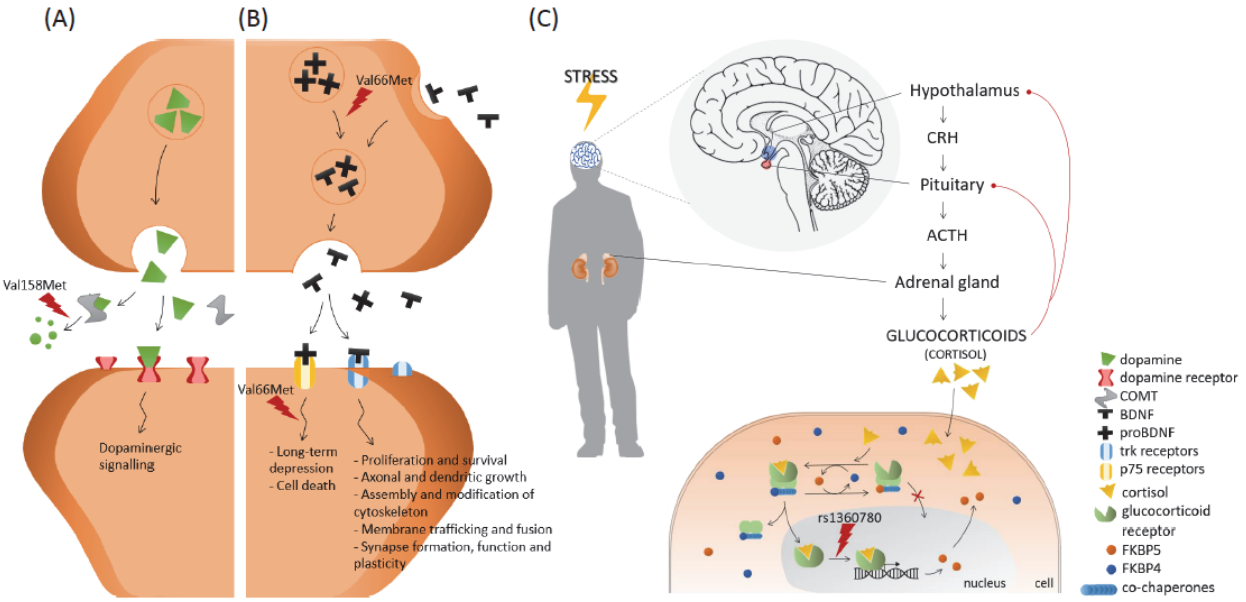

Figure 1 Schematic representation of the pathways the *COMT* (A), *BDNF* (B) and *FKBP5* (C) genes are involved in.

(A) The *COMT* gene codes for a methyltransferase that catabolizes catechol substrates such as dopamine and thus plays a key role in dopamine catabolism, thereby modulating the amount of dopamine in the synaptic clefts. The *Val158Met* polymorphism (rs4680, G>A) produces a valine (Val) to methionine (Met) substitution at codon 158 that results in enzymatic differences. Activity is three- to fourfold lower with the low activity allele (*Met*) than with the high activity allele (*Val*). Thus, dopamine signalling is likely to be enhanced in *Met* carriers compared to *Val* carriers.

(B) BDNF is synthesized as a precursor protein that is cleaved into proBDNF, which can then be further cleaved into mature BDNF. ProBDNF activates different intracellular signalling pathways from mature BDNF, and has different effects on the central nervous system. ProBDNF signals through the low-affinity receptor p75, which is believed to be involved in apoptosis. BDNF signals through the high-affinity tropomyosin-related kinase B (TrkB) receptor, activating several intracellular signalling cascades that lead to proper axonal and neurite growth, and the development and survival of neurons during development, as well as synaptic plasticity and neurotransmitter release in the mature brain. The *Val66Met* polymorphism (rs6265, *C>T*), resulting in Val to Met substitution at codon 66, is related with disrupted trafficking, packaging and release of both proBDNF and BDNF, and affects the signalling pathways they activate.

(C) The *FKBP5* gene codes for a co-chaperone involved in the stress reactivity system via the HPA axis. FKBP5 binds to a complex of proteins consisting of co-chaperones and glucocorticoid receptors (GRs), promoting a conformation that has lower affinity for cortisol and inhibits GR activity (Zannas & Binder, 2014). The binding of cortisol promotes the exchange of FKBP5 for FKBP4, activating GR signalling favouring the synthesis of FKBP5 which, in turn, will bind to and inactivate the complex, thereby regulating its own activity via a negative feedback loop (Zannas, Wiechmann, Gassen, & Binder, 2016). The *T* allele of the rs1360780 polymorphism modifies the affinity of the transcription start of FKBP5, enhancing the transcription start, and thus increasing the levels of FKBP5 (Klengel et al., 2013).

the phenotype (G x E → P). The met1hodology used in the studies reviewed and the results reported are described in the next section.

3.1 Summary of the methodology used in the studies reviewed

3.1.1. Assessment of psychopathology

The diagnosis of the clinical samples included in the studies reviewed was based on the Diagnostic and Statistical Manual of Mental Disorders (DSM) or the International Statistical Classification of Diseases and Related Health Problems (ICD). The Structured Clinical Interview for DSM Disorders (SCID) was used for DSM diagnoses.

As regards to clinical symptoms, the Diagnostic Interview for Psychosis (DIP; Castle et al., 2005) was used for clinical and diagnostic information in Green et al. (2014), in which the authors used specific DIP items to determine the severity of positive symptoms (i.e. lifetime hallucination and delusion) and negative symptoms (i.e. restricted affect, blunted affect and negative formal thought disorder, as well as indices of social functioning obtained from subject interviews). In the study by Collip and colleagues (Collip et al., 2013), they assessed psychotic symptoms using the Brief Psychiatric Rating Scale (BPRS; Thomas et al., 2004).

The reviewed studies analysing psychotic-like experiences, mostly used the Community Assessment of Psychic Experiences (CAPE; Stefanis et al., 2002), which measures the lifetime prevalence of subclinical positive, negative and depressive symptoms as well as distress related to the symptoms. Ramsay and colleagues (2013) used the psychosis section of the Schedule for Affective Disorders and Schizophrenia for School-aged Children (K-SADS; Kaufman et al., 1996) to assess psychotic-like experiences in their young adolescent sample.

Schizotypy or schizotypal personality traits was also analysed as the outcome for G x E interaction effects. These characteristics were measured in Savitz et al. (2010) using the Schizotypal Personality Scale (STA; Broks et al., 1984). Collip and colleagues explored the positive schizotypy subscale of the Structured Interview for Schizotypy (SIS-R) (Kendler et

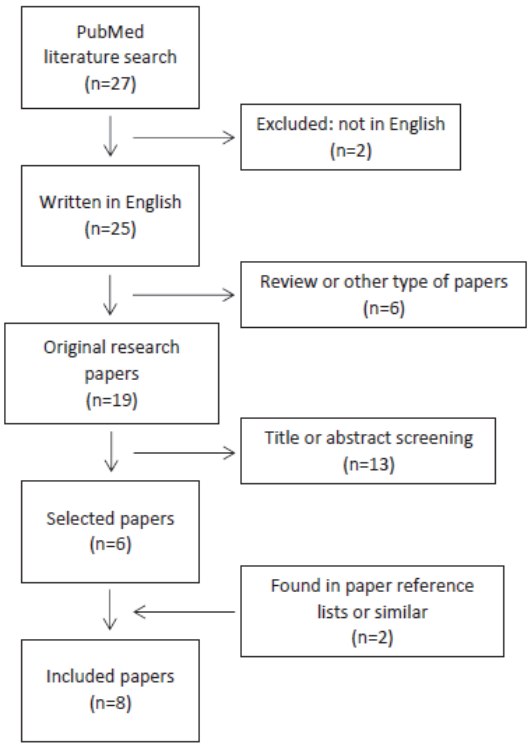

Figure 2 Flow chart for selection of studies included in this review.

Table 1 Summary of gene–environment (G x E) interaction studies analysing the modulating role of *COMT*, *BDNF* or *FKBP5* genetic variation on the association between childhood trauma (E, environment) and schizophrenia or psychosis proneness (P, phenotype). Results are given for the significant associations between childhood trauma and the phenotype (E→P), the gene variation and the phenotype (G→P) and the G x E interaction effect on the phenotype (G x E→P). Statistical significance was fixed at 0.05, non-significant results are specified as "n.s."

Study	Sample characterization and study design	Clinical outcome or intermediate phenotype (P)	Childhood trauma measure (E)	Gene and polymorphisms studied (G)	Results
Savitz *et al.,* 2010 Behav Genet PubMed	n = 222 bipolar disorder patients and relatives Subjects with bipolar disorder (SCID) and affected (bipolar or other psychiatric conditions) or unaffected relatives Mean age: 48.77 years Males: 44% Ethnicity: white - South Africa [1] Cross-sectional study	Schizotypal personality traits (STA)	Childhood trauma (CTQ)	***COMT*** rs4680 (Val158Met)	· E→P: n.s. · G→P: n.s. · G x E→P: significant interaction effect between total CTQ and COMT on schizotypal personality traits. Val/Val showed a pattern of increasing STA score with increasing total CTQ score.
Ramsay *et al.,* 2013 PLOS ONE [2] PubMed	n = 237 healthy subjects General population subjects Mean age: not specified, range 11-15 years Males: 54% Ethnicity: white - European Cross-sectional study	Psychotic-like experiences (K-SADS)	Traumatic experiences, physical abuse, sexual abuse and exposure to domestic (interparental) violence (Measure not specified)	***COMT*** rs4680 (Val158Met)	· E→P: n.s. · G→P: n.s. · G x E→P: n.s.
Green *et al.,* 2014 J Psychiatr Res PubMed	n = 429 patients Subjects with schizophrenia or schizoaffective disorder (ICD-10) (cohort ASRB) Mean age: 39.3 years Males: 65.7% Ethnicity: not specified – Australian Cross-sectional study	Positive and negative symptom severity (DIP + indices of social functioning)	Physical abuse, Emotional abuse, Emotional neglect, Total childhood adversity (CAQ [3])	***COMT*** rs4680 (Val158Met)	· E→P: main effect of physical abuse on positive symptoms and of emotional neglect on negative symptoms. · G→P: main effect of genotype on negative symptoms. · G x E→P: Met homozygotes showed more severe negative symptoms in the presence of emotional neglect, and more severe positive symptoms in the presence of physical abuse, than those Met/Met subjects without a history of these types of trauma.
Alemany *et al.,* 2011 BJPsych PubMed	n = 533 healthy subjects General population subjects Mean age: 22.9 years Males: 45.4% Ethnicity: white - European Cross-sectional study	Psychotic-like experiences (CAPE)	Childhood abuse, Childhood neglect (CTQ)	***BDNF*** rs6265 (Val66Met)	· E→P: Association found between childhood abuse and positive psychotic-like experiences. · G→P: n.s. · G x E→P: Met carriers showed higher scores for positive PEs when childhood abuse was present than Val/Val subjects.
Ramsay *et al.,* 2013 PLOS ONE [2] PubMed	n = 237 healthy subjects General population subjects Mean age: not specified, range 11-15 years Males: 54% Ethnicity: white - European Cross-sectional study	Psychotic-like experiences (K-SADS)	Traumatic experiences, physical abuse, sexual abuse and exposure to domestic (interparental) violence (Measure not specified)	***BDNF*** rs6265 (Val66Met)	· E→P: n.s. · G→P: n.s. · G x E→P: n.s.
de Castro-Catala *et al.,* 2016 JPsychRes PubMed	n = 799 healthy subjects General population subjects Mean age: 20.79 years Males: 23% Ethnicity: not specified – 93% European Cross-sectional study	Psychotic-like experiences (CAPE)	Childhood trauma, Childhood abuse, Childhood neglect (CTQ)	***BDNF*** rs6265 (Val66Met)	· E→P: Association found between childhood trauma, abuse and neglect in positive and negative psychotic-like experiences · G→P: n.s. · G x E→P: Val/Val subjects, especially males, showed more positive psychotic-like experiences than Met carriers, when exposed to childhood trauma or neglect.
	n = 460 healthy subjects General population female twins (cohort EFPTS) Mean age: 27.8 years Males: 0 Ethnicity: white - European Cross-sectional study	Psychotic-like experiences (CAPE)	Childhood trauma (adapted CTQ without questions regarding physical and sexual abuse)	***BDNF*** rs6265 (Val66Met)	· E→P: Association found between childhood trauma and positive and negative psychotic-like experiences · G→P: n.s. · G x E→P: Met carriers showed higher positive and negative psychotic-like experiences when exposed to childhood trauma than Val carriers.

Collip *et al.*, 2013 BJPsych PubMed	n = 401 healthy subjects General population female twins (cohort EFPTS) Mean age: 27.7 years Males: 0 Ethnicity: white - European Cross-sectional study	Psychotic-like experiences (CAPE, positive items subscale)	Childhood trauma (CTQ)	***FKBP5*** rs9296158 rs1043805 rs1360780 rs4713916	· E→P: Association found between childhood trauma and psychotic-like experiences. · G→P: n.s. · G x E→P: significant interaction found with rs9296158 (A), rs1043805 (A) and rs1360780 (T).
	n = 175 healthy subjects General population subjects (controls) Mean age: 30.7 years Males: 32% Ethnicity: 93% white – presumed European Cross-sectional study	Positive schizotypy (SIS-R)	Childhood trauma (CTQ)	***FKBP5*** [4] rs9296158 rs992105 rs3800373 rs4713916	· E→P: n.s. · G→P: n.s. · G x E→P: n.s.
	n = 200 healthy relatives Unaffected siblings of patients with non-affective psychotic disorder Mean age: 26.58 years Males: 44% Ethnicity: 81% white – presumed European Cross-sectional study	Positive schizotypy (SIS-R)	Childhood trauma (CTQ)	***FKBP5*** [4] rs9296158 rs992105 rs3800373 rs4713916	· E→P: n.s. · G→P: n.s. · G x E→P: Significant interaction with trauma and rs992105 (C) and rs4713916 (A) in positive schizotypy
	n = 195 patients Subjects with non-affective psychotic disorder (DSM-IV) Mean age: 28.12 years Males: 73.3% Ethnicity: 87% white - presumed European Cross-sectional study	Psychotic symptoms (BPRS)	Childhood trauma (CTQ)	***FKBP5*** [4] rs9296158 rs992105 rs3800373 rs4713916	· E→P: Association found between childhood trauma and psychotic symptoms. · G→P: n.s. · G x E→P: rs9296158 (A) moderated the effect of trauma on positive psychotic symptoms.
Alemany *et al.*, 2016 Psych Med PubMed	n = 437 healthy subjects General population subjects Mean age: 22.9 years Males: 45.4% Ethnicity: not specified – presumed European Cross-sectional study	Psychotic-like experiences (CAPE)	Childhood abuse (CTQ)	***FKBP5*** rs1360780	· E→P: Association found between childhood abuse, and positive and negative psychotic-like experiences. · G→P: Main genetic effects found for negative psychotic-like experiences. · G x E→P: T homozygotes presented higher scores of positive psychotic-like experiences when exposed to childhood abuse than C homozygotes.
	n = 305 healthy subjects General population subjects Mean age: 21.8 years Males: 40.1% Ethnicity: not specified – presumed European Cross-sectional study	Psychotic-like experiences (CAPE)	Childhood abuse (CTQ)	***FKBP5*** rs1360780	· E→P: Association found between childhood abuse, and positive and negative psychotic-like experiences. · G→P: n.s. · G x E→P: T homozygotes presented higher scores of positive psychotic-like experiences when exposed to childhood abuse than C homozygotes.
de Castro-Catala *et al.*, 2017 Psychoneuro-endocrinology (submitted)	n = 808 healthy subjects General population subjects Mean age: 20.79 years Males: 23% Ethnicity: not specified – 93% European Cross-sectional study	Schizotypy (WSS) Psychotic-like experiences (CAPE)	Childhood trauma, Childhood abuse, Childhood neglect, Emotional abuse, Physical abuse, Sexual abuse, Emotional neglect Physical neglect (CTQ)	***FKBP5*** rs3800373 rs9296158 rs1360780 rs9470080 rs4713916	· E→P: Association found between all childhood trauma variables and positive and negative schizotypy and positive, negative and depressive CAPE dimensions, except for sexual abuse. · G→P: n.s. · G x E→P: Subjects TA carriers (rs9470080- rs4713916) showed lower negative and depressive CAPE dimensions when exposed to childhood abuse.

[1] All the subjects were Caucasian and from South Africa; 55% were of Afrikaner ancestry and 41% of British ancestry. [2] Note that Ramsay *et al.* (2013) is duplicated in the table because two different genes were analysed in the same paper. [3] Information on two additional items concerning experiences of parental loss and sibling loss were added to the CAQ score in this study. [4] In these studies, the polymorphisms rs992105 and rs3800373 were genotyped to capture the effect of rs1043805 and rs1360780, respectively. *Cohort abbreviations*: ASRB, Australian Schizophrenia Research Bank; EFPTS, East Flanders Prospective Twin Survey. *Measure abbreviations*: BPRS, Brief Psychiatric Rating Scale (Thomas et al., 2004); CAPE, Community Assessment of Psychic Experiences (Stefanis et al., 2002); CAQ, Childhood Adversity Questionnaire (Rosenman and Rodgers, 2004); CTQ, Childhood Trauma Questionnaire (Bernstein et al., 2003); DIP, Diagnostic Interview for Psychosis (Castle et al., 2005); ICD-10, International Statistical Classification of Diseases and Related Health Problems version 10 (World Health Organization); K-SADS, Schedule for Affective Disorders and Schizophrenia for School-aged Children (Kaufman J, Birmaher B, Brent D, Rao U, 1996); SCID: Structured Clinical Interview for DSM Disorders (First et al., 1999); SIS-R: Structured Interview for Schizotypy – Revised (Kendler et al., 1989); STA: Schizotypal Personality Scale (Broks et al., 1984); WSS: Wisconsin Schizotypy Scales (Chapman et al., 1978, 1976; Eckblad et al., 1982; Eckblad and Chapman, 1983; Ros-Morente et al., 2010).

al., 1989). The Wisconsin Schizotypy Scales (WSS) were used in the third study (de Castro-Catala et al., 2017) for the evaluation of schizotypy (Chapman et al., 1978, 1976; Eckblad et al., 1982; Eckblad and Chapman, 1983).

3.1.2. Assessment of childhood trauma

To assess childhood trauma, most of the studies included in the present review used the Childhood Trauma Questionnaire (CTQ) (Bernstein and Fink, 1998). Some of the studies included here used a global measure of trauma (by summing the scores for all the items), whereas others used the subscale scores and/or abuse and neglect scores (by summing up the items corresponding to each subscale, to abuse or neglect, respectively). The Childhood Adversity Questionnaire (CAQ; Rosenman and Rodgers, 2004) was used by Green and colleagues, who computed a total score by summing the values of the CAQ items and adding two items referring to experiences of parental loss and sibling loss (Green et al., 2014). Ramsay and colleagues did not use a specific questionnaire to assess childhood trauma; as part of the assessment interview, both parent and child were asked about a range of traumatic experiences, including instances of physical abuse, sexual abuse and exposure to domestic (inter-parental) violence (Ramsay et al., 2013).

3.1.3. Genes analysed

The genotyping was performed using standard methodologies in all studies. The SNPs within the *COMT*, *BDNF* and *FKBP5* genes analysed in the reviewed studies are shown in Figure 3.

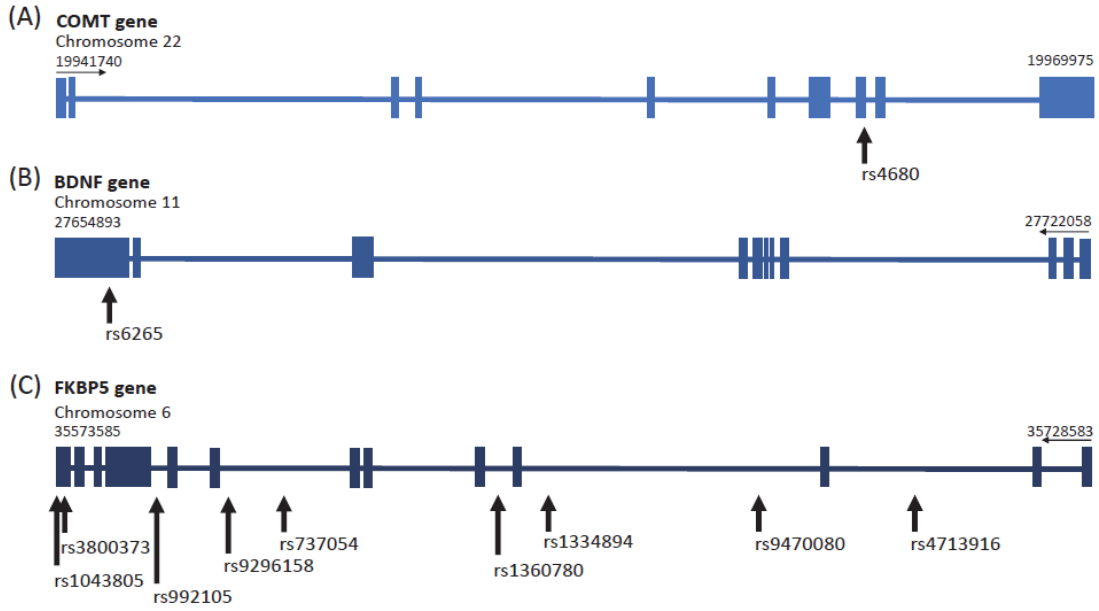

Figure 3 Single nucleotide polymorphisms (SNPs) within the *COMT* (A), *BDNF* (B) and *FKBP5* genes (C) studied in the papers included in this review.

3.2 Results reported in the studies reviewed

3.2.1. *COMT*, childhood trauma and psychosis proneness

Three of the studies reviewed analysed the *COMT* x childhood trauma interaction. Savitz and colleagues (2010) found an interaction effect in their bipolar patients and relatives' sample, with Val/Val subjects who had been exposed to childhood trauma showing a pattern of increased schizotypy. In the study by Ramsay and colleagues (2013) in which they analysed this G x E interaction in a sample of healthy young teenagers, they could not detect any significant interaction effect. However, a trend was observed for Val/Val subjects who had been exposed to childhood trauma to be more likely to report psychotic experiences than those with the other genotypes. The third study exploring this G x E interaction included patients with schizophrenia or schizoaffective disorders. They showed that Met/Met subjects reported more severe negative symptoms in the presence of emotional neglect. In addition, Met carriers with a history of physical abuse were found to have greater positive symptoms (Green et al., 2015).

3.2.2. BDNF, childhood trauma and psychosis proneness

Three papers explored the effect of the *BDNF* x childhood trauma interaction on psychosis proneness, one of them analysing two independent samples. The first study, by Alemany and colleagues, reported that Met carriers were showing higher scores for positive psychotic-like experiences in the presence of childhood abuse than Val/Val subjects (Alemany et al., 2011). Ramsay and colleagues also explored this interaction in their young sample, but they could not find any interaction effect for this gene on psychotic-like experiences (Ramsay et al.,2013). The last study analysing this G x E interaction included two healthy samples: a predominantly female, general population sample and a female twins sample (de Castro-Catala et al., 2016). In that study, the authors reported an interaction effect on psychotic-like experiences in both samples, but whereas in the first sample they observed that Val/Val subjects (particularly males) showed more positive psychotic-like experiences than Met carriers when exposed to childhood trauma, in the second sample (all females) the effect was detected with the Met allele and on both positive and negative psychotic-like experiences.

3.2.3. FKBP5, childhood trauma and psychosis proneness

The effect of the interaction between *FKBP5* and childhood trauma on psychosis proneness was explored in three papers, comprising seven independent samples. The first study explored the effect of this interaction in four samples with different levels of psychosis severity and genetic predisposition (Collip et al., 2013). In the sample of healthy female twins, the authors observed a significant interaction effect between childhood trauma and rs9296158 (A allele), rs1043805 (A allele) and rs1360780 (T allele) on positive psychotic-like experiences.

In the healthy controls sample, they could not detect any G x E interaction effect on schizotypy. A significant interaction effect between childhood trauma and rs992105 (C allele) and rs4713916 (A allele) on schizotypy was reported in the sample of unaffected siblings of patients. In the patients sample, rs9296158 (A allele) was shown to moderate the effect of childhood trauma on positive psychotic symptoms. The second paper analysed this G x E interaction in two independent general population samples (Alemany et al., 2016). In both samples, they showed that rs1360780 T homozygotes presented higher positive psychotic-like experience scores when exposed to childhood abuse. The last study examined five makers in the *FKBP5* using a diplotypic approach (de Castro-Catala et al., 2017). They showed that subjects who were TA carriers for the rs9470080-rs4713916 diplotype showed lower psychotic-like experience scores when exposed to childhood abuse, particularly in the negative and depressive dimensions.

4. Discussion

In the present review, we summarize the studies published to date exploring the G x E interaction effect between the *COMT*, *BDNF* or *FKBP5* genes and childhood trauma on schizophrenia and related attenuated phenotypes.

The studies reviewed that analyse the *COMT* gene seem to show that the Val158Met polymorphism is moderating the association between childhood trauma and schizotypy and symptom severity in adults with psychosis and their relatives (Green et al., 2014; Savitz et al., 2010). This was not observed in relation to psychotic-like experiences in a sample of young healthy subjects from the general population (Ramsay et al., 2013). These studies suggest that the interaction between Val158Met and childhood trauma may be relevant only in subjects presenting a higher risk for psychosis, i.e. subjects who already developed the illness and their relatives. However this has to be considered with caution for several reasons. First, although two studies show an interaction effect, the direction of such an interaction is not consistent between studies in terms of the allele that confers risk. Second, some of the studies have analysed samples sizes that may be limited for exploring G x E interactions ($n \approx 200$). Finally, although the three studies included white subjects, the population origin is diverse (i.e. South African, European and Australian) which could have influenced the results observed in terms of population stratification.

The *COMT* gene has largely been studied in relation to psychosis and, although the first meta-analysis revealed a lack of association (Munafò et al., 2005), a new meta-analysis showed that this gene does indeed play a role in schizophrenia (Costas et al., 2011). Several authors have shown an association between this gene and some schizophrenia endophenotypes, such as some neuropsychological functions, brain structures or cognitive domains (e.g. Ira et al., 2013; Lindenmayer et al., 2015; Tosato et al., 2015; Twamley et al., 2014). A sex-dependent effect has also been found for *COMT*

in some studies analysing psychosis proneness (de Castro-Catala et al., 2015), prefrontal inhibitory control (White et al., 2014) or other psychosis-related phenotypes (Carmichael and Lockhart, 2012; Harrison and Tunbridge, 2008). Other studies have shown a triple interaction effect between *COMT*, another gene (e.g. *MTHFR* or *OXTR*) and childhood trauma on psychosis proneness (Cristóbal-Narváez et al., 2017; Peerbooms et al., 2012). Genotypic differences in this gene have largely been related to cannabis use and psychotic symptoms. From the first study by Caspi and colleagues (Caspi et al., 2005) to the present, there is a growing body of evidence supporting the claim C*O*MT plays a moderating role in the relationship between cannabis use and psychosis (Halldorsdottir and Binder, 2017), including three-way interactions *COMT* x cannabis x childhood trauma (Alemany et al., 2014; Vinkers et al., 2013). In general, Val homozygous subjects were more likely to develop psychotic symptoms in adulthood after using cannabis in adolescence. Along the same lines, cannabis use and a history of childhood trauma were associated with increased psychotic symptoms in Val carriers. Taking into account all the mentioned findings with this gene, it seems that it is indeed influencing the risk for psychosis but as part of a complex network including not only environmental influences such as childhood trauma or cannabis use, but also other genes and probably sexual or related hormones.

Regarding *BDNF*, all the reviewed studies explored *BDNF* Val66Met x childhood trauma in healthy subjects of European origin using similar (or identical) methodology, but they do not show consistent findings. Although studies point toward an interaction effect on psychotic-like experiences in young adults, they are discordant regarding the allele associated with an increased risk. Also, the specific type of maltreatment leading to an effect differs between studies, as does the trait dimension. Two of the studies analysed large samples that seem to be appropriate for exploring such G x E. However, the third study included a relatively small sample of teenagers (n=237) that could have influenced the negative results found.

In the literature, the effect of this G x E interaction has also been examined in relation with cognition and brain abnormalities in clinical samples. Met carriers with childhood trauma exposure were reported to show poorer cognitive functioning, reduced hippocampal volumes and larger ventricles (Aas et al., 2013). However, these results were not replicated in a later study (Hernaus et al., 2014). The different findings regarding the allele conferring risk for this polymorphism have also been observed in schizophrenia case–control and family studies (Kheirollahi et al., 2016; Neves-Pereira et al., 2005; Rosa et al., 2006), but also in relation to other psychiatric phenotypes (Lohoff et al., 2005; Oswald et al., 2004). The reason for the inconsistencies observed is not clear, but some authors have pointed out that the expression of psychopathology in relation to *BDNF* may be explained by the variability within this gene and environmental factors, but also by the cumulative and/or epistatic effects of other

genes (Dougherty et al., 2010; Gutiérrez et al., 2015; Han et al., 2008), epigenetic factors (Boulle et al., 2012), sexual hormones (de Castro-Catala et al., 2016) or other still unknown factors.

Regarding *FKBP5*, overall, the reviewed studies point to an increased vulnerability to psychopathology dependent on *FKBP5* and environmental influences. Specifically, they show that the variability within this gene interacts with childhood trauma to increase the risk for psychotic-like experiences in non-clinical subjects, schizotypy in patient's relatives and positive symptoms in psychotic patients. However, there are some differences between studies regarding the associated polymorphisms and the alleles conferring risk. These risk-allele differences –which cannot be related to population stratification as subjects are mainly European– should be explored further. From all studies including healthy samples, only one of the two analysed by Collip and colleagues did not show an interaction effect. This could be attributed to the reduced number of subjects analysed (n = 175), which may be unpowered to detect such G x E interaction. Also the studies in patients and relatives included relatively small samples (n ≤ 200).

The *FKBP5* x childhood trauma interaction has been detected also in other schizophrenia-related traits such as cognition (Green et al., 2015) and neuroticism (Mihaljevic et al., 2016), and also in relation to other psychopathology-related endophenotypes and psychiatric disorders (Isaksson et al., 2016; Scheuer et al., 2016; VanZomeren-Dohm et al., 2015), thereby supporting the idea that *FKBP5* plays a role in psychosis and psychosis proneness. Similarly, interaction effects between the *FKBP5* gene and other stressful life events (e.g. bullying) have been found (Cristóbal-Narváez et al., 2016). As shown in Figure 1, *FKBP5* is importantly involved in the regulation of the HPA axis, although the exact mechanism by which this gene interacts with early stressing events is still unclear. In this regard, a recent study showed that an epigenetic mechanism could be mediating the combined effects of environmental exposure in early life and *FKBP5* genetic variation on the risk of developing stress-related disorders (Klengel et al., 2013). Specifically, an excessive cortisol release following early life stress exposure in rs1360780-A carriers would lead to epigenetic changes in the *FKBP5* glucocorticoid response elements, resulting in disruptions of the negative feedback that balances the stress response causing a dysregulation of the stress hormonal system and ultimately increasing the risk for certain psychiatric disorders. In this sense, and in light of the different *FKBP5* polymorphisms associated with psychosis proneness in the literature, further work is needed in relation to this gene to unravel whether other variants could be adding additional risk.

In conclusion, although few papers were found analysing these specific interactions, in general, the presence of a G x E interaction effect seems to be confirmed for the three genes explored,

despite being some nuances between the studies, and point towards a complex network of multiple factors interacting underlying psychosis proneness. Given the multifactorial aetiology of schizophrenia and the related endophenotypes, the exploration of all the mechanisms involved require complex numerical approaches leading to intricate results that would be difficult to interpret. In this sense, we believe that less complex approaches such as the exploration of the effect of positive environmental factors, as well as the use of polygenic approaches including epistatic and G x E interaction effects in large samples may shed further light on the mechanisms that shape the risk for psychopathology. The present review shows that there may be a shared underlying aetiology for schizophrenia spectrum disorders and the phenotypes studied at the different levels of severity, and it supports future research on the continuum hypothesis of psychosis in this G x E interaction causal model.

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Supervisor's report on the contribution of the PhD candidate to the article.

Dr. Araceli Rosa de la Cruz, Senior Lecturer at the Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals of the Facultat de Biologia (Universitat de Barcelona) and supervisor of the present doctoral thesis by Marta de Castro i Català, hereby certifies that none of the co-authors of the article "*COMT*, *BDNF* and *FKBP5* genes as moderators of the association between childhood trauma, schizophrenia and psychosis proneness: a systematic review of gene-environment studies" have used this publications for a doctoral thesis, and that the participation of the applicant in this article included the following tasks:

- Participation in the conception and design of the review.
- Identification and selection of papers
- Summary of the evidence and interpretation of data.
- First drafting of the manuscript.
- Critical revision of the article for intellectual content.

Signed by Dr. Araceli Rosa

Barcelona, May 31st 2017

4. Global Summary of Results

The specific objectives of section I, which aimed to explore three genes previously associated with schizophrenia (i.e. *COMT*, *RGS4* and *ZNF804*) in relation to psychosis-proneness (G→P), resulted in three publications:

- de Castro-Catala et al., 2016. *COMT-by-Sex Interaction Effect on Psychosis Proneness*. Biomed Research International; 2015:829237.
- de Castro-Catala et al., 2017. *Association between RGS4 variants and psychotic-like experiences in nonclinical individuals*. European Archives of Psychiatry and Clinical Neurosciences; Feb;267(1):19-24.
- de Castro-Catala et al. *The genome-wide associated candidate gene ZNF804A and psychosis-proneness: evidence of sex-modulated association.* PLoS One (submitted).

The results obtained in these studies are the following:

In the first study, we explored the relation between the ***COMT*** *Val158Met* genetic variability and psychosis-proneness in a sample of 808 nonclinical subjects. We observed that there was no direct association with the positive or negative dimension of schizotypy and psychotic-like experiences (all $p>0.05$).

When this was explored in males and females independently, the lack of associations held in the case of schizotypy ($p>0.05$). Regarding psychotic-like experiences, no association was found in females ($p>0.05$); however, in males, there was an effect on the negative dimension: those individuals with the *Val/Met* genotype were reporting higher scores than *Val/Val* or *Met/Met* ($F=3.6$ $p=0.03$).

When we analysed the presence of a sex x *Val158Met* interaction, we observed a significant effect on the negative dimension of both schizotypy ($F_{(2, 772)}=3.9$ $p=0.02$) and psychotic-like experiences ($F_{(2, 772)}=3.9$ $p=0.02$), but not on the positive ($p>0.05$). Further analyses revealed that these significant effects resulted mostly from significant effects of the *Val* allele in males: males carrying the *Val* allele showed higher scores for the negative dimension of both schizotypy ($F_{(1, 772)}=5.1$ $p=0.02$) and psychotic-like experiences ($F_{(1, 772)}=8.3$ $p=0.004$) than *Met/Met*, whereas this pattern was not observed in females.

In the second study, we analysed the genetic variability in the ***RGS4*** gene and its association with psychotic-like experiences in a sample of 808 nonclinical subjects. The analyses showed a significant association for the two SNPs explored (SNP4 or rs951436, and SNP18 or rs2661319). We observed that subjects with the *T* allele (SNP4) and the *A* allele (SNP18) were reporting higher scores on both positive (SNP4 $F=5.89$ $p=0.003$ and SNP18 $F=3.99$ $p=0.02$) and negative psychotic-like experiences (SNP4 $F=4.86$ $p=0.008$ and SNP18 $F=4.90$ $p=0.008$). Post hoc analyses indicated that subjects with the *TT* genotype in SNP4

or the *AA* genotype in SNP18 were showing higher scores in both dimensions ($p<0.05$). Similarly, analyses using a haplotypic approach (SNP4-SNP18) showed a significant association with positive ($F=5.35$ $p=0.005$ and negative psychotic-like experiences ($F=5.36$ $p=0.005$), in which subjects carrying at least one *TA* haplotype were scoring higher for both dimensions than the other haplotypes ($p<0.05$).

In the third study, the association between the variability within the genome-wide associated ***ZNF804A*** gene and the positive dimension of the psychosis-proneness phenotype was explored in this sample of 808 nonclinical subjects. These analyses showed a significant association between schizotypy and rs7597593, but not rs1344706, showing that subjects with the *TT* genotype were reporting lower scores than the other genotypes. Further analyses exploring by-sex differences in this association revealed that the effect detected was driven by females ($F=3.98$ $p=0.02$), but not males ($F=0.49$ $p=0.62$), showing that those carrying the *TT* genotype were reporting lower scores compared to *C* carriers ($t=2.54$ $p=0.006$). In the case of positive psychotic-like experiences, no association was found neither with rs7597593 nor rs1344706. However, when exploring sex specific associations, a trend was observed in females ($F=3.05$ $p=0.05$) but not males ($F=1.96$ $p=0.14$). Further explorations revealed that females with the TT genotype were reporting lower scores compared to those carrying the C allele ($t=2.37$ $p=0.009$).

The specific objectives of section II aiming to explore the association between childhood trauma and psychosis-proneness phenotypes and the modulating role of genes involved in neurotransmission, neurodevelopment or stress-related systems in this association (GxE→P), resulted in three publications, including two original research articles and a systematic review:

- de Castro-Catala et al., 2016. *Childhood trauma, BDNF Val66Met and subclinical psychotic experiences. Attempt at replication in two independent samples.* Journal of Psychiatric Research; Dec;83:121-129.
- de Castro-Catala et al. *Interaction effect of FKBP5 gene and childhood trauma on psychosis, depression and anxiety symptoms: study in a non-clinical sample.* Psychoneuroendocrinology (first revision submitted).
- de Castro-Catala et al. *COMT, BDNF and FKBP5 genes as moderators of the association between childhood trauma, schizophrenia and psychosis proneness: a systematic review of gene-environment studies.* Neuroscience & Biobehavioral reviews (submitted).

The results obtained in these studies are the following:

In the first study of this section, we explored the interaction effect of childhood trauma and ***BDNF*** *Val66Met* on psychosis-proneness in two nonclinical samples: 808 subjects (sample 1) and the 621 female twins (sample 2). No differences were observed between samples in childhood trauma scores, nor in the genotype frequencies. However, differences in psychotic-like experiences' scores were detected, with sample 1 showing higher mean scores than sample 2.

The main effects of childhood trauma and *BDNF Val66Met* were first explored. Childhood trauma was associated to positive and negative psychotic-like experiences in both samples (all $p<0.001$). Abuse and neglect, studied only in sample 1, was also associated to both dimensions of these two psychometric measures (all $p<0.001$). No main effect of *BDNF Val66Met* was detected in either sample ($p>0.05$).

In sample 1, the interaction analyses showed a significant *BDNF Val66Met* x childhood trauma effect on positive psychotic-like experiences (B= –0.08, s.e.=0.04 p=0.036) –but not negative ($p<0.05$)–, by which *ValVal* were showing higher scores than *Met* carriers when exposed to childhood trauma. When exploring childhood trauma further as abuse and neglect, similar results were obtained (abuse B= –0.13 s.e.=0.07 p=0.06 and neglect B= –0.15 s.e.=0.07 p=0.03).

In sample 2, we observed a significant interaction between *BDNF Val66Met* and childhood trauma on both positive (B=0.05 s.e.=0.03, p=0.045) and negative psychotic-like experiences (B=0.13 s.e.=0.04 p=0.001). In both cases *Met* carriers exposed to childhood trauma were reporting higher scores than those *ValVal*.

Given the different composition of the sample, in terms of sex proportions (sample 1 comprising both males and females and sample 2 only females), sample 1 was re-analysed exploring males and females separately. This showed that the effects observed in the whole sample were significant only in males (all $p<0.04$), but not females ($p>0.05$).

The second study, aimed to explore the interaction effect of childhood trauma and ***FKBP5*** genetic variability in a broad phenotype of psychosis-proneness, including psychotic-like experiences, schizotypy and depressive and anxiety symptoms, in the sample of 808 nonclinical subjects. Five SNPs were studied: rs3800373, rs9296158, rs1360780, rs9470080 and rs4713916. Two haplotypic blocks were observed, block 1: rs3800373-rs9296158-rs1360780 and block 2: rs9470080-rs4713916. For each haplotype block, subjects were classified in carriers of the risk haplotype and non-carriers, according to the risk alleles previously described in the literature.

In this study, the association between childhood trauma and psychosis-proneness was explored in deep by using both the childhood trauma subscales (i.e. emotional, physical and sexual abuse and emotional and physical neglect) as well as the overall scores (i.e. abuse, neglect and total trauma). These analyses showed that all childhood trauma subscales, except sexual abuse in some cases ($p>0.05$), were associated with schizotypy, psychotic-like experiences and depressive and anxiety symptoms ($p<0.01$). *FKBP5* genetic variability did not show a main effect on any of the outcomes (all $p>0.05$).

The interaction analyses between childhood trauma and block 1 did not show a significant effect on the psychotic-like experiences and schizotypy dimensions and depressive symptoms. In the case of anxiety, although when analysing the overall scores we could not detect any effect, the analyses with the trauma subscales revealed a significant interaction between this block and both emotional (β=-0.18 s.e.=0.11 p=0.04) and physical abuse (β=-0.33 s.e.=0.24 p=0.01). The effect observed for physical abuse remained significant after permutation analyses ($p<0.05$). Specifically, subjects carrying the *CAT* haplotype were showing lower anxiety scores than non-carriers when exposed to physical abuse.

The interaction analyses between childhood trauma and block 2 did not show any significant effect for the childhood trauma variables on schizotypy dimensions and positive psychotic-like experiences (all $p>0.05$). In the case of negative psychotic-like experiences, an interaction effect with the diplotypic groups was found for abuse (β=-0.31 s.e.=0.07 p=0.03) and total trauma (β=-0.32 s.e.=0.04 p=0.02), this effect that was driven by emotional abuse (β=-0.21 s.e.=0.11 p=0.02). Subjects *TA* carriers were reporting lower scores than non-TA carriers when exposed to these adverse experiences. However, none of these results were significant after permutations ($p>0.05$). In the case of the depressive dimension of psychotic-like experiences, an interaction with abuse was detected (β=-0.29 s.e.=0.04 p=0.03), which reflected the effect of emotional abuse (β=-0.18 s.e.=0.06 p=0.04). Those individuals carrying the TA haplotype were reporting lower scores when exposed to this type of abuse. These results were not significant after permutations ($p>0.05$).

On depressive symptoms, an interaction effect between block 2 and childhood abuse was observed (β=-0.33 s.e.=0.12 p=0.01), which was driven by emotional (β=-0.19 s.e.=0.18 p=0.03) and physical abuse (β=-0.34 s.e.=0.38 p=0.009). In this sense, individuals with the *TA* haplotype showed lower depressive symptoms than non-carriers, when exposed to this types of trauma. After permutation analyses, only the interaction with abuse and physical abuse remained significant ($p<0.05$). In the case of anxiety symptoms, abuse showed an interaction effect with this block (β=-0.29 s.e.=0.08 p=0.03), reflecting the effect of emotional (β=-0.20 s.e.=0.11 p=0.02) and physical abuse (β=-0.12 s.e.=0.25 p=0.02). Only the effect detected with physical abuse remained significant after permutations ($p>0.05$).

Specifically, carriers of the *TA* haplotype were presenting lower anxiety symptom scores than those non-carriers when exposed to this type of abuse.

The third study included in this section aimed to review systematically all the GxE studies examining the interaction effect between *COMT*, *BDNF* or *FKBP5* genetic variants and childhood trauma on psychosis or psychosis-proneness phenotypes. Eight papers encompassing 14 studies based on independent samples fulfilled our inclusion criteria.

Three of the studies reviewed analysed the interaction between *COMT Val158Met* and childhood trauma. In the first study, *ValVal* bipolar patients and relatives that had suffered childhood trauma were showing increased schizotypy (Savitz *et al*, 2010). No GxE was observed in young teenagers, although a trend was observed for *ValVal* subjects exposed to childhood trauma to be more likely to report psychotic-like experiences (Ramsay *et al*, 2013). The third study explored this GxE in patients with schizophrenia or schizoaffective disorder and showed that *MetMet* subjects were reporting more severe negative symptoms in the presence of emotional neglect. Also, *Met* carriers with a history of physical abuse were observed to have greater positive symptoms (Green *et al*, 2014).

Three papers explored the *BDNF* x childhood trauma effect on psychosis proneness, one of them including two independent samples. The first one, observed higher positive psychotic-like experiences in *Met* carriers in the presence of childhood abuse (Alemany *et al*, 2011). The second study was that by Ramsay and colleagues, who also explored this interaction with *BDNF*, but could not find any interaction effect in their young sample (Ramsay *et al*, 2013). The third study exploring this was the study included in the present dissertation exploring the interaction of *BDNF* and childhood trauma in two independent samples (de Castro-Catala *et al*, 2016), which results are shown above.

The interaction between *FKBP5* and childhood trauma on psychosis proneness was explored in three papers including 7 independent samples. The first study analysed this GxE in four samples with different levels of psychosis severity (Collip *et al*, 2013). They found a significant interaction effect between childhood trauma and rs9296158 *A* allele, rs1043805 *A* allele, and rs1360780 *T* allele on positive psychotic-like experiences in a sample of healthy female twins. In controls, in which they explored this GxE on schizotypy, no effect could be detected. In unaffected siblings of patients, an interaction effect was observed showing increased schizotypy scores in subjects with childhood trauma exposition and rs992105 *C* allele or rs4713916 *A* allele. Patients with the rs9296158 *A* allele that reported childhood trauma were shown to have increased positive psychotic symptoms. The second study exploring this, included two independent nonclinical samples in which they observed higher positive psychotic-like experiences in those subjects that were *T* homozygotes in rs1360780 and had experienced childhood abuse (Alemany *et al*, 2016). The third study exploring this

is the second study of the section II of the present thesis (de Castro-Catala *et al*, 2017b), whose results have been previously commented.

5. Discussion

The present thesis aimed to explore the involvement of genetic and environmental risk factors for psychosis on the development of psychotic-related traits or symptoms in nonclinical subjects. The hypothesis tested followed two objectives that aimed to investigate the influence of (i) genetic variants and (ii) mechanisms of gene-environment interactions on psychosis-proneness phenotypes, and resulted in five articles and a systematic review.

One of the main assumptions of this thesis is the existence of psychosis as a continuum of characteristics, rather than a dichotomized entity considering individuals as healthy or ill. According to this continuum model, psychosis-related traits or symptoms are present in the population ranging from nonclinical and subclinical manifestations to the clinical phenotype. In the present thesis we have studied two nonclinical samples (subjects without a diagnostic of psychosis) consisting in 808 students and 621 female twins. In line with the continuum of psychosis, in these two healthy samples we have observed a distribution of the scores studied (mainly schizotypy and psychotic-like experiences) with subjects showing low (most of them), medium and high scores. Although this was not specifically assessed, in general, the experience of these symptoms did not affect daily life functioning among the participants since all of them were working or studying and socially well adapted. According to previous observational studies, very few of the individuals who score high on psychosis-proneness scales will go on to develop psychotic disorders (Chapman *et al*, 1994), with subclinical negative psychotic symptoms (e.g. physical and social anhedonia) carrying stronger predictive power than others (Valmaggia *et al*, 2013).

Additionally, in our students' sample we detected that male subjects were reporting slightly higher scores than females in all variables, although these differences were small or not statistically significant. This finding is similar to previous investigations reporting sexual differences within psychotic patients and in psychosis-proneness scores, showing a worse prognosis in males (e.g. Kremen *et al*, 1998; Leung and Chue, 2000; Maric *et al*, 2003).

In line with previous investigations, the studies comprising this thesis point towards this continuum framework in which there are some shared etiological factors between both the extreme phenotype (i.e. clinical psychosis) and psychosis-proneness endophenotypes in nonclinical subjects. Current understanding of the genetics underlying psychosis-proneness in the general population derives principally from traditional quantitative genetic methods, applied to phenotypic data alone, and obtained from close relatives such as twins or parents-offspring. These, have offered heritability estimates ranging from 15 to 65% depending upon symptom evaluated or population age (Hay *et al*, 2001; Kendler and Hewitt, 1992; Zavos *et al*, 2014). Given the relevance of these genetic effects on psychosis-proneness in the general population, the current looking for specific genes and markers affecting these traits was a fundamental and sensible action at the first conception of this thesis, in the context of a funded project that started in 2012.

Following this strategy, the variability within the three genes explored in the studies of section I has been associated with increased scores in the psychosis-related analysed traits and, thus, an increase of psychosis vulnerability.

The first gene studied here is the *COMT*, a classic candidate gene for schizophrenia encoding for an enzyme that degrades dopamine in the synaptic clefts. The functional polymorphism *Val158Met* results in a different COMT enzymatic activity, with the Val-containing protein showing higher activity than the Met. This polymorphism has received great attention in relation to schizophrenia (and other psychoses as well), since the large amount of evidences relating dopaminergic abnormalities to psychosis (e.g. Davis *et al*, 1991; Maia and Frank, 2017). The association between *Val158Met* and schizophrenia was analysed in multiple studies relating inconsistently both *Val* and *Met* alleles with an increased risk for the illness (e.g. Chen *et al*, 2004a; van Winkel *et al*, 2008). These studies culminated in different meta-analyses that showed non-conclusive results (Costas *et al*, 2011; Munafò *et al*, 2005). The *Val158Met* polymorphism has also been studied in relation to schizophrenia endophenotypes, with results associating the high activity *Val* allele with higher schizotypy scores in male conscripts (Avramopoulos *et al*, 2002; Smyrnis *et al*, 2007; Stefanis *et al*, 2004), healthy controls and first-degree relatives of patients with psychosis (Schürhoff *et al*, 2007), although the *Met* allele was the allele conferring risk in a Chinese sample (Ma *et al*, 2007). In our study, we detected a sex-modulated association between *COMT* and the negative dimension of both schizotypy and psychotic-like experiences, showing that male subjects carrying the *Val* allele tended to score higher on negative schizotypy and psychotic-like experiences (de Castro-Catala *et al*, 2015). Our results with schizotypy are consistent with those previously reported in male conscripts as well as in other samples (i.e. Ma *et al*, 2007; Schürhoff *et al*, 2007), despite sex-specific associations were not specifically addressed in these studies. In the case of psychotic-like experiences, its association with *Val158Met* has been examined in GxE studies, showing that *Val* allele constitutes a genetic risk factor for this phenotype in the context of exposure to childhood maltreatment and/or cannabis use (e.g. Alemany *et al*, 2014). The *COMT*-by-sex increase of risk for psychosis detected is in line with several studies reviewed by Godar and Bortolato (2014) that showed sex-specific associations of certain dopaminergic genes (as *COMT*) with schizophrenia.

Although the biological mechanisms underlying these different associations between males and females are not clear, there are some plausible hypotheses. Males have been reported to show higher COMT activity than females, which seems to be caused by the ability of testosterone to increase *COMT* expression (Godar and Bortolato, 2014). This, in combination with the *Val* high activity allele, could lead to an important decrease of dopamine in the synaptic clefts and cause a deficit in dopamine neurotransmission, that is believed to underlie negative symptomatology (Laruelle, 2014). An additional mechanism

that may predict the different COMT activity in males and females may be afforded by the function of catechol-estrogens on modulating the turnover of catecholamines. The finding of an association between *Val158Met* and the negative dimension of the psychosis-proneness phenotype provides evidence that the biological effects of *COMT* may be relevant to the pathophysiology of schizophrenia and encourages further research in the complex neural dopaminergic system and the consideration of sex-specific mechanisms underlying the development of psychosis and psychosis-related traits.

Another interesting positional candidate gene that has been related to psychosis is the *RGS4*, located in a region (1q23) highly linked with psychosis (Brzustowicz *et al*, 2002). It encodes a protein that modulates the intensity and duration of signal transduction via G proteins of a variety of signalling cascades, including some related with neurotransmission (Paspalas *et al*, 2009). This gene emerged from an expression study showing a consistent reduced expression in schizophrenia over thousands of genes explored (Mirnics *et al*, 2001). After this, *RGS4* has been widely explored in relation to schizophrenia, including three meta-analysis, showing either modest effects for rs951436 and rs2661319 or no-association (e.g. Talkowski *et al*, 2006; Vilella *et al*, 2008). Studies in schizophrenia endophenotypes revealed associations between the *A* allele of these two polymorphisms and structural and functional brain alterations (Buckholtz *et al*, 2007; Prasad *et al*, 2005), and negative schizotypal personality traits (Stefanis *et al*, 2008). In a similar way, we detected an association between the same allele of both polymorphisms and positive and negative psychotic-like experiences in our healthy students' sample (de Castro-Catala *et al*, 2017a). Note that results for rs951436 were reported with the complementary allele (i.e. *T* allele).

Despite the contradictory results found with *RGS4* in relation to schizophrenia, studies in endophenotypes have sown consistent findings supporting the utility of studying these intermediate phenotypes that allow a more specific definition of the phenotype under study. The association reported in our study and in Stefanis' regarding negative psychotic-like symptoms suggest a contribution of *RGS4* variants to the alterations associated to these symptoms (e.g. lower prefrontal volumes, decreased metabolism or lower dopaminergic transmission; Galderisi *et al*, 2015). In fact, lower specific brain volumes have been observed in healthy subjects and first episode patients carrying the *A* alleles of rs951436 and/or rs2661319 (Buckholtz *et al*, 2007; Prasad *et al*, 2005). Positive psychotic-like experiences were also associated with *RGS4* variants in our study. Positive symptoms, as mentioned before, have been related to a hyperdopaminergic state (Kapur, 2003). Interestingly, expression of RGS4 was found to match closely dopaminergic receptors (Erdely *et al*, 2004) suggesting, as in other independent studies, a role for this gene in dopamine signalling (Lerner and Kreitzer, 2012). Additionally, it has been related to motor behaviour, synaptic plasticity and several neurodevelopmental processes such as neuronal differentiation or axon

development (Cheng *et al*, 2013; Grillet *et al*, 2003). Also, functional studies have linked its expression to the regulation of signalling systems related to psychosis as the opioid, cholinergic and serotoninergic (Gerber *et al*, 2016). All these evidences point towards an involvement of *RGS4* on several pre- and postnatal brain processes and that variants within this gene may lead to an abnormal function and/or structure causing an increased predisposition to develop psychotic symptomatology. Further studies are needed to better elucidate how this gene is implicated in mental health.

The third gene studied in the present thesis, the *ZNF804A*, is the first gene that achieved genome-wide level of statistical significance in schizophrenia GWAS (O'Donovan *et al*, 2008; Ripke *et al*, 2013). This gene has been related to neurodevelopment and plasticity processes (e.g. Deans *et al*, 2016). It seems to influence the expression of several genes involved in these processes and some schizophrenia-associated genes as well (Girgenti *et al*, 2012; Hill *et al*, 2012). Moreover, the two GWAS-associated polymorphisms (rs7597593 and rs13344708) may modify the affinity of the gene sequence to DNA- and/or RNA-binding proteins, altering the expression levels of the gene itself (Hess and Glatt, 2014). In relation to the clinical phenotype of schizophrenia, the rs1344706 *A* allele has been associated with more and severe symptoms and poorer outcome in patients (Cummings *et al*, 2010; Wassink *et al*, 2012; Wickramasinghe *et al*, 2015), as well as with brain structural alterations in different samples across the broad continuum of psychosis (e.g. (Wei *et al*, 2015). A sex-specific association was reported for this gene, associating rs7597593 and schizophrenia in women but not men (Zhang *et al*, 2011), although another study did not find this sex moderation (Riley *et al*, 2010). Studies on *ZNF804A* genetic variability and schizophrenia endophenotypes have reported association between the *A* allele on rs1344706 and impaired neuroimaging and neurocognitive phenotypes (Walters *et al*, 2010; Wassink *et al*, 2012), as well as higher schizotypy in a Japanese nonclinical subjects (Yasuda *et al*, 2011). However, another study exploring this gene and psychosis-proneness observed higher schizotypy scores in *C* carriers of rs7597593 and rs1344706 and also higher psychotic-like experiences in *C* carriers of rs7597593 (Stefanis *et al*, 2013). Our results in the whole sample seemed to be in the same direction as Stefanis and colleagues, showing higher schizotypy scores in rs7597593 *C* carriers (i.e. lower scores in *TT*), although we could not detect an association with rs1344706. However, sex-stratified analyses showed that the effect detected was driven by females' subjects –but not males–, whereas Stefanis' sample comprised only males. Our results are not in line with those by Yasuda and colleagues, although their results were observed in a Japanese sample and thus may not be comparable, as shown in a study reporting differences between European and Asian populations in the genomic region covering *ZNF804A* (Li et al, 2012).

The associations observed in psychosis-proneness in relation to *ZNF804A* also support the idea of shared genetic factors along the continuum and show the interest of studying the variants obtained from GWAS in relation to schizophrenia endophenotypes. After the first GWAS, given that the genes emerged were not the classic candidate genes and seemed unrelated to psychopathology, some researchers were a bit sceptical about this approach. However, independent replications and further functional analyses of genes, like the *ZNF804A*, pointed out that they indeed could be playing a role on psychosis despite showing low effect sizes, which in fact supported the polygenic aetiology of the illness. In the case of *ZNF804A*, molecular studies have shown a possible implication of this gene on neurodevelopment and brain plasticity, given its described function on gene transcription, translation, mRNA trafficking, cytoskeletal organization, protein folding and chromatin remodelling. Moreover, given its influence on the expression of dopamine-related genes (e.g. *COMT*), it is tempting to speculate that this gene may be involved in psychosis vulnerability through a dopaminergic pathway. Also, the evidences of sex-specific associations with both *ZNF804A* and *COMT* (discussed above) suggest a common pathway that may be influenced by sexual hormones.

At this point, the abovementioned studies support the existence of allelic variants in different genes coding for proteins involved on several neurobiological or related pathways that may impact this neural circuits and lead to the development of psychopathology at different levels of severity (i.e. clinical or nonclinical level), depending on the relevance of the pathway and the degree of affectation. Some of our results could seem contradictory in terms of risk allele or sex-specific associations when compared with others. Nevertheless, contradictory and non-replicated findings are also observed from candidate-gene studies in the literature, including those in classic schizophrenia candidate genes with highly plausible aetiological relevance. This suggest caution when considering these findings and point towards the existence of methodological inconsistences across studies (e.g. different definitions of the outcome), the use of underpowered samples, or the lack of consideration of other factors that may be also involved. Regarding this last point, and taking into account the polygenic nature of psychosis, research on this field is moving towards genome-wide approaches. In this sense, GWAS on schizophrenia have identified several common variants that are believed to contribute to the risk for schizophrenia (O'Donovan *et al*, 2008; Ripke *et al*, 2013), although they explain only a small proportion of the genetic risk for schizophrenia and large number of common risk alleles (with small effects) remain to be identified. This genome-wide strategy has been applied in some recently published studies in psychosis-proneness phenotypes. One of these studies provide suggestive evidence of a genomic link between psychosis-proneness in healthy adults and schizophrenia/bipolar disorder and identified a loci that explained up to 14.1% of the estimated trait heritability

(e.g. Ortega-Alonso *et al*, 2017). In contrast, another one could not detect any association in an adolescent sample, and variants known to be associated with schizophrenia were far from significant (Zammit *et al*, 2014). Another study also provided positive evidence for this genomic link using polygenic risk scores for schizophrenia and bipolar disorder in relation to nonclinical adolescent psychotic experiences (Sieradzka *et al*, 2014). In light of the conflicting results observed and the lower variances explained by the promising genome-wide approaches, several researchers pointed out that the environment and gene-gene interactions should be considered in the future studies, emphasizing that they may explain an important part of the illness (Woo *et al*, 2017).

In this sense, the involvement of environmental risk factors for psychosis has consistently been reported in the literature. Those include obstetric complications, childhood adversity, urbanicity, migration, socio-economic status and cannabis (van Os *et al*, 2010). Regarding childhood trauma, many lines of evidence have shown that people who have suffered adversity at early ages are at increased risk for psychosis, and tend to present a worse illness course and outcome (e.g. Read *et al*, 2005). Also, childhood trauma has been related to intermediate phenotypes of psychosis, showing an increased expression of these traits for those exposed to such experiences (e.g. Velikonja *et al*, 2015). Among the nonclinical samples analysed in our studies, we observed that some subjects had suffered those adverse experiences during childhood, showing in the sample of 808 individuals a prevalence between 8-36% depending on the type of trauma, being the sexual abuse levels the less reported. In line with previous evidences, we observed an association between these traumatic experiences and psychotic-like experiences in the two independent samples analysed (808 young adults and 621 twins), using an overall score of childhood abuse, neglect as well as with a total trauma score. Moreover, in another study, we explored in deep the effects of trauma over a broader psychosis-proneness phenotype including depressive and anxious measures using these 'global' scores, as well as considering different subtypes of trauma (i.e. emotional, physical and sexual abuse, and emotional and physical neglect). All subtypes of trauma were strongly associated to higher schizotypy scores, psychotic-like experiences, and depression and anxiety symptoms, except sexual abuse. However, this lack of association regarding sexual abuse should be considered with caution, since participants reported very low levels of this specific type of abuse with the consequences it implies regarding statistical power in our samples. This detected association with childhood trauma supports the existence of key windows of vulnerability in which environmental factors may impact subjects' neurobiology. Thus, the effect of this adverse event is strongly influenced by the age at which the stressor occurs, the nature and intensity of the stressor, past experience and, especially, by the genetic vulnerability of the individual.

Given the consistent evidence that environmental factors confer substantial risk in addition to the individual common genetic risk variants, the exploration of the effect of GxE seems to be worthy of investigation as it optimizes our chances of mapping the risk landscape (McGrath *et al*, 2013). The GxE approach posits that the effect of environmental exposures will be modulated by the individual genotype. In this sense, regarding the modulator role of genes in this association between childhood trauma and psychopathology, there are evidences involving genes such as *COMT*, *BDNF*, *SERT* or *FKBP5* in samples along the psychosis continuum (e.g. Alemany *et al*, 2011; Collip *et al*, 2013; Karg *et al*, 2011). We have explored the possible interplay between *BDNF Val66Met* and childhood trauma on psychotic-like experiences in two independent nonclinical samples. The BDNF is a neurotrophin that plays important roles in several neurodevelopmental processes, plasticity and also some neurotransmitter systems (Buckley *et al*, 2011). The *Val* allele of the *BDNF Val66Met* polymorphism has been associated with risk for psychosis in case-control and family-based studies (e.g. Lohoff *et al*, 2005; Rosa *et al*, 2006). The role of this polymorphism in psychosis-proneness has only been studied in the context of GxE interaction studies. In this regard, two studies have examined this in relation to psychotic-like experiences and, whereas one found and effect for *Met* allele x childhood abuse on positive psychotic-like experiences in young adults (Alemany *et al*, 2011), a later study could not detect this effect in adolescents (Ramsay *et al*, 2013). In our study, we observed a GxE effect on psychotic-like experiences in both samples, although results were inconsistent in terms of the allele conferring a major risk for psychotic-like experiences (de Castro-Catala *et al*, 2016). Specifically, in the students' sample (i.e. sample 1) we observed that *ValVal* subjects exposed to childhood trauma were reporting more positive psychotic-like experiences, and this was detected in males, but not in females. In the female twins' sample (i.e. sample 2), *Met* carriers with childhood trauma exposure were those showing higher psychotic-like experiences (positive and negative). These results suggest sex-specific associations that are challenging to interpret, since none of the mentioned previous studies examined sex differences, and highlight the importance of replication attempts in independent samples. Other researchers explored this GxE in schizophrenia neuroimaging endophenotypes but although an effect was observed in one study showing *Met* carriers with childhood trauma associated to risk-related phenotypes (Aas *et al*, 2013), this was not replicated in a later study (Hernaus *et al*, 2014).

Converging evidence shows that the *Met* allele of *Val66Met* affects intracellular processing and secretion of the mature protein (e.g. Egan *et al*, 2003) which may influence the different processes in which this protein is involved, such as neurogenesis, neurotransmission and plasticity. Although the underlying processes mediating the effect of this polymorphism on the brain are unclear, it seems reasonable that altered neurotrophic support during brain

development could underlie neuronal structural and functional disorganization leading to an impaired brain unable to respond adequately according to the inputs received (e.g. childhood trauma). In this sense, the *Met* allele has been related to core features of schizophrenia such as reduced brain volumes, memory dysfunctions, learning and memory impairment and cognition (e.g. Altmann *et al*, 2016; Chen *et al*, 2004; Pezawas *et al*, 2004; Soliman *et al*, 2010). However, contradictory findings have been observed regarding the allele of risk associated with the illness or relate phenotypes across case-control, family and association studies, including ours. The reason for these inconsistencies is unclear, but they seem to suggest that, together with *BDNF*, cumulative and/or interactive effects of other genetic, epigenetic or environmental factors are operating in the expression of psychopathology or related traits (e.g. Boulle *et al*, 2012; Gutiérrez *et al*, 2015; Mané *et al*, 2017). Moreover, in this study we observed, as in others, that sex should be taken into account in mental health research, which is supported by multiple studies exploring sex differences for example in brain structure, function and neurotransmission, risk for psychopathology, age of onset, clinical prognosis or genetic effects (e.g. Carbone and Handa, 2013; Cosgrove *et al*, 2007; Godar and Bortolato, 2014).

The HPA axis is one of the biological systems hypothesised to underlie stress-related psychopathology. In this sense, an impairment on the negative feedback regulation of this systems has been proposed to be a potential risk factor for these illnesses (Binder, 2009). Genes as the *FKBP5*, involved in the stress response driven by the HPA axis, are thus interesting candidate genes in this regard. FKBP5 is part of a complex of proteins including other co-chaperones and a GR and modulates the GR affinity to cortisol and signalling (Zannas and Binder, 2014). A series of studies by Binder and collaborators, showed a functional haplotype related with a differential *FKBP5* mRNA and protein induction by GR activation, and also to a variation in GR sensitivity (Binder *et al*, 2004; Zannas and Binder, 2014). Given the implication of *FKBP5* in the stress-response system, the genetic variability within this gene has been mostly studied in relation to stressing environmental factors. In this sense, several authors have provided independent evidences of GxE in depression and/or anxiety, showing higher risk in nonclinical, subclinical and clinical subjects carrying the minor alleles of rs1360780, rs9470080, rs9394309, rs9296158 or rs4713916 that were exposed to different early adversities (e.g. separation from parents, abuse, peer victimization or violence) (Appel *et al*, 2011; Isaksson *et al*, 2016; Lahti *et al*, 2016; Scheuer *et al*, 2016; VanZomeren-Dohm *et al*, 2015). This has been scarcely studied in psychosis or related phenotypes. Specifically, Collip and colleagues observed that, in the presence of childhood trauma exposition, minor alleles of rs9296158 and rs1360780, and major allele of rs1043805 were associated to higher risk in nonclinical subjects; minor alleles of rs992105 and rs4713916 were associated to higher schizotypy in patients' relatives; and

minor allele of rs9296158 was associated to higher positive psychotic symptoms in patients (Collip *et al*, 2013). A more recent study reported increased positive psychotic-like experiences in nonclinical subjects exposed to childhood abuse that were carriers of the minor allele of rs1360780 (Alemany *et al*, 2016). In our study, we explored the interaction effect of *FKBP5* genetic variants and childhood trauma across a broad phenotype of psychotic, depressive and anxiety symptoms in nonclinical young adults (de Castro-Catala *et al*, 2017b). Our results showed a GxE in the variables tapping the depressive-anxious symptoms, but not the psychotic symptoms. Specifically, we observed that those subjects that were not carrying any copy of the *CAT* haplotype in block 1 (i.e. *C*, *A* and *T* are the minor alleles of rs3800373, rs9296158 and rs1360780, respectively) and had been exposed to childhood abuse were reporting higher nonclinical anxiety scores. In the case of block 2, in our sample, those subjects that were not carrying any copy of the *TA* haplotype (i.e. *T* and *A* are the minor alleles of rs9470080 and rs4713916, respectively) and reported exposition to childhood physical abuse were showing increased depression and anxiety scores. Our results support the evidences suggesting a role of *FKBP5* on the risk for mental disorders, specifically depression, in subjects that have suffered childhood adverse experiences such as abuse. However, they are contradictory with those in the literature regarding the alleles conferring an increased risk. Such an inconsistency between previous results and ours is difficult to explain and suggest that further analyses are required.

In light of the available literature studying *FKBP5* in mental health, which has been mostly mentioned above, it seems clear that this gene is involved in the development of psychopathology in adulthood after exposure to childhood trauma. Also it is interesting to see that evidences associate this gene to psychosis, depression and anxiety, pointing towards an extended phenotype including affective and non-affective psychosis or related traits with a shared underlying genetic and environmental vulnerability. From a biological point of view, this extended phenotype with shared aetiology is reasonably justifiable since the affectation of one pathway (for example, in this case, the HPA axis) could lead to an aberrant signalling that would result in a more depressive or more psychotic phenotype depending on other individual characteristics (e.g. other altered pathways).

Our GxE studies seem to support, thus, a role for these two genes on the development of psychopathology in relation of childhood trauma exposure. Considering the lack of an existing review providing an update of the available investigations of the modulating role of these genes on the association between childhood trauma and psychosis, and the interest of having an overview of the effect of these GxE on the continuum psychotic phenotype, we performed a systematic review of all the published evidences in this regard. Since a modulator role has also been found in some studies for the *COMT* gene, evidences on this

GxE effect on psychosis were also explored. In general, few studies were found analysing this and some studied sample sizes that may not be well powered to explore GxE.

The studies exploring *COMT* x childhood trauma suggested that this interaction may be relevant only in subjects at high risk for psychosis, such as those who already developed the illness and their relatives, since no GxE effect was observed in a study analysing nonclinical subjects (Ramsay *et al*, 2013). However, this has to be considered with caution given the studies finding an effect report contradictory results regarding the allele conferring a major risk. Moreover, they have analysed small samples with different population origins, which could have influenced the results observed. As shown at the beginning of the discussion section, evidence of the involvement of the *COMT* gene in psychosis has been largely provided in relation to schizophrenia and related endophenotypes in clinical, subclinical and nonclinical samples. Besides the GxE reviewed, several studies have shown that this gene may be involved in the development of psychopathology under childhood exposure together with other genes (e.g. *MTHFR* or *OXTR*; Cristóbal-Narváez *et al*, 2017; Peerbooms *et al*, 2012) or also when there is exposure to environmental risk factors during adolescence (e.g. cannabis abuse; Alemany *et al*, 2014; Vinkers *et al*, 2013). Taking into account this and the results on psychosis showing a sex differential association mentioned above, it seems that *COMT* is indeed influencing the risk for psychosis as part of a complex network including different adverse environmental factors, other genes and probably sexual or related hormones.

In the case of *BDNF* and *FKBP5*, some of the reviewed studies analysing this gene have been already cited in the discussion in the context of our GxE explorations. Briefly, the reviewed studies that fulfilled inclusion criteria were showing an interaction effect on psychotic-like experiences in young adults, but were discordant regarding the polymorphisms (in the case of *FKBP5*) and alleles associated with an increased risk. Also, the type of maltreatment (e.g. abuse, neglect) and the trait dimension (e.g. positive, negative) associated differed between studies. Similarly, as in the *COMT*, studies point towards an involvement of these genes on risk for psychosis as part of an intricate network of multiple factors interacting between them.

The results of the present thesis support a multifactorial aetiology of schizophrenia involving genes, environment and possibly other factors (e.g. hormones or personal factors) that are active agents in the formation of an individuals' level of vulnerability. Moreover, they provide evidence supporting the hypothesis that some schizophrenia candidate genes are also underlying the development of non-clinical endophenotypes. Ideally, the exploration of these phenotypes and the mechanisms involved should integrate all these multiple factors. However, this would require complex numerical approaches leading to intricate results difficult to interpret and probably would not be much helpful. During the last years, several

approaches have been developed to better consider the multiple factors that underlie these phenotypes. One of these strategies are polygenic risk scores, which intend to represent better the polygenic nature of the illness by providing a score accounting for all the risk alleles of one person weighted by the ORs estimated for each one for a given locus of interest (Iyegbe *et al*, 2014). Moreover, polygenic risk scores allow the study of genes of one specific biological pathway (pathway analyses) and enables the consideration of gene-gene or gene-environment interactions. In this sense, a recent study has shown the interest of polygenic-risk scores observing that they predicted the development of schizophrenia or not in European first-episode psychosis patients (Vassos *et al*, 2017). For example, another study explored whether polygenic-risk score for schizophrenia moderated the effect of childhood adversity on psychosis, but could not find evidence for an interaction effect (Trotta *et al*, 2016). Another strategy that is still in development is genome-wide environment interaction studies (GWEIS), which considers GxE on a genome-wide basis. Although some researches have started to use GWEIS for example in relation to schizophrenia and maternal infection during pregnancy (Avramopoulos *et al*, 2015; Børglum *et al*, 2014) or also in depression and social support and stressful life events (Dunn *et al*, 2016), others recommend caution in the use of this perhaps still immature strategy (Iyegbe *et al*, 2014).

The study of the implications of genetic and environmental factors on psychosis liability, which at this moment is experiencing a shift regarding the approaches used (from single candidate gene studies to genome-wide or polygenic studies), is still an open field worthy of further investigation. In this sense, future studies should be done in samples across the psychosis continuum considering the multifactorial nature of the disorder and related traits by implementing the new developed methodologies and considering adequate sample sizes, appropriate statistical models, accurate phenotypic and environmental measures and also sexual differences. Moreover, findings should be replicated in well powered independent samples to ensure the reliability of the findings.

Limitations

Several limitations should be taken into account when considering the present work. First, in our study we have explored nonclinical subjects from the general populations, which is really interesting regarding the analyses of nonclinical phenotypes related with psychosis. However, the inclusion of subclinical and clinical samples would have allowed the study of the explored mechanisms across the whole continuum of psychosis. Second, our data from the nonclinical samples studied might be biased since the 808 subjects are all undergraduates or technical school students and may not represent well the general population. Moreover, all subjects volunteered to participate in the study and, normally, the type of individuals

who are willing to participate in scientific research will probably present low levels of psychotic traits (e.g. lower suspicion levels) or also childhood trauma (e.g. some subjects abandoned the study because they did not want to answer personal questions regarding childhood trauma). Also, our sample is more represented by female subjects because they tend to volunteer more than males, and also because part of the sampling was performed in first courses of psychology, degree that is studied more predominantly by females. Because of this, the sample principally studied, of more than 800 subjects accurately assessed, although as a whole is well powered, it may be limited for the sex-stratified or interaction analyses. Third, the number of genes (and polymorphisms), environmental factors and outcome phenotypes studied is reduced. In case of the polymorphisms, we studied a small number of markers that did not allow the study of polygenic-risk scores or pathway analyses, which may reflect better the likely genetic structure of the traits studied and would have been of great interest. Regarding the environment, only childhood trauma has been explored, which could have led to observed associations that may be influenced by other environmental factors that have not been considered (e.g. cannabis abuse, bullying). Also, only negative or adverse environmental factors have been taken into account, while considering also the positive "side" may be essential for obtaining a complete and adequate characterization of the environment surrounding each subject (e.g. secure maternal attachment may compensate paternal neglect). Regarding the outcomes, only self-reported measures were analysed. In this regard, it would have been interesting to explore also interview-based measures to compare the results observed. Despite all the mentioned limitations, the choice of the genes, polymorphisms, environmental factors and outcomes analysed in each study was made considering a priori hypotheses based on previous evidences or in plausible neurobiological explanations.

6. Conclusions

The results of the studies presented in this thesis provide new and independent evidence of the involvement of genetic and environmental factors underlying the non-clinical phenotype of the continuum of psychosis. Overall, the main conclusions are:

1. Psychosis-related traits or symptoms are present in the nonclinical population in an attenuated form.

2. Polymorphic variants within the *COMT*, *RGS4* and *ZNF804A* genes are associated with an increased risk for psychosis-related phenotypes in nonclinical subjects. Specifically (i) the *Val* allele on *COMT Val158Met* increase the vulnerability to negative schizotypy and psychotic-like experiences in male subjects, (ii) the rs951436 *T* allele and rs2661319 *A* allele increase the vulnerability to positive and negative psychotic-like experiences and (iii) the *C* allele in rs7597593 increase the vulnerability to positive schizotypy and psychotic-like experiences in females.

3. Exposition to childhood trauma is present in the general population and nonclinical subjects who experienced this adverse events are more likely to develop schizotypy, psychotic-like experiences and depressive and anxiety symptoms.

4. The gene *BDNF* modulate the development of psychosis-related phenotypes in nonclinical subjects who suffered childhood trauma. Specifically, males carrying the *Val66Met Val* allele and females carrying the *Met* allele were more vulnerable to the effects of childhood trauma regarding psychotic-like experiences.

5. The gene *FKBP5* modulate the development of depressive-anxious phenotypes in nonclinical subjects who suffered childhood trauma. Specifically, nonclinical subjects carrying the *CAT* haplotype (rs3800373-rs9296158-rs1360780) or the *TA* haplotype (rs9470080-rs4713916) were at lower risk of presenting depressive and/or anxiety symptoms when exposed to childhood physical abuse.

5. According to the existing evidences published to date, the genes *BDNF*, *COMT* and *FKBP5* modulate the development of psychosis and psychosis-related phenotypes in subjects who suffered childhood trauma. The inconsistencies regarding the alleles conferring a major risk for this phenotypes show that they are likely involved in psychosis as part of a complex framework.

6. Sexual factors (i.e. being male or female) modulate how genes –specifically *COMT*, *BDNF* and *ZNF804A*– impact the psychosis-proneness phenotype.

7. References

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Zhang F, Chen Q, Ye T, Lipska BK, Straub RE, Vakkalanka R, *et al* (2011). Evidence of sex-modulated association of ZNF804A with schizophrenia. *Biol Psychiatry* **69**: 914–917.

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Zubin J, Spring B (1977). Vulnerability - a new view of schizophrenia. *J Abnorm Psychol* **86**: 103–26.

8. Curriculum vitae

Marta de Castro-Catala
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Pre-doctoral researcher
Secció de Zoologia i Antropologia Biològica
Dept. de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia
Universitat de Barcelona
Avinguda Diagonal 643 2on A
08028 Barcelona, Spain

EDUCATION

2008 – 2012 BSc, Biology – Universitat de Barcelona
Final Project: "Study of genetic association in 1q21-23 locus, a candidate region for psychosis", supervised by Dr. Araceli Rosa. Qualification: 9.2

2012 – 2013 MSc, Genetics and Genomics – Universitat de Barcelona
Final Project: "Impact of schizophrenia candidate genes on schizotypy: study of genetic variants in a sample of healthy undergraduates", supervised by Dr. Araceli Rosa. Qualification: 8.7

2013 – present Doctorate in Biomedicine – Universitat de Barcelona

SCIENTIFIC ACTIVITY

2011 – 2013 Collaboration in the project "Genetic Variability in Emotion Regulation, Social Bonding and Hypothesised Candidate Pathopysiological Mechanisms in Psychosis: Relationship with Daily-life Stress-Sensitivity and Expression of the Psychosis Continuum Phenotype" (PSI2011-30321-C02-02), IP: Dr. Araceli Rosa.

2012 – 2013 Collaboration in the project "The Interaction between Daily-Life Stressors and Subjective Appraisals of Psychotic-Like Symptoms in the Psychosis Prodrome during One Year Follow-up: Ecological and Dynamic Evaluation with the Experience Sampling Methodology and Analysis of Gene-Environment (Stress) Interactions" (091110, Fundació La Marató de TV3), IP: Dr. Neus Barrantes-Vidal.

2012 – 2015 Collaboration in the project "EU-GEI, European Network of National Schizophrenia Networks Studying Gene-Environment Interactions" (HEALTH-F2-2010-241909), general coordinator: Prof. Jim van Os, IP: Prof. Phillip McGuire and Prof. Luccia Valmaggia (Institute of Psychiatry, London, UK); coordinator in Barcelona: Prof. Dra. Neus Barrantes-Vidal.

2014 – 2016 Member of the research group recognised by the Generalitat de Catalunya "Gens i ambient en la comprensió de la diversitat de la conducta humana i

de la etiopatogenia de la malaltia mental" (2014SGR1636). IP: Dra. Lourdes Fañanás.

PUBLICATIONS

Articles published in scientific journals

1. Marta de Castro-Catala, Neus Barrantes-Vidal, Tamara Sheinbaum, Artal Moreno-Fortuny, Thomas R. Kwapil, Araceli Rosa. *COMT-by-sex interaction effect on psychosis proneness.* **BioMed Research International** (Journal of Biomedicine and Biotechnology); 2015; 2015:829237. doi: 10.1155/2015/829237

2. Jordi Soler, Salvador Miret, Luisa Lázaro, Mara Parellada, Manuel Martín, Sara Lera-Miguel, Araceli Rosa, Marta de Castro-Catala, Manuel Cuesta, Lourdes Fañanás, Marie-Odile Krebs, Mar Fatjó-Vilas. *Influence of DAOA and RGS4 genes on the risk for psychotic disorders and their associated executive dysfunctions: a family-based study.* **European Psychiatry**; 2016; 32:42-7. doi: 10.1016/j.eurpsy.2015.11.002

3. Paula Cristóbal-Narváez, Tamara Sheinbaum, Araceli Rosa, Sergi Ballespí, Marta de Castro-Catala, Elionora Peña, Thomas R. Kwapil, Neus Barrantes-Vidal. *The Interaction between Childhood Bullying and the FKBP5 Gene on Psychotic-Like Experiences and Stress Reactivity in Real Life.* **PLoS ONE**; 2016; 11(7):e0158809. doi:10.1371/journal.pone.0158809

4. Marta de Castro-Catala, Martine van Nierop, Neus Barrantes-Vidal, Paula Cristóbal-Narváez, Tamara Sheinbaum, Thomas R. Kwapil, Elionora Peña, Nele Jacobs, Catherine Derom, Evert Thiery, Jim van Os, Ruud van Winkel, Araceli Rosa. *Childhood trauma, BDNF Val66Met and subclinical psychotic experiences. Attempt at replication in two independent samples.* **Journal of Psychiatric Research**; 2016; 83:121-129. doi: 10.1016/j.jpsychires.2016.08.014

5. Marta de Castro-Catala, Elionora Peña, Paula Cristóbal-Narváez, Tamara Sheinbaum, Thomas R. Kwapil, Neus Barrantes-Vidal, Araceli Rosa. *RGS4 variants and psychosis proneness: association at the population level.* **European Archives of Psychiatry and Clinical Neuroscience**; 2017; 267(1):19-24. doi:10.1007/s00406-016-0676-7

6. Marta de Castro-Catala, Elionora Peña, Thomas R. Kwapil, Sergi Papiol, Tamara Sheinbaum, Paula Cristóbal-Narváez, Sergi Ballespí, Neus Barrantes-Vidal, Araceli Rosa. *Interaction effect of FKBP5 gene and childhood trauma on psychosis, depression, and anxiety symptoms: study in a non-clinical sample.* **Submitted to Psychoneuroendocrinology** (ID manuscript: PNEC_2017_214).

7. Marta de Castro-Catala, Lourdes Fañanás, Neus Barrantes-Vidal, Araceli Rosa. *COMT, BDNF and FKBP5 genes as moderators of the association between childhood*

trauma, schizophrenia and psychosis proneness: a systematic review. **Submitted to Neuroscience & Biobehavioral reviews** (ID manuscript: EURPSY-D-17-00129).

8. Marta de Castro-Catala, Aurea Mora-Solano, Thomas R. Kwapil, Paula Cristóbal-Narváez, Tamara Sheinbaum, Anna Rcioppi, Neus Barrantes-Vidal, *Araceli Rosa. The genome-wide candidate gene ZNF804A and psychosis-proneness: evidence of sex-modulated association.* **Submitted to PLoS One** (ID manuscript: PONE-D-17-19137).

9. Paula Cristóbal-Narváez, Tamara Sheinbaum, Inez Myin-Germeys, Thomas R. Kwapil, Marta de Castro-Catala, Tecelli Domínguez-Martínez, Anna Racioppi, Manel Monsonet, Lídia Hinojosa-Marqués, Ruud van Winkel, Araceli Rosa, Neus-Barrantes-Vidal. *The role of stress-regulation genes in moderating the real-world association of stress and psychotic experiences in early-psychosis*. **Acta Psychiatrica Scandinavica** (**submitted**, ID manuscript: ACP-2017-6560).

10. Paula Cristóbal-Narváez, Tamara Sheinbaum, Araceli Rosa, Marta de Castro-Catala, Tecelli Domínguez-Martínez, Thomas R. Kwapil, Neus Barrantes-Vidal. *The Interaction of Both Positive and Negative Daily-Life Experiences with FKBP5 Haplotype on Psychosis Risk*. **The British Journal of Psychiatry** (**submitted**, ID manuscript: BJP/2017/203943).

11. Paula Cristóbal-Narváez, Tamara Sheinbaum, Araceli Rosa, Sergi Ballespí, Marta de Castro-Catala, Elionora Peña, Thomas R. Kwapil, Neus Barrantes Vidal. *Interplay between childhood trauma, COMT and OXTR genes on psychotic-like reactivity in real life*. In preparation.

12. Beatriz Pérez-Pérez, Paula Cristóbal-Narváez, Tamara Sheinbaum, Thomas R. Kwapil, Sergi Ballespí, Elionora Peña, Marta de Castro-Catala, Maria Dolors Riba, Araceli Rosa, Neus Barrantes-Vidal. *Interaction between FKBP5 Variability and Recent Life Events in the Anxiety Spectrum: Evidence for the Differential Susceptibility Model*. In preparation.

Publications in dissemination journals

1. de Castro i Català, Marta. "Global Questions on Advanced Biology". Experiència d'un estudiant. Omnis Cellula, December 2012 (No. 29), p. 48 (ISSN: 1696-8107).

2. de Castro-Català, Marta. La recerca de les bases genètiques de les psicosis: un viatge a la complexitat. Treballs de la Societat Catalana de Biologia. Vol. 65 (2014), pp. 68-69 (ISSN: 0212-3037).

Conference communications published on indexed journals

1. P. Cristóbal-Narváez, A. Rosa, M. Castro-Català, T. R. Kwapil, N. Barrantes-Vidal. *COMT Moderation of the Association between Momentary Stress and Psychotic-Like Experiences in Daily Life*. Schizophrenia Research, 153: 316. 4th Schizophrenia International Research Society Conference (Florence, 5-9 April 2014). **Poster presented by P. Cristóbal-Narváez.**

2. A. Rosa, E. Peña, M. de Castro-Català, T. Kwapil, P. Cristóbal- Narváez, N. Barrantes-Vidal. *P250GAP a new candidate gene for schizophrenia and psychosis-proneness?* Schizophrenia Research, 153: 249. 4th Schizophrenia International Research Society Conference (Florence, 5-9 April 2014). **Poster presented by A. Rosa.**

3. M. de Castro-Catala, N. Barrantes-Vidal, T.R. Kwapil, P. Cristóbal-Narváez, A. Rosa. *Relationship between the BDNF Val66Met Polymorphism, Childhood Trauma, Schizotypy and Psychotic-Like Experiences*. Schizophrenia Research, 153: 318. 4th Schizophrenia International Research Society Conference (Florence, 5-9 April 2014). **Poster.**

4. M. de Castro-Catala, N. Barrantes-Vidal, E. Peña, T.R. Kwapil, P. Cristóbal-Narváez, A. Rosa. *Impact of the schizophrenia candidate gene RGS4 on psychosis-proneness.* Schizophrenia Research, 153: 146. 4th Schizophrenia International Research Society Conference (Florence, 5-9 April 2014). **Poster.**

5. de Castro-Català M, van Nierop M, Barrantes-Vidal N, Kwapil TR, Cristóbal-Narváez P, Van Winkel R, Rosa A. *Does the BDNF Val66Met polymorphism play a role on predicting psychotic-like experiences by childhood trauma? American Journal of Medical Genetics* (in press). XXIInd World Congress of Psychiatric Genetics (Copenhagen, 12-16 October 2014). **Poster.**

6. de Castro-Català M, Peña E, Cristóbal-Narváez P, Kwapil TR, Barrantes-Vidal N, Rosa A. *Psychotic-like experiences in non clinical samples and its association with several candidate genes for psychosis*. American Journal of Medical Genetics (in press). XXIInd World Congress of Psychiatric Genetics (Copenhagen, 12-16 October 2014). **Poster.**

7. Cristóbal-Narváez P, Sheinbaum T, Rosa A, Ballespí S, Mitjavila M, de Castro-Catala M, Peña E, Kwapil TR, Barrantes-Vidal N. *The interaction between bullying and FKBP5 haplotype on psychotic-like experiences and reactivity to stress: Does it matter in real life?* Schizophrenia Research (in press). 5th Schizophrenia International Research Society Conference (Florence, 2-6 April 2016). **Poster presented by P. Cristóbal-Narváez.**

Conference communications published in abstract books

1. de Castro M, Fatjó-Vilas M, Arias B, Miret S, Muñoz MJ, Cuesta MJ, Peralta V, Fañanas L, Rosa A. *Genetic screening of the 1q21-23 region confirms the association between UHMK1 gene and schizophrenia spectrum psychoses in a family-based association study.* Abstract book from the VIII Edició dels Premis Gemma Rossell Romero / IV Setmana de la Recerca, organised by the Associació d'Estudiants de Ciències de la Salut (AECS). Facultat de Medicina, Universitat de Barcelona (May 8-10th, 2012). **Oral communication.**

2. de Castro M, Fatjó-Vilas M, Arias B, Miret S, Muñoz MJ, Cuesta MJ, Peralta V, Fañanás L, Rosa A. *Study of genetic association in 1q21-23 locus, a candidate region for psychosis.* Abstract book from I Congrés Internacional de Biologia de Catalunya: "Global Questions on Advanced Biology", Organised by the Societat Catalana de Biologia (SCB) from the Institut d'Estudis Catalans (Barcelona, July 9-12nd, 2012). **Oral communication.**

3. Peña E, de Castro-Català M, Burela PA, Barrantes-Vidal N, Rosa A. *Association between the candidate gene for schizophrenia p250GAP and schizotypy: study in healthy population.* Abstract book from the IX Edició dels Premis Gemma Rossell Romero / V Setmana de la Recerca, organised by the Associació d'Estudiants de Ciències de la Salut (AECS). Facultat de Medicina, Universitat de Barcelona (May 7-9th, 2013). **Oral communication presented by E. Peña.**

4. de Castro-Català M, Moreno A, Ros-Morente A, Peña E, Burela PA, Kwapil TR, Barrantes-Vidal N, Rosa A. *Estudio de la variabilidad del gen de la COMT y su asociación con rasgos esquizotípicos y síntomas psicóticos en población sana.* Abstract book from the XVIII Congreso Internacional de la Sociedad Española de Antropología Física: Una mirada al futuro (Bilbao, June 19-21st, 2013). **Oral communication.**

5. Rosa A, Escarré-Vinyeta J, de Castro-Català M, Ros-Morente A, Kwapil TR, Barrantes-Vidal N. *Interés del estudio de genes candidatos para esquizofrenia en endofenotipos basados en rasgos esquizotípicos medidos en individuos sanos de la población general.* Abstract book from the XVIII Congreso Internacional de la Sociedad Española de Antropología Física: Una mirada al futuro (Bilbao, June 19-21st, 2013). **Oral communication presented by A. Rosa.**

6. Cristóbal-Narváez P, Rosa A, de Castro-Català M, Kwapil TR, Barrantes-Vidal N. *COMT Moderation of the Association between Momentary Stress and Psychotic-Like Experiences in Daily Life.* Abstract book from the Lemanic workshop on Schizotypy (Geneva, December 5-7th, 2013). **Poster presented by P. Cristóbal-Narváez.**

7. de Castro-Catala M, Peña E, Mora A, Sheinbaum T, Cristóbal-Narváez P, Kwapil TR, Barrantes-Vidal N, Rosa A. *Efecto Moderador de los genes en la expresión del fenotipo humano bajo el efecto de factores ambientales estresantes.* Abstract book from the XIX Congreso de la Sociedad Española de Antropología Física "Poblaciones humanas, genética, ambiente y alimentación" (Madrid, June 23-26th, 2015). **Oral communication.**

8. Rosa A, Samper B, Herranz M, de Castro-Catala M, Martín M, Peña E, Torche F. *Estudio de la asimetría fluctuante en niños sometidos a estrés prenatal: el terremoto de tarapacá.* Abstract book from the XIX Congreso de la Sociedad Española de Antropología Física "Poblaciones humanas, genética, ambiente y alimentación" (Madrid, June 23-26th, 2015). **Oral communication presented by A. Rosa.**

9. de Castro-Catala M, Peña E, Sheinbaum T, Cristóbal-Narváez P, Kwapil TR, Barrantes-Vidal N, Rosa A. *The FKBP5 and its moderating role on the psychosis-inducing effects of childhood trauma: new evidences from GxE studies.* 5th European Conference on Schizophrenia Research (Berlin, September 24-26th, 2015). **Poster.**

10. Peña P, de Castro-Catala M, Cristóbal-Narváez P, Sheinbaum T, Kwapil TR, Barrantes-Vidal N, Rosa A. *Moderating effect of the candidate gene p250GAP in the association between childhood trauma and psychosis liability.* 5th European Conference on Schizophrenia Research (Berlin, September 24-26th, 2015). **Poster presented by E. Peña.**

11. Cristóbal-Narváez P, Sheinbaum T, Rosa A, de Castro-Catala M, Kwapil TR, Barrantes-Vidal N. *Adverse childhood experiences, COMT and BDNF genes: An examination of gene-environment interplay on psychosis proneness.* 5th European Conference on Schizophrenia Research (Berlin, September 24-26th, 2015). **Poster presented by P. Cristóbal-Narváez.**

12. de Castro-Catala M, Mora-Solano A, Peña E, Cristóbal-Narváez P, Barrantes-Vidal N, Rosa A. *Uso de polygenic risk scores para el studio de factores genéticos implicados en fenotipos no clínicos* XX Congreso de la Sociedad Española de Antropología Física "La Antropología Física en la era de la Genómica" (Barcelona, July 12-14th, 2017). **Oral communication presented by A. Rosa.**

13. de Castro-Catala M, Mora-Solano A, Peña E, Cristóbal-Narváez P, Barrantes-Vidal N, Rosa A. *Utilidad de lose studios de asociación del genoma complete para la comprensión de los fenotipos complejos en la población sana.* XX Congreso de la Sociedad Española de Antropología Física "La Antropología Física en la era de la Genómica" (Barcelona, July 12-14th, 2017). **Oral communication.**

14. Planas S, Martín M, de Castro-Catala M, Peña E, Bastons-Compta A, Vall O, García-Algar O, Rosa A. *Estudio de las alteraciones de la asimetría fluctuante en niños expuestos a alcohol dorante su desarrollo prenatal.* XX Congreso de la Sociedad Española de Antropología Física "La Antropología Física en la era de la Genómica" (Barcelona, July 12-14th, 2017). **Oral communication presented by S. Planas.**

15. Peña E, de Castro-Catala M, Rivera M, Gutiérrez B, Cardoner N, Rosas A. *Depresión y envejecimiento premature: implicación de los telómeros.* XX Congreso de la Sociedad Española de Antropología Física "La Antropología Física en la era de la Genómica" (Barcelona, July 12-14th, 2017). **Oral communication presented by E. Peña.**

Book chapters

1. de Castro-Català M, Moreno-Fortuny A, Ros-Morente A, Peña E, Burela PA, Kwapil TR, Barrantes-Vidal N, Rosa A. *Estudio de la variabilidad del gen de la COMT y su asociación con rasgos esquizotípicos y síntomas psicóticos en población sana.* La investigación en antropología física: una mirada al futuro, p. 239-255 (ISBN: 978-84-9082-034-6).
2. Rosa A, Escarré-Vinyeta J, de Castro-Català M, Ros-Morente A, Kwapil TR, Barrantes-Vidal N. *Interés del estudio de genes candidatos para esquizofrenia en endofenotipos basados en rasgos esquizotípicos medidos en individuos sanos de la población general.* La investigación en antropología física: una mirada al futuro, p. 281-296 (ISBN: 978-84-9082-034-6).
3. de Castro-Catala M, Peña E, Mora A, Sheinbaum T, Cristóbal-Narváez P, Kwapil TR, Barrantes-Vidal N, Rosa A. *Moderating effect of genes in the human phenotype expression under the effect of environmental stressors.* Poblaciones Humanas, genética, ambiente y alimentación, p. 238-252 (ISBN: 978-84-617-4098-7).
4. Rosa A, Samper B, Herranz M, Martín M, de Castro-Catala M, Peña E, Torche F. *Study of fluctuating asymmetry in children exposed to prenatal stress: The earthquake of Tarapacá.* Poblaciones Humanas, genética, ambiente y alimentación, p. 47-64 (ISBN: 978-84-617-4098-7).

ATTENDANCE TO CONFERENCES AND WORKSHOPS

1. *VIII Edició dels Premis Gemma Rossell Romero / IV Setmana de la Recerca* (May 2012). Organised by the Associació d'Estudiants de Ciències de la Salut (AECS). Facultat de Medicina (UB), Barcelona, Spain.
2. I Congrés Internacional de Biologia de Catalunya: *Global Questions on Advanced Biology* (July 9-12nd, 2012). Organised by the Societat Catalana de Biologia (SCB) from the Institut d'Estudis Catalans. Barcelona, Spain.
3. DNA Anniversary qPCR User Meeting (May 21st, 2013). Organised by the Life Technologies. Centre Esther Koplowitch, Barcelona, Spain.
4. XVIII Congreso Internacional de la Sociedad Española de Antropología Física: Una mirada al futuro (June 19-21st, 2013). Organised by the Sociedad Española de Antropología Física (SEAF), Bizkaia Aretoa, Universidad del País Vasco, Bilbao, Spain.
5. 4rth Schizophrenia International Research Society Conference (April 5-9th, 2014). Organised by the Schizophrenia International Research Society (SIRS), Florence, Italy.
6. 10th Workshop on Genomics and Proteomics (October 3rd, 2014). Organised by the Societat Catalana de Biologia. Institut d'Estudis Catalans (IEC), Barcelona, Spain.

7. *Jornada Institucional de Recerca* (March 13rd, 2015). Organised by the Fundació per a la Investigació i la Docència María Angustias Giménez (FIDMAG) Germanes Hospitalàries. Hospital Sant Rafael, Barcelona, Spain.

8. XIX Congreso de la Sociedad Española de Antropología Física "Poblaciones humanas, genética, ambiente y alimentación" (June 23-26th, 2015). Organised by the Sociedad Española de Antropología Física (SEAF), Universidad Autónoma de Madrid, Madrid, Spain.

9. IV Encontre Ment – Cervell i Societat "Prevenció en Salut Mental" (November 5th, 2015). Organised by the Fundació Parc Taulí – Institut Universitari UAB. Parc Taulí Sabadell, Sabadell, Spain.

8. XX Congreso de la Sociedad Española de Antropología Física "La Antropología Física en la era de la Genómica" (Barcelona, July 12-14th, 2017). Organised by the Sociedad Española de Antropología Física (SEAF), Universidad Autónoma de Barcelona, Barcelona, Spain.

ATTENDANCE TO COURSES AND SEMINARS

1. *III Curso Intensivo de Introducción a la Investigación Básica en Neurociencias: el Cerebro en la Esquizofrenia* (November 18th, 2011). Organised by the Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM). Facultat de Biologia (UB), Barcelona, Spain.

2. *Avenços metodològics en l'estudi de la interacció gens-ambient en la malaltia complexa* (January 16-20th, 2012), module *Gens-Ambient i malaltia* from the Master in Human Biology. Facultat de Biologia (UB), Barcelona, Spain.

3. *Bases fisiopatològiques de les malalties neurològiques i psiquiàtriques* (March 5-9th, 2012), from the Master in Neurosciences. Facultat de Medicina (UB), Barcelona, Spain.

4. *IV Curso Intensivo de Introducción a la Investigación Básica en Neurociencias: el Cerebro en la Depresión* (November 16th, 2012). Organised by the Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM). Facultat de Biologia (UB), Barcelona, Spain.

5. *Advances in the Research of Intellectual Disabilities and Autism* (November 23rd, 2012). Organised by the Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER). Aula Novartis, Barcelona, Spain.

6. *Curso de Formación de Personal Investigador Usuario de Animales de Experimentación* (May 6-27th, 2013). Organised by the Servei d'Experimentació Animal from the Centres Científics i Tecnològics, Universitat de Barcelona. Facultat de Medicina (UB), Barcelona, Spain.

7. *VII Intensive Course of Introduction to Neurosciences: An update in Autism Spectrum Disorders (ASD) research* (July 3rd, 2013). Organised by the Centro de Investigación

Biomédica en Red de Salud Mental (CIBERSAM). Facultat de Biologia (UB), Barcelona, Spain.

8. *Simposio Internacional: Discapacidad intelectual: desafíos diagnósticos en los array de CGH y la secuenciación de nueva generación* (October 3-4th, 2013). Organised by the Fundació Ramón Areces, Hospital Universitari Clínic, Barcelona, Spain.

9. *VIII Jornadas Científicas – Trastornos del Neurodesarrollo* (October 10-11th, 2013). Organised by the Fundació Alícia Hoplowitz. Hospital General Universitario Gregorio Marañón, Madrid, Spain.

10. *VIII Curso Intensivo de Introducción a la Investigación en Neurociencias: Interacción Gen-Ambiente en la causalidad de la enfermedad mental* (November 22nd, 2013). Organised by the Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM). Facultat de Biologia (UB), Barcelona, Spain.

11. *IX Curso Intensivo de Introducción a la Investigación en Neurociencias: Buscando el origen temprano de los trastornos mentales de la edad adulta* (June 27th, 2014). Organised by the Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM). Facultat de Biologia (UB), Barcelona, Spain.

12. *10th Workshop on Genomics and Proteomics* (October 3rd, 2014). Organised by the Societat Catalana de Biologia. Institut d'Estudis Catalans (IEC), Barcelona, Spain.

13. *X Curso Intensivo de Introducción a la Investigación en Neurociencias: Familia, Genes y Enfermedad Mental: Mecanismos etiopatogénicos y avances metodológicos en su investigación* (February 6th, 2015). Organised by the Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM). Facultat de Biologia (UB), Barcelona, Spain.

14. *XI Curso Intensivo de Introducción a la Investigación en Neurociencias: Psicopatología y neurobiología del estrés psicosocial* (June 26th, 2015). Organised by the Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM). Facultat de Biologia (UB), Barcelona, Spain.

15. *Curs d'estadística aplicada a ciències biològiques* (January 25-27th, 2016). Organised by the representative of the doctorate students from the Departament de Biologia Animal. Facultat de Biologia (UB), Barcelona, Spain.

16. *VIII Jornada d'Actualització sobre el Transtorn Psicòtic Incipient: Estrès i Psicosi* (April 15th, 2016). Organised by the Comissió Pedagògica del Pla Director de Salut Mental i Addiccions. Hospital del Mar, Barcelona, Spain.

17. *XII Curso Intensivo de Introducción a la Investigación básica en Neurociéncias: Síntomas, genes y cerebro: nuevos paradigmes en la investigación de la enfermedad mental grave* (April 22nd, 2016). Organised by the Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM). Facultat de Biologia (UB), Barcelona, Spain.

18. *XIII Curso Intensivo de introducción a la Investigación básica en Neurociéncias: The early origin of adult mental health* (June 2nd, 2016). Organised by the Centro de

Investigación Biomédica en Red de Salud Mental (CIBERSAM). Facultat de Biologia (UB), Barcelona, Spain.

19. *XIV Curso Intensivo de introducción a la Investigación básica en Neurociéncias: Cannabis y Enfermedad Mental* (January 27th, 2017). Organised by the Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM). Facultat de Biologia (UB), Barcelona, Spain.

AWARDS RECEIVED

Name:	**Premi per a Estudiants de la Societat Catalana de Biologia (50ª Edició).**
Title:	Estudi d'associació genètica al locus 1q21-23, una regió candidata per les psicosis.
Organisation:	Societat Catalana de Biologia (Institut d'Estudis Catalans).
Place and date:	Institut d'Estudis Catalans, Barcelona, Spain. April 22nd 2013.

Name:	**Premi a l'Excel · lència per treballs de Màster.**
Title:	Impact of schizophrenia candidate genes on schizotypy: study of genetic variants in a sample of healthy undergraduates.
Organisation:	Facultat de Biologia (Universitat de Barcelona).
Place and date:	Facultat de Biologia, Barcelona, Spain. April 17th 2013.

FELLOWSHIPS AND FINANTIAL SUPPORT

Personal fellowships

Name:	**Beca de col · laboració en departaments per a estudiants d'últim curs.**
Organisation:	Ministeri d'Educació.
Place:	Unitat d'Antropologia. Dept. Biologia Animal. Facultat de Biologia. Universitat de Barcelona, Spain.
Period:	February 13th – June 30th 2012.
Activity:	Participation in the project *Genetic Variability in Emotion Regulation, Social Bonding and Hypothesised Candidate Pathophysiological Mechanisms in Psychosis: Relationship with Daily-life Stress-Sensitivity and Expression of the Psychosis Continuum Phenotype* (PSI2011-30321-C02-02).

Name:	**Beca de col · laboració en projectes de recerca.**
Organisation:	Fundació Bosch i Gimpera. Universitat de Barcelona
Place:	Unitat d'Antropologia. Dept. Biologia Animal. Facultat de Biologia. Universitat de Barcelona, Spain.

Period: March 1st – June 30th 2014.
Activity: Collaboration in the project 307651 "Anàlisi de marcadors de risc pre i perinatal en mostres de població sana exposada a estrès intrauterí" directed by Dr. Araceli Rosa de la Cruz.

Name: **Beca de col · laboració amb el Comissionat del Rector par a Participació, Ocupabilitat i Emprenedoria Social.**
Organisation: Universitat de Barcelona
Place: Facultat de Biologia, Universitat de Barcelona, Spain.
Period: February 16th – March 31st 2015.
Activity: Support to the student and any faculty membership in the scope of occupation and startups.

Name: **Beca de col · laboració amb la Secretaria de la Facultat de Biologia**
Organisation: Universitat de Barcelona
Place: Facultat de Biologia, Universitat de Barcelona, Spain.
Period: May 1st 2015 – April 30th 2016.
Activity: Support to general administration tasks from the Secretary of the Faculty, support to the Final Degree Projects administration and dissemination and management software.

Name: **Beca de col · laboració en projectes de recerca.**
Organisation: Fundació Bosch i Gimpera. Universitat de Barcelona
Place: Secció de Zoologia i Antropologia Biològica. Dept. Biologia Evolutiva, Ecologia i Ciències Ambientals. Facultat de Biologia. Universitat de Barcelona, Spain.
Period: May 1st – December 31st 2016.
Activity: Collaboration in the project 305661 "Assessorament i la Investigació aplicada en el camp de la Genètica dels caràcters complexes a les poblacions humanes i de l'Etiopatogènia de les malalties mentals" directed by Dra. Lourdes Fañanás.

Study grants

Name: **Borsa d'estudis Abelard Fàbrega**
Organisation: Fundació Abelard Fàbrega (Institut d'Estudis Catalans)
Place: Unitat d'Antropologia. Dept. Biologia Animal. Facultat de Biologia. Universitat de Barcelona, Spain.
Period: May 5th 2014 – May 5th 2015.
Activity: Execution of the project "Estudi de marcadors biològics de risc prenatal en una cohort de nens catalans amb exposició intrauterina a drogues o alcohol".
Aid granted: 3.000€

Travel grants

Name:	**Grant for conference participation**
Organisation:	Facultat de Biologia (Universitat de Barcelona)
Description:	Participation to the 4th Schizophrenia International Research Society Conference, Florence April 5-9th 2014.
Aid granted:	380 €

Name:	**Grant for conference participation**
Organisation:	Facultat de Biologia (Universitat de Barcelona)
Description:	Participation to the XIX Congreso de la Sociedad Española de Antropología Física "Poblaciones humanas, genética, ambiente y alimentación", Madrid June 23-26th 2015.
Aid granted:	260 €

Name:	**Grant for a predoctoral stay**
Organisation:	Health Universitat de Barcelona Campus (HUBc, Universitat de Barcelona)
Description:	Stay of 3 months at the Institute of Psychiatric Phenomics and Genomics (IPPG), Medical Center of the University of Munich (Ludwig-Maximilians Universität), München, September 23rd – December 23rd 2016.