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Preschool psychopathology using a gene-X-environment model: contributions of immune-related genes and maternal care

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Universidade do Minho
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Professora Doutora Ana Mesquita
e da
Professora Doutora Isabel Soares

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Statement of Integrity

I hereby declare having conducted my thesis with integrity, I confirm that I have not used plagiarism or any form of falsification of my results in the process of the thesis elaboration. I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

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Abstract

Children's emotional and behavioural functioning is increasingly recognized as critical for children's success, in school as well as in other contexts, and in later phases of life into adulthood. Emotional and behavioural disorders during early childhood has been vastly studied, with recent considerable interest in expanding the research from environmental (e.g., maternal care and contextual experience), to genetic predictors (e.g., polymorphisms in neurotransmission system, given its link to behaviour). Despite this growing interest, research is still in many ways preliminary.

The goal of the present doctoral dissertation was to study preschoolers' emotional and behavioural disorders using a Gene-X-Environment interaction (GxE) approach, combining environmental variables and child's genetic background related to the immune system. Specifically, its two main aims were to investigate environmental determinants and their action mechanisms on child's internalizing and externalizing problems; and, on the other hand, to examine whether an immune-related gene interact with environment in the prediction of withdraw behaviour (internalizing symptoms).

Therefore, Chapter 1 introduced the state of the art behind the proposed field of study. Chapter 2 then focus the first aim of this dissertation: to examine the effect of family risks and maternal care and their action mechanisms on child's internalizing and externalizing problems in preschool age. Specifically, Chapter 2 analyzed whether the relationship between family risk and child's internalizing and externalizing problems is differentially mediated by maternal insensitivity and intrusiveness. Based on a sample of 205 children, their mothers and their preschool teachers, results revealed that only maternal intrusiveness mediated the relation between family risk and children's internalizing behaviours, in way that children display higher internalizing symptoms when mothers were more intrusive particulatly in the case of higher risk families.

Chapter 3 provided a systematic review on GxE research focused on internalizing or/and externalizing problems in early childhood in order to provide a critical overview of the literature. Based on a sample of 14 studies, results concluded that the prevalence of G×E effects in predicting early emotional and behaviour problems is salient, with

parental variables, namely, parental psychopathology and parental interactive behaviours, as important moderators. This review also revealed a prevalence of 'usual suspect' candidate genes, mostly integrated in the dopaminergic and serotonergic systems (neurotransmission-related genes), disregarding other putatively relevant systems, such as the immune system.

Finally, Chapter 4 took into consideration the link between the immune system and some psychiatric conditions, and analyzed the role of the child's polymorphism (rs2430561) on the Interferon Gamma Gene (*IFNG*) on withdrawn behaviour, testing maternal psychological distress as a possible moderator of such association. Results proved consistent with the differential susceptibility model of person-X-context interaction.

This dissertation underlines the relevance of considering the interaction between individual and environmental factors in shaping emotional and behavioural functioning in a preschool age. Building on this work, future studies should consider larger scale samples, and follow approaches which include different biological, psychological and environmental aspects for a broader comprehension of early emotional and behavioural problems.

Psicopatologia no período pré-escolar através do modelo gene-X-ambiente: contribuições de genes imunes e dos cuidados maternos

Resumo

O funcionamento emocional e comportamental infantil tem sido amplamente reconhecido como critício para o sucesso das crianças, no contexto escolar, mas também em outros contextos, e em fases posteriores do ciclo de vida, até à idade adulta. As perturbações emocionais e comportamentais durante a infância têm sido muito estudadas, recentemente com considerável interesse na expansão da investigação de preditores ambientais (e.g., cuidados maternos e experiências contextuais), para preditores genéticos (e.g., polimorfismos em genes implicados no sistema de neurotransmissão, dada a sua relação com o comportamento). Apesar deste interesse crescente, a investigação é ainda preliminar em múltiplos aspetos.

O objetivo da presente dissertação de doutormaneto foi estudar as perturbações emocionais e comportamentais em idade pré-escolar, usando uma abordagem de intereção entre Genes e Ambiente (Gene-X-Ambiente) combinando variáveis ambientais e informação genética relacionada com o sistema imunitário da criança. Especificamente, os dois objetivos principais foram estudar determinantes ambientais e os seus mecanismos de interação relativamente a problemas de internalização e externalização da criança; e, por outro lado, investigar se um gene relacionado com o sistema imunitário interage com o ambiente na predição de comportamento de isolamento (sintomas de internalização).

Assim, o Capítulo 1 introduz o estado da arte acerca da área de estudo proposta. O Capítulo 2 foca o primeiro objetivo desta dissertação: estudar o efeito do risco familiar e dos cuidados maternos e os seus mecanismos de interação nos problemas de internalização e externalização em idade pré-escolar. Especificamente, o Capítulo 2 analisa se a relação entre risco familiar e problemas de internalização e externalização é mediada de forma diferencial pela insensibilidade ou intrusividade maternas. Baseado numa amostra de 205 crianças, as suas mães e professores, os resultados revelaram que a relação entre o risco familiar e os problemas de internalização foi apenas mediada pela inrusividade, de forma que as crianças apresentaram mais problemas de internalização

quando as suas mães eram mais intrusivas, particularmente no caso de elevado risco familiar.

O Capítulo 3 forneceu uma revisão sistemática da literatura sobre estudos de interação Gene-X-Ambiente focados em problemas de internalização e/ou externalização na infância, de forma a oferecer uma visão crítica da literatura neste âmbito. Baseado numa amostra de 14 estudos, os resultados concluiram que a prevalência do efeito de interação Gene-X-Ambiente na predição de problemas emocionais e comportamentais é saliente entre os estudos, com variáveis parentais a funcionarem como moderadores importantes, nomeadamente a psicopatologia parental e os comportamentos interativos parentais. Esta revisão sistemática também realçou a prevalência dos genes candidatos 'suspeitos do costume', integrados maioritariamente nos sistemas dopaminérgico e serotonérgico (genes relacionados com a neurotransmissão), desconsiderando outros sistemas putativamente relevantes, como o sistema imunitário.

Finalmente, o Capítulo 4 teve em consideração a relação entre o sistema imunitário e algumas condições psiquiátricas, e analisou o papel de um polimorfismo (rs2430561) no Gene do Interferon Gama (*IFNG*) no comportamento de isolamento da criança, testando o bem-estar psicológico materno como possível moderador. Os resultados provaram consistência com o modelo da susceptibilidade diferencial.

Esta dissertação sublinha a relevância de considerar a interação entre fatores individuais e ambientais no funcionamento emocional e comportamental em idade pré-escolar. Com base neste trabalho, estudos futuros deverão considerar amostras de maior escala e seguir abordagens que incluam diferentes aspectos biológicos, psicológicos e ambientais, para uma compreensão mais ampla dos problemas emocionais e comportamentais manifestados em idades tão precoces.

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LIST OF ABBREVIATIONS

5-HTTLPR	Serotonin Transporter-Linked Polymorphic Region
ADRA2A	Adrenoceptor Alpha 2A
AMP	*Adenosine Monophosphate*
BDNF	Brain Derived Neurotrophic Factor
BSI	Brief Symptom Inventory
CBCL	Child Behaviour Checklist
CRH	Corticotrophin Releasing Hormone
CRP	C-reactive protein
CTRF	Caregiver-Teacher Report Form
DNA	Deoxyribonucleic Acid
DRD2	Dopamine Receptor D2
DRD4	Dopamine Receptor D4
fMRI	Functional Magnetic Resonance Imaging
GR	Glucocorticoid Receptor
GXE	Gene-X-environment
HPA	Hypothalamic–pituitary–adrenal
HTR2A	Hydroxytryptamine Receptor 2A
IDO	Indoleamine-2,3-dioxygenase
IL-1α	Interleukin-1α
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IFN-γ	Interferon-γ
KYNA	kynurenic
LPS	Lipopolysaccharide
MAO-A	Monoamine Oxidase A
mRNA	Messenger Ribonucleic Acid
NMDA	*N* -methyl- D -aspartate
PSDI	Positive Symptoms Distress Index, Brief Symptom Inventory
QUIN	Quinolinic
rGE	Gene-Environment correlation
SNP	Single Nucleotide Polymorphism
TNF-α	Tumor Necrosis Factor-α
TRP	Tryptophan
VNTR	*Variable Number Tandem Repeat*
WB	Withdrawn Behaviour

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CHAPTER 1

INTRODUCTION

CHAPTER 1

INTRODUCTION

The view that developmental processes and outcomes are influenced by a dynamic interplay between individuals and the environment, operating at multiple levels of influence, including neurobiological, psychological, and social, contributing to variations in biological and psychological processes that affect functioning and can lead to psychopathology, is central in Developmental Psychopathology (see Lewis & Rudolph, 2014) and it is incorporated into this dissertation. Within this conceptual framework, the main aim is to study preschoolers' emotional and behavioural disorders using a Gene-Environment interaction (GxE) approach, combining environmental variables and child's genetic factors related to the immune system.

This first chapter is organized into three sections. The first focuses on key concepts and issues in the current understanding of psychopathology during preschool years. In the second section the environmental influences are approached by examining the role of differential environments, from more distal to more proximal ones. The final section provides a gene-environment framework and neuro-immune hypotheses for emotional and behavioural symptoms.

I. PSYCHOPATHOLOGY DURING PRESCHOOL YEARS

In applying a developmental framework to the study of preschooler's emotional and disorders problems, it is important to understand key developmental processes relevant to mental health issues. The preschool years represent a time of expansive psychological growth, with the initial expression of many psychological abilities that will

continue to be refined into young adulthood (Troller-Renfree & Fox, 2017). Likewise, brain development is characterized by its "blossoming" nature, showing some of its most dynamic and elaborative anatomical and physiological changes which are the basis of the sophistication of mental processes underlying cognition and social-emotional development (Brown & Jernigan, 2012). In addition to this biological capacity for the sophistication of mental processes, the environmental experiences and expectations associated with the preschool context and the upcoming primary school reinforce other highly important developmental acquisitions at this age, such as an increased level of autonomy, emotional regulation abilities, and more advanced problem-solving strategies that utilize internal and external resources to adjust to new environments and situations.

Because children develop very rapidly in this period, both normal and pathological mental phenomena may be only transiently observed, being often unclear whether a disorder is best interpreted as an expression of a problematic environmental dynamics or, alternatively, as a potential first sign of individual psychopathology. Many symptoms cause clinically significant impairment at subliminal levels reinforcing the importance of evaluating psychological symptoms using a dimensional approach, which could facilitate the identification of children who are at risk for more severe or pernicious courses prior to the full presentation of symptoms (Drabick, 2009). For the purposes of this dissertation we define early psychopathology as the lack of social and emotional competence, which according to Yates and colleagues (2008) corresponds to the ability of young children to form close and secure adult and peer relationships, experience, regulate, and express emotions in appropriate socially and culturally ways, as well as to explore the environment and learn in family, community and cultural contexts. Among the many foundational psychopathology signs in early childhood, researchers consistently consider the internalizing and externalizing behaviour problems (e.g. Early Childhood

Longitudinal Program and the NICHD Study of Early Child Care and Youth Development).

One of the best established dimensional systems for classifying the signs and symptoms of preschool psychopathology is the Achenbach System of Empirically Based Assessment (ASEBA; Achenbach & Rescorla, 2000). This system is now the gold standard in the field and has been already used in around 104 societies and cultural groups (Bérubé & Achenbach, 2014). The forms for preschool children – Child Behaviour Checklist – CBCL 1½-5 for parents and the Caregiver-Teacher Report Form – CTRF 1½-5 for teacher - are validated measures and have been psychometrically examined across 15 different societies (Rescorla et al., 2012). The CBCL 1½–5 and C-TRF 1½–5 are both scored on broadband Internalizing and Externalizing scales, each one comprising four (Emotionally reactive, Anxious/Depressed, Somatic complaints, and Withdrawn) and two (Attention problems and Aggressive behaviour) syndrome scales, respectively. This measure is particularly useful in early childhood because emergent psychopathology may be milder, and distinctions from normative misbehaviours may be best captured along a dimensional scale (Wakschlag et al., 2015). Additionally, this system has proven to differentiate among clinical populations and unaffected individuals (Smith & Corkum, 2007).

In this early age it is particularly difficult to determine the prevalence rates of emotional and behavioural problems. Nevertheless, epidemiological studies have reported an estimated prevalence of 7-26% (Rescorla et al., 2011; Wichstrøm, Berg-Nielsen, Angold, Egger, Solheim & Sveen, 2012). These rates are similar to prevalence rates for older children, suggesting that emotional and behavioural problems in preschool age children occur at comparable levels. Research has also shown that when internalizing and externalizing problems in childhood are not resolved, emotional and behavioural

difficulties tend to persist in school age and adolescence (Lane, Barton-Arwood, Nelson, & Wehby, 2008; Nelson, Benner, Lane, & Smith, 2004).

Thus, understanding the influences that contribute for early internalizing and externalizing problems in preschool allows improving the knowledge about the sources of vulnerability and support that can promote children mental health. Given the major impact of early intervention related to greater neuroplasticity during this period (Troller-Renfree et al., 2017), it is important to identify markers of risk and protective mechanisms as the earliest as possible.

Decades of research have established that a wide array of contextual characteristics serve as critical precursors of children's mental health outcomes (Evans, Li, & Whipple, 2013; Shaw & Shelleby, 2014). Reviews of the literature have specifically documented that family and parenting contexts qualify as predictors for the emergence and persistence of both internalizing and externalizing behaviour problems throughout childhood (Repetti, Robles, & Reynolds, 2011). In the next section we present a summary of key environmental factors, with a focus on two facets of early environmental factors that are particularly prominent during childhood: at more proximal level, the maternal care and, at a more distal level, the family context.

II. Environmental Influences: From Proximal To Distal Experiences

Research on developmental psychopathology has emphasized the importance of considering a bioecological model, proposed by Bronfenbrenner (1999; Bronfenbrenner & Morris, 2006), which asserts that process, person, context, and time are crucial for the understanding of human development. According to this model, human development takes place in a progressively more complex series of reciprocal interactions between the

biopsychological human organism and the different elements in the proximal and distal external contexts. Context in development is characterized by a series of systems, defined hierarchically, from the most proximal to the most distal. At the center are the child's individual characteristics (biological background, such as genetics and temperament), then the child's immediate/proximal environment (family, school, neighborhood), then the surrounding social and economic context and the cultural context, providing additional sources of influence. The comprehension of child's development requires an understanding of the influences of all these contexts proving different physical, cognitive, and social experiences (Bronfenbrenner & Morris, 2006).

In Western cultures, parents typically play the major role in structuring young children's environments and experiences. Therefore, parent-child relationship constitutes the first environment in which development unfolds and the earliest foundation for risk and resilience. Theoretical conceptualizations of parent-child dynamics have predominantly focused on the effects by which parenting influences children's development, with a general tendency to give greater emphasis to maternal care.

It is widely accepted that maternal care has a profound influence on the child development as the relational patterns experienced early in life are "internalized" to become part of the developing child's functioning (Bowlby, 1969). It is well established that mother-child interactions serve as a regulatory mechanism by which the infant develops adaptive emotion regulation patterns and problem-solving skills and facilitates a series of developmental acquisitions such as autonomy, exploration, and self-control (Dagne & Snyder, 2011; Lunkenheimer, Kemp, & Albrecht, 2013). The processes through which stable child characteristics are shaped by the relational environment have been framed in terms of "entrained self-regulation" (Dishion & Patterson, 2006), wherein entrainment refers to the role of environments in structuring neural pathways implicated

in automatic and overlearned behaviour patterns. For instance, research on developmental neuroscience has suggested that the limbic system, associated with emotion processing and self-regulation, appears to be influenced by early maternal deprivation and suboptimal caregiving (Gee, 2016).

At preschool age, children have not yet fully developed the ability to regulate their emotions by their own, and therefore, benefit from the relationship with their caregivers through the process of dyadic regulation. In this regard, it has been widely described that prompt maternal responsiveness to distress (Del Carmen, Pedersen, Huffman, & Bryan, 1993), appropriate stimulation (e.g., Belsky, Rovine, & Taylor, 1984), mutually interactional synchrony (Isabella & Belsky, 1991), and autonomy support (Bernier, Matte-Gagne, Belanger, & Whipple, 2014) promote child's secure attachment relationships. In contrast, controlling interactional styles, excessively stimulating and intrusive have been associated with insecure attachment relationships (Bureau, Easlerbrooks, & Lyons-Ruth, 2009). Research has also shown that securely attached children develop more social skills and higher emotional understanding of others, show more advanced moral development, have a more positive self-concept, exhibit better cognitive performance and language development and show higher achievement in school (O'Connor, Matias, Futh, Tantam, & Scott, 2013; Thompson, 2013). Likewise, consistency and stability of maternal warmth and supportive care have been speculated to have effect on structural brain development, namely in amygdala and prefrontal cortex, highly related to emotion regulation and executive functions (Whittle et al., 2014).

Maternal care has been investigated in light of a broad set of constructs, including affect attunement (Stern, 1985), sensitivity (Ainsworth, Blehar, Waters, & Wall, 1978), emotional availability (Source & Emde, 1981), mutually responsive orientation (Kochanska, 1997), reflective function (Fonagy & Target, 1997), mind-mindedness

(Meins, 1997), and insightfulness (Oppenheim & Koren-Karie, 2002). Despite these constructs differing in substantive ways, they converge on the focus on the emotional qualities of dyadic interactions and stress the importance of the mother's responsiveness to the child's needs, both physical and psychologically (O'Connor, 2002). The maternal interactive behaviours – sensitivity and cooperation – as described by Ainsworth and colleagues (Ainsworth et al., 1978) have been considered as valuable contributions to the field of parenting and child development (Cassidy, 2016).

Sensitivity is defined as the parent's ability to notice child signals, interpret these signals correctly, and respond to these signals promptly and appropriately (Ainsworth et al., 1978). These components of parental behaviour refer to universally relevant aspects of caregiving, including proximity to the child (necessary for protection and meeting basic needs), contingent responding (promoting social development), and appropriateness of interventions based on the child's responses rather than on a fixed list of specific parenting behaviours (Mesman, van IJzendoorn, & Bakermans-Kranenburg, 2012). Maternal sensitivity has been considered a significant predictor of attachment security (de Wolff & van IJzendoorn, 1997), with meta-analysis showing that improvements in parental sensitivity induced by parenting interventions improve child attachment quality (Bakermans-Kranenburg, van IJzendoorn, & Juffer, 2003). Recent work on sensitivity has found relations with a large variety of developmental outcomes, namely with internalizing and externalizing behaviour problems in preschool age (e.g., Kok et al., 2013) and with externalizing problems during childhood and adolescence (e.g., Garai et al., 2009). Additionally, empirical studies have also demonstrated that parenting interventions focused on promoting parental sensitivity decreases emotional and behavioural problems (Juffer, Struis, Werner, & Bakermans-Kranenburg, 2017).

Maternal cooperation has been defined as the extent to which the mother's interventions break into, interrupt or cut cross the child's ongoing activity rather than being geared in both timing and quality of the child's state, mood and current interests. The degree of interference may be evaluated according the extent of actual physical interference with the child's activity and the sheer frequency of interruptions (Ainsworth et al., 1978). According to Ainsworth (1978), some mothers are highly interfering in a physical sense such as snatche the child up, move about, confine, and, indeed, release with utter disregard for the child's activity-in-progress. Some mothers also try to use force in instances in which the child's cooperation is required if the intervention is to be effective. Other mothers, whose intrusiveness does not so conspicuously, emphasize physical sense nevertheless but are "at" the child most of the time, in diverse instances such as instructing, training, eliciting, directing, controlling. Mothers at the other end of this continuum rather than to control child's activity, seem to guide, integrating her functioning with the child's functioning in way that their interactions seem co-determined.

Both sensitivity and cooperation have been found to capture vast individual differences in quality of parenting within a wide array of populations (Dexter, Wong, Stacks, Beeghly, & Barnett, 2013), however there are other variables directly linked to maternal care which are important consider, as is the case of maternal psychopathology, socioeconomic status, lack of parental support, and other family stressors (Weitzman, Edmonds, Davagnino, Briggs-Gowan, 2013).

Maternal psychopathology has gained much attention and several reasons may explain its importance. First, parents pass their genes to their offspring, which makes their child more or less susceptible to a particular disorder. Second, mothers may use strategies that contribute to the development of emotional and behavioural problems. For example, maternal control has been found to be associated with childhood anxiety (van Der

Bruggen, Stams, & Bogels, 2008), maternal hostility has been found strongly correlated with child depressive behaviours (McLeod, Wood, & Wood, 2007), and physical punishment has been associated with aggressive behaviour (Gershoff, 2002).

During the childbearing years, maternal psychopathological conditions, especially depression and anxiety symptoms, tend to affect 10-30% of mothers (Dekel, Stuebe, & Dishy, 2017; Lyons-Ruth, Wolfe, Lyubchik, & Steingard, 2002) with significant impact on child development, being associated with internalizing and externalizing problems, that persist throughout childhood and adolescence (Maughan et al., 2007; Murray et al., 2011). Most research to date has focused on the effects of maternal depression on child outcomes, with several evidence showing the adverse effects of depression exposure during early childhood with lasting consequences over and above current parental depressive symptoms (Pearson et al., 2013). For example, parental depression is associated with difficulties in affect regulation and emotional adjustment (Kerr et al., 2013; Maughan, Cicchetti, Toth, & Rogosch, 2007), social acceptance (Maughan et al., 2007), and cortisol regulation (Laurent et al., 2013) in young children, all of which may be precursors to later psychopathology.

It is consensual that maternal psychopathological symptoms predict elevated children's emotional and behavioural problems, particularly by disrupting mother-child interactions (Elgar, Mills, McGrath, Waschbusch, & Brownridge, 2007). Psychopathological conditions exacerbate maternal caregiving pivotal to children's behavioural adjustment, thus contributing to self-regulatory difficulties and internalizing and externalizing behaviour problems (Berg-Nielsen, Vikan, & Dahl, 2002). For example, mothers reporting elevated levels of psychological distress were evaluated as being more negative, intrusive and hostile toward children's attempts for autonomy and social behaviour, and less sensitive and responsive in their face-to-face interactions

(Campbell, Mastetic, von Stauffenberg, Mohan, & Kirchner, 2007). Although important, maternal care does not appear to be the exclusive factor for the development of child's psychopathology. Family stress, such as socioeconomic difficulties, affect child development also.

Socioeconomic context is one of the most widely studied constructs in the social sciences. Research shows that socioeconomic risks are associated with a wide array of health, cognitive and emotional outcomes in children, with effects beginning prior to birth and continuing into adulthood. In child development literature it is recognized that children facing multiple family risks fare less well developmentally in a wide variety of areas, from emotional development to academic performance (Burchinal, Roberts, Zeisel, Hennon, & Hooper, 2006; Rouse & Fantuzzo, 2009). Within the socioeconomic context, some of the most prominent risk factors to the psychological development arise surprisingly diverse, including economic disadvantage (Mistry, Biesanz, Taylor, Burchinal, & Cox, 2004), low social support (Thompson, Flood, & Goodwin, 2006), low parental education (Carter, Wagmiller, Gray, McCarthy, Horwitz, & Briggs-Gowan, 2010), higher marital conflict (Zimet & Jacob, 2001), and lower parental mental and physical health (Anderson, Huth, Garcia, & Swezey, 2014; Mensah & Kierman, 2010; Micali et al., 2014).

Thus, the socioeconomic environment has a substantial impact on the quality of relationships between parents and on the quality of their parenting. The stress of socioeconomic difficulties can directly impact on parent's ability to care for children optimally and have been associated with punitive parenting and increased child neglect (McLoyd, Aikens, & Burton, 2006). Socioeconomic stress not only affects each parent's behaviour directly, it also reduces mutual supportiveness and adversely affects the quality of parenting behaviour. However, socioeconomic difficulties affect child development in

ways that go beyond parenting (McLoyd et al., 2006). Comparing different socioeconomic environments, children living in high risk families experience lower levels of emotional and verbal responsivity, fewer opportunities for variety of daily stimulation, fewer appropriate play materials, and more disorganized and unstructured environments (Garret, Ng'andu & Ferron, 1994; Letourneau, Duffett-Leger, Levac, Watson, & Young-Morris, 2011).

In summary, family socioeconomic context exerts direct and indirect influences on child development. The links between environmental factors and children's risk for a full range of adjustment problems including social and academic difficulties and internalizing and externalizing behaviour problems have been clearly established, the mechanisms by which this occurs are complex and are less understood. Several conceptual models have been proposed emphasizing the utility of a multi-level approach which accounts for influences at multiple levels including child-level factors (Cicchetti & Toth, 2009), such genetic ones. Consistent with recent theoretical frameworks suggesting that children with certain biological characteristics may be more susceptible than others to the effects of the caregiving environment (Boyce & Ellis, 2005), it is important to consider the contribution of individual variables in understanding the effects of environmental experiences on development of children's internalizing and externalizing problems.

III. GENE-X-ENVIRONMENT INTERACTION

In the past decades, the study of emotional and behavioural disorders has focused on its multifactorial nature, accounting for the individual variability in the expression of different traits. In order to address the complexity of emotional and behavioural

functioning, developmental research has been guided by an integrated approach looking to the contribution of both genetics underpinning neural circuits and hormonal signaling, and environment-dependent experience on shaping these pathways (Sharma, Powers, Bradley & Ressler, 2016). This multilevel perspective considering the interplay between distinct environmental and individual characteristics, such as the contribution genes and brain, has been adopted to a better understanding of the etiological mechanisms of emotional and behavioural symptoms.

Gene-X-Environment (GxE) approach emphasizes the transactional nature of environmental experience and the genome in the development of behavioural profiles, and specifically in the occurrence of numerous common diseases. G×E interaction occurs when the relationship between an environmental experience and the emergence of a phenotype is contingent with individual differences in genetic makeup, or, conversely, when the effect of an individual genotype on a phenotype is conditional on an environmental experience (Rutter, Moffitt, & Caspi, 2006).

Following this perspective, the next section will address a brief theoretical and empirical background of GxE approach followed by the current conceptual models of GxE and, by testing the neuro-immune hypothesis, addressing the putative role of immune genes on emotional and behavioural symptoms.

Theoretical and empirical background of GxE approach

According to developmentalists (e.g., Rutter et al., 2006) there are some plausible reasons why GxE was expected to be common. First, the evolutionary perspective is based on the idea that the differential response to the environment is genetically influenced. Therefore, rejecting GxE would be equivalent to rejecting the keystone of evolutionary thinking (Rutter, 2014). Additionally, a wide range of experimental studies have shown

a great heterogeneity in response to environmental features, which seems to imply a genetic influence. So, the idea that both genetic and environmental influences contribute to differential behavioural outcomes has been widely recognized and, conceptually, GxE research has been an attractive model (Dick et al., 2015).

Particularly in the field of developmental psychopathology, where although recent in history, important advances have been made so far. Seminal work from Caspi and colleagues (2002) hypothesized that a functional polymorphism in the promoter region of the gene encoding the enzyme monoamine oxidase A (MAO-A) moderates the relationship between childhood maltreatment and antisocial behaviour. Results showed that maltreated children carrying a genotype conferring low levels of MAO-A expression developed more antisocial behaviour, increasing the likelihood to commit violent crimes as adults, than children carrying a genotype conferring high levels of MAO-A. One year later, and by using a longitudinal design, the same team demonstrated that stress and subsequent depression were contingent with a functional polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR). Individuals carrying one or two copies of the 5-HTTLPR short allele exhibited more depressive symptoms than individuals homozygous for the long allele (Caspi et al., 2003). In fact, empirical evidence, with replication, has shown that environmental experiences can differently impact the development of psychopathology according to individual genetic variations (e.g. Kendler et al., 2005). These findings supported the G×E hypothesis in the study of emotional and behavioural symptoms, leading to an exponential increase in the number of studies in this area. Understanding how genetic predispositions interact with environmental influences is critical and may guide the development of tailored intervention and prevention efforts for those at greatest risk.

Models of Gene-X-Environment interaction

Two main conceptual models have been used to study G×E interaction and to describe how individual and environmental characteristics interact in shaping development. The model that initially guided GxE research was the Diathesis-Stress model, which stipulate that individuals carrying genetic-risk variants are more vulnerable to the effects of environmental adversity or suboptimal environment and thus more prone to develop psychopathology. However, a pertinent evolutionary question challenges the sensitivity of this model, i.e., why genetic vulnerability factors persist over the evolution? From this evolutionary perspective, Belsky and colleagues (2007; 2009) proposed an alternative model, a differential-susceptibility hypothesis. This model establishes that individuals with different genetic background should differ in the degree to which they are affected by the whole environmental spectrum (from positive to negative) and not only by the degree in which they are affected by adverse environments, as predicted by the diathesis-stress hypothesis. Therefore, some individuals are more developmentally plastic "for better and for worse", meaning that more plastic individuals are expected to be more susceptible to both the negative effects of adverse environments and the beneficial effects of positive environments, while less plastic individuals are expected to be less affected by the environment (Belsky et al., 2007; 2009). This way of conceptualizing GxE derives from an evolutionary analysis of human development (Belsky & Pluess, 2009; Ellis, Boyce, Belsky, Bakermans-Kranenburg, & van Ijzendoorn, 2011), explicitly acknowledging that there are evolutionary benefits for which some alleles remain along evolution, since the same allelic variant can express itself as either a risk factor or a promoting factor depending on environmental experience. Therefore, rather than conceptualizing some polymorphic variants as "risk alleles", genetic

polymorphisms should be conceptualized as “plastic”, emphasizing the lack of a genetic determinism as well as the importance of the quality of environmental context.

Differential susceptibility offers an exciting new perspective in the study of development and psychopathology (Ellis et al., 2011). This model has major implications in the understanding how genetic variations lead to differential child’s responses to all types of environmental conditions and interventions. Therefore, the differentiation between diathesis-stress and differential-susceptibility models has critical implications, as it could lead to an inadequate description of the etiology of psychopathological disorders, resulting in interventions that are based on an incomplete or even erroneous comprehension of human development, which in turn can produce inefficacy, iatrogenic effects, and unnecessary economical costs (Pérez-Pérez et al., 2018).

Despite the fast accumulating evidence of GxE interaction on the etiology of child psychopathology, most studies have focused on a restricted pool of genes, most of them involved in neurotransmission. More specifically, genes implicated in the dopaminergic (MAOA, DRD2, DRD4) and the serotoninergic (5-HTT) transmission, still yielding a limited understanding on etiological mechanisms underlying children’s psychopathological disorders. Thus, it is important to extend GxE research to other putative relevant systems.

Neuro-immune hypothesis

The immune system, which has a bi-directional communication with the central nervous system influencing several aspects of brain functioning relevant to behaviour regulation (Capuron & Miller, 2011; O’Connor, Moynihan & Caserta, 2014), is a likely candidate to address the role of other genes in the development of child psychopathology. Interestingly, the immune and the central nervous systems have been classified as the

main regulatory systems in the organism having specialized cells to "sense" the environment.

There is now substantial evidence that stress exposure can trigger a significant increase in inflammatory activity (Bottaccioli, Bottaccioli, & Minelli, 2018; Calcia, Bonsall, Bloomfield, Selvaraj, Barichello, & Howes, 2016; O'Connor et al., 2014). Several studies in adults associate multiple markers of neuro-inflammation with psychiatric symptoms (Dantzer, O'Connor, Lawson, & Kelly, 2011; Howren, Lamkim, & Suls, 2009), with special emphasis on the cytokine network (for review see Capuron & Miller, 2011; Felger & Lotrich, 2013). Indeed, there has been a great interest in the effects of cytokines on brain and behaviour in recent years.

Cytokines are small secreted proteins that immune cells use to communicate. They are present in central nervous system, both during development and adulthood, being produced by microglia and astrocytes as early as week five of gestation in humans, suggesting their important role in development (Ellenbroek & Youn, 2016). They are important for brain development and can promote healthy brain function by supporting neuronal integrity, neurogenesis, and synaptic remodeling (Yirmiya & Goshen, 2011). Based upon their molecular structure and physiological action, cytokines can be classified as either pro- or anti-inflammatory molecules (Khairova, Machado-Vieira, Du & Manji, 2009). Interleukin-1α, β and 6 (IL-1α, IL-1β and IL-6), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) are examples of pro-inflammatory cytokines that are involved in the crosstalk between peripheral immune function and the brain (Brebner, Hayley, Zacharko, Merali, & Anisman, 2000).

A growing body of evidence has demonstrated an important link between pro-inflammatory activity and psychiatric symptoms. Additionally, studies have also shown that patients with different inflammatory diseases have greater probability to exhibit

psychiatric symptoms, being inflammatory abnormalities frequently observed in several psychiatric disorders, such as schizophrenia or bipolar disorder (Drexhage, Knijff, Padmos, Heul-Nieuwenhuijzen, Beumer, Versnel, & Drexhage, 2010), with strong evidence especially linking them to depression (Capuron, Miller, & Irwin, 2007). Meta-analyses have reported that depressed patients have elevated levels of pro-inflammatory cytokines including IL-1β, IL-6 and TNFα circulating peripherally (Dowlati, Herrmann, Swardfager, Liu, Sham, Reim, & Lanctôt, 2010; Howren, Lamkin, & Suls, 2009; Liu, Ho, & Mak, A, 2012). IL-6 has been the most consistently elevated cytokine in depressed patients and the presence of elevated IL-6 and C-reactive protein (CRP) can predict a subsequent development of depression over the course of a decade even in individuals with no history of depression when samples were taken (Gimeno et al., 2009). Furthermore, there is also evidence demonstrating that patients with increased levels of pro-inflammatory cytokines due to a variety of medical diseases have increased rates of depression compared to the general population (Yirmiya et al., 2000). On the other hand, some patients with major depression but without co-morbid conditions also exhibit increased circulating levels of pro-inflammatory cytokines (Dowlati et al., 2010). Moreover, at least some types of depression may be causally mediated by chronic increases in pro-inflammatory cytokines (Raison & Miller, 2011), affecting brain function and leading to the expression of a set of depressive-like behaviour, including fever, fatigue, anhedonia, and decreased activity. Evidence has shown that the peripheral administration of cytokines, such as IL-1β or TNF-α, or their inducers (for example LPS) both in animals (Bay-Richter, Janelidze, Hallberg, & Brundin, 2011) and humans (Bonaccorso et al., 2002; Harrison, Brydon, Walker, Gray, Steptoe, & Critchley, 2009) produces neuropsychiatric symptoms and behavioural alterations consistent with depression, including decreased motor activity, reduced food and water intake, increased

slow-wave sleep, withdrawal from the physical and social activities, and altered cognition. Additionally, evidence linking altered levels of IFN-γ and suicide (Gabbay et al., 2009; Janelidze, Mattei, Westrin, Traskman-Bendz, & Brundin, 2011), including in the adolescent population (Gabbay et al., 2009), reinforces the potential role of these cytokines in emotional and behavioural functioning.

Evidence linking abnormalities in immune function and emotional and behavioural symptoms in children is far more limited. A recent review on the cytokines and depressive symptoms in pediatric populations (Mills et al., 2013), has had some difficulties in summarizing pediatric research findings, that contrasts with the existence of meta-analysis of the adult literature. Nonetheless, it is clear that a number of specific and plausible mechanisms throughout dysregulation of the immune system may be connected with emotional and behavioural symptoms in childhood.

Numerous pathophysiologic mechanisms involved in psychopathology and specifically in depression phenotype have been demonstrated to be associated with pro-inflammatory cytokines (Haroon et al., 2012; Miller et al., 2009; Mills, Scott, Wray, Cohen-Woods & Baune, 2013; Pace, Hu, & Miller, 2007). The elucidation of these mechanisms is of interest and includes cytokine effects on neurotransmitter metabolism, neuroendocrine function and neuronal plasticity. Such mechanisms have been reviewed extensively elsewhere (Anisman, 2009; Miller et al., 2009). From these mechanisms, considerable attention has recently been paid to the ability of pro-inflammatory cytokines such as IFN-γ to enhance the activity of the enzyme indoleamine-2,3-dioxygenase (IDO). IDO breaks down tryptophan, the primary amino acid of serotonin synthesis, into kynurenine. The breakdown of tryptophan is believed to contribute to reduced serotonin availability and increased kynurenine levels. The degradation of tryptophan into kynurenine and related metabolites such as quinolinic (QUIN) and kynurenic (KYNA)

acids with effects at the *N* -methyl- D -aspartate (NMDA) receptors could account for changes in serotonin transmission which promote the emergence of depression related behaviours (Muller & Schwarz, 2007). In support of this, administration of kynurenine to mice induces depression-like behaviour in a dose dependent manner (O'Connor et al., 2009). Additionally, in human studies correlations between depression-like behaviour, decreased plasma tryptophan, and increased KYN and/or the KYN/tryptophan ratio, have been reported (Bonaccorso et al., 2002), suggesting that the up-regulation of TRP-KYN pathway is one of the putative mechanisms linking pro-inflammatory cytokines to the onset of depression-like behaviours (Oxenkrug, Turski, Zgrajka, Weinstock, Ruthazer & Summergrad, 2014).

Due to the ability of pro-inflammatory cytokines to activate the hypothalamic pituitary adrenal (HPA) axis (Pace et al., 2007), non-serotonergic mechanisms such as hyperactivity of the HPA axis may also be significant in mediating depression-like behaviour and inflammatory activity. A recent study has shown that depressed patients have lower expression of glucocorticoid receptor (GR) which is strongly correlated with increased levels of pro-inflammatory cytokines (Carvalho, Bergink, Sumaski, Wijkhuijs, Hoogendijk, Birkenhager, & Drexhage, 2014). Furthermore, depressed patients show elevated levels of corticotrophin releasing hormone (CRH), a hypothalamic neuropeptide under the regulation of glucocorticoids that drives HPA axis activation and plays an important central role in behavioural, endocrine and immune responses to stress (Pace et al., 2007).

It is also of significance that immunological stimulation with agents such as LPS provokes a reduction in the expression of brain derived neurotrophic factor (BDNF) and in neurogenesis in the hippocampus (Ormerod, Hanft, Asokan, Haditsch, Lee, & Palmer, 2013). Such reports of reduced BDNF expression and neurogenesis may contribute to

reductions in hippocampal volume as indicated in numerous neuroimaging studies in depressed patients (Chen, Dowlatshahi, MacQueen, Wang & Young, 2001). Moreover, it is proposed that reduced hippocampal volume may reflect neuronal atrophy being related to memory and social learning deficits (Omizzolo et al., 2013).

Furthermore, and supporting the relationship between cytokines and psychiatric conditions several single-nucleotide polymorphisms (SNPs) of functional allelic variants of genes encoding inflammatory molecules have been identified in association with depression-like phenotypes (Bufalino, Hepgul, Aguglia & Pariante, 2012; Raison & Miller, 2013), as it is the case of INF-γ, a pro-inflammatory cytokine encoded by the *IFNG* gene.

In vitro studies have reported an association between the *IFNG* gene SNP in position +874 (rs2430561; T/A) and cytokine production. More specifically, AA genotype has been associated with low INF-γ production, AT with intermediate production and TT with high production of this pro-inflammatory cytokine (Matos et al., 2007). Particularly relevant for depressive phenotype is evidence showing that the distribution of *IFNG* (+874) genotypes was different between depressed and not depressed subjects, with higher frequency of T carriers among depressed subjects than among not depressed subjects, suggesting that the presence of high producer (T) allele might be a genetic risk factor for the development of depression (Oxenkrug, 2011). More recently, a study focused on personality traits also found an effect of T allele in relation with low extraversion (MacMurray et al., 2014). However, in another study, no differences were found regarding genotype or allele distribution of the *INFG* (+874) in major depression patients (Clerici et al., 2009).

Considering the role of gene-environment interactions in psychiatric disorders, these inconsistent results found in the literature may be explained by the lack of a GxE

approach. In fact, more than purely genetic mechanisms, the effects of some SNPs may only become evident in the presence of specific environmental experiences. For instance, Tartter and colleagues (2015) found that a specific SNP of the *IL1β gene (*rs16944), associated with higher expression of this pro-inflammatory cytokine, was correlated with greater depressive symptoms in adulthood following interpersonal stress in childhood. On the other hand, Kovács and collaborators (2016) found that, in the same SNP, the minor (A) allele interacted with childhood adversity increasing depressive and anxiety symptoms. Even with the plausibility of these models, the interaction between genetic variants of immune system and environmental factors has not been considered yet in original studies including children, so little is known about the effects of the interaction between early experiences and specific cytokine genes. Although interesting, all these models addressing adult psychopathology rely on retrospective data of early adversity; therefore, it might be important to understand if this GXE interaction already explain child psychopathology.

IV. THE PRESENT DOCTORAL DISSERTATION

Thus, and taking into account all of these theoretical and empirical review, the present doctoral dissertation aims to (i) determine the effect of family risks and maternal care and their action mechanisms on child's internalizing and externalizing problems in preschool age; (ii) review the literature on GxE research regarding the study of child's internalizing and externalizing problems is preschool age; and (iii) determine whether an immune-related gene interact with environment in the prediction of preschool internalizing problems.

The first aim is addressed in the first article entitled **Links between family risks and child's internalizing and externalizing problems in preschool context: maternal**

sensitivity and intrusiveness as specific mediators (Chapter 2), which explores the differential role of maternal sensitivity and maternal nonintrusiveness interactive behaviour in relation to children's internalizing and externalizing behaviours problems in preschool context, assessed by teachers. The pertinence of this article lies on the analysis of the different maternal interactive behaviours and in its differential effect on child functioning in a context beyond the familiar context.

The second aim is addressed in the second article entitled **Gene–environment interactions in psychopathology throughout early childhood: a systematic review** (Chapter 3) where studies using a G×E approach to examine children emotional and behavioural problems at preschool age were systematized in order to provide a critical overview on the current state of art. The relevance of this review is based on the fact that up until today no revisions have been made on this subject, despite the increase in the number of studies using GxE model.

The third aim is addressed in the third article of this dissertation entitled **Mothers' psychological distress and children's internalizing behaviour problems – a moderating role for the *IFNG* gene** (Chapter 4) where we examined whether the *IFNG* functional polymorphism (rs2430561) moderates the association between maternal psychological distress and children internalizing behaviours at preschool age.

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Chapter 2

LINKS BETWEEN FAMILY RISKS AND PRESCHOOLERS' EMOTIONAL/ BEHAVIOURAL FUNCTIONING: MATERNAL SENSITIVITY AND INTRUSIVENESS AS SPECIFIC MEDIATORS

A version of this Chapter is currently under review in Attachment & Human Development

CHAPTER 2

LINKS BETWEEN FAMILY RISKS AND PRESCHOOLERS' EMOTIONAL/BEHAVIOURAL FUNCTIONING: MATERNAL SENSITIVITY AND INTRUSIVENESS AS SPECIFIC MEDIATORS

I. INTRODUCTION

The preschool years represent a time of extensive psychological growth, with great sophistication of mental processes underlying cognition and socioemotional development (Troller-Renfree & Fox, 2017). In fact, the preschool context is critical for the development of socioemotional skills that play an important role in other important milestones, being directly linked to school readiness (Baptista, Osório, Martins, Veríssimo, & Martins, 2016) and academic achievement in formal school (Hair, Halle, Terry-Humen, Lavelle, & Calkins, 2006). In this context, socioemotional skills, including emotion understanding, social problem-solving skills, and positive social behaviour, promote peer acceptance, positive relationships with teachers and learning processes (e.g., Denham, 2006; Downer, Sabol, & Hamre, 2010; Garner & Waajid, 2008). On the other hand, low socioemotional competence, translated into internalizing and externalizing behaviour problems are linked with more difficulties in school readiness (Doctoroff, Greer, & Arnold, 2006; Brennan, Shaw, Dishion, & Wilson, 2012), and academic difficulties in school age (see meta-analysis in Riglin, Petrides, Frederickson, & Rice, 2014). Thus, understanding the mechanisms that contribute to early internalizing and externalizing problems in preschool context is crucial to identify risk and protective markers at this developmental stage.

One of the contexts where it is important to look at these markers is the family context. In fact, it is well established that children facing multiple family risks fare less

well developmentally in a wide variety of areas, being disproportionately affected by internalizing and externalizing problems (Bradley & Corwyn, 2002; Goodnight et al., 2012).

Within the family context, several risk factors to children's emotional/behavioural functioning have been identified, including economic disadvantage (Conger, Conger, & Martin, 2010), absence of social support (Taylor, Conger, Robins, & Widaman, 2015), low parental education (Carter et al., 2010), marital conflict (Zimet & Jacob, 2001), and poor parental mental or physical health (Mensah & Kierman, 2010). Nevertheless, little is known regarding the specific mechanisms through which family adversity impacts on the development of child's psychopathology (Zeanah & Sonuga-Barke, 2016).

It has been suggested that one important way in which the family context influences children's emotional/behavioural functioning is through parenting. Family stress model (Conger & Conger, 2002; Conger et al., 2010) is a useful framework for understanding the processes by which family risks exacerbate the child's difficulties through parenting. According to this model, family adversities deplete the psychological and relational resources of parents, resulting in negative parenting behaviour (Masarik & Conger, 2017). Indeed, several studies provide support to the hypothesis that low quality parenting practices are prospectively linked to internalizing and externalizing problems in early childhood (Neppl, Senia, & Donnellan, 2015), as well as problems with preschoolers' literacy and math performance (Iruka, LaForett, & Odom, 2012). For example, mothers reporting elevated levels of contextual stress were observed to be more negative, intrusive, and hostile toward children's attempts at autonomy and social behaviour, and less sensitive and responsive in their face-to-face interactions (Campbell, Mastetic, von Stauffenberg, Mohan, & Kirchener, 2007; Emmen et al., 2013; Newland, Crnic, Cox, & Mills-Koonce, 2013).

In accordance, studies have evinced the relevant role of parenting as a mediator in the relations between family risks and child emotional/behavioural functioning. For instance, previous research has shown an indirect effect of family risk on externalizing and internalizing problems through non-nurturant and less involved parenting (Trentacosta et al., 2008) and through parenting stress and harsh disciplining (Rijlaarsdam et al., 2013). There is also evidence demonstrating that family risk has a negative influence on children's preschool achievement, self-regulatory skills, and social behaviour through the parental warmth and responsiveness (Mistry, Benner, Biesanz, & Howes, 2010).

Despite these evidences on the mediating role of parental behaviours, less attention has been given to the specific effect of distinct parenting behaviours, which have varied considerably between studies (O'Connor, 2002). Considering the limitations of using more global constructs (Turiel, 2010), the models of child psychopathology that have emerged recently owe much to research concerned with "unpacking" the various dimensions that comprise broader parenting dimensions (Hawes, 2017). Indeed, process models and evidence-based interventions have focused on specific – and not broad – parent-child behaviours (Dishion & Stormshak, 2007).

Following this rational, the present inquiry will focus two specific maternal interactive behaviours – sensitivity and intrusiveness.

Maternal interactive behaviours: differential role of sensitivity and intrusiveness

Sensitivity-insensitivity and cooperation-intrusiveness, as described by Ainsworth and colleagues (Ainsworth, Bell, & Stayton, 1974) have been considered as valuable contributions to the field of parenting and child development (Cassidy, 2016). However, there has been a tendency for research to focus exclusively on sensitivity

(Bernard, Meade, & Dozier, 2013) or to create a composite measure in which both behaviours are considered (e.g., Luijik et al., 2011). Indeed, little is known about the differential effect of these behaviours on the emotional/behavioural functioning of preschool children.

Maternal sensitivity is defined as the mother's ability to notice child signals, interpret these signals correctly, and respond to these signals promptly and appropriately (Ainsworth et al., 1974). It is a well-recognized predictor of attachment behaviour (de Wolff & Van IJzendoorn, 1997), especially at very early ages. Recent work on sensitivity has found links to a large variety of developmental outcomes, namely with internalizing and externalizing behaviour problems in preschool age (e.g., Kok et al., 2013) and with externalizing problems during childhood and adolescence (e.g., Garai et al., 2009).

On the other hand, maternal intrusiveness, defined as an exertion of parental control over the child that preempts the development of child autonomy (Ainsworth et al., 1974) has also an important impact at this developmental stage. There is evidence suggesting that controlling parenting behaviours, such as noncontingent physical behaviour or verbal directives, limit the child's activities and foster passivity and inhibition (Clincy & Mills-Koonce, 2013). In fact, the lack of autonomy experienced by the child in this kind of interactions with the parent hinders his/her assertiveness in establishing positive relationships with peers (Clark & Ladd, 2000), and tends to promote inhibition and anxiety (Negreiros & Miller, 2014).

The quality of parent-child interactions and the family environment play key roles in social-emotional development, including school adjustment (Lengua, Honorado, & Bush, 2007). However, very few studies have addressed how parenting behaviours are linked to children's emotional/behavioural functioning in other contexts beyond family, such as preschool. In fact, preschool is one of the first extra-family contexts that the child

needs to adapt, being an interesting marker of socioemotional development. Research suggests that teachers' perceptions of children's emotional/behavioural functioning may be of particular interest for understanding preschool experience and subsequent school adjustment (Baker, Tichovolsky, Kupersmidt, Voegler-Lee, & Arnold, 2014). Indeed, in addition to their privilege in observing the child in the classroom and at the playground, practicing emotional and behavioural skills, teachers also have the ability to compare a particular child with other children of the same age and developmental level.

Following this empirical evidence, by using a cross-sectional design, the present study aimed to examine whether the link between family risk and children's adjustment in the preschool evaluated by the teacher was differentially mediated by maternal sensitivity and intrusiveness. More specifically, it was hypothesized that sensitivity would have a more prominent effect on externalizing problems, since maternal behaviours marked by lack of ability to respond appropriately and promptly to signals of the child may foster overactivity and impulsivity as well as defiant and oppositional behaviour (e.g., Wang, Christ, Mills-Koonce, Garrett-Peters, & Cox, 2013). Additionally, it was expected that maternal intrusive behaviour would have a more evident effect on internalizing problems, considering previous reports on the association between parental control over the child, through physical or verbal directives, and the child's withdrawn behaviour (e.g., Clincy & Mills-Koonce, 2013).

II. METHODS

PARTICIPANTS

Participants included 205 children (113 girls, 54.3%) and their biological mothers as well as their preschool teachers. At the time of assessment, the mean age of the children was 58 months (M = 58.12, SD = 7.63). Exclusion criteria for participants were prior institutionalization, physical growth below the 5th percentile, and the presence of mental or physical impairments, genetic syndromes or autism spectrum disorders.

The age of the child at admission in the preschool ranged from 3 to 60 months (M = 24.19, SD = 14.86). Most children were Caucasian (98.6%). Mothers were aged from 20 to 48 years (M = 33.28, SD = 5.70), all being the primary caregiver, most of them (53.4%) had less than secondary education, 26.5% were unemployed, and 18.3% were single.

PROCEDURE

The participants were recruited from 34 Portuguese preschool classrooms. Firstly, parents and teachers were explained the purposes of the study, as well as the detailed procedure, and gave their written informed consent for their children's and their own participation. Two trained experimenters administered the tasks. Data collection was carried out in the preschool setting or at the child's home, according to mother's preference.

MEASURES

Children's internalizing and externalizing problems

To assess children's internalizing and externalizing problems, teacher-reports were collected using the Portuguese version of the Caregiver Teacher Report Form for ages 1 ½-5 (CTRF; Achenbach & Rescorla, 2000; Achenbach et al., 2014). The CTRF consists of 100 items that describe emotional/behavioural difficulties, which are rated by

teachers on a 3-point-scale (0 = not true, 1 = sometimes/somewhat true or 2 = very/frequently true), with well-established psychometric properties (Achenbach et al., 2014). Internalizing and externalizing scales were used in the present inquiry. Their Chronbach Alphas were .914 and .878, respectively.

Family risks

A socio-demographic questionnaire based on risk research (e.g., Weitzman, Edmonds, Davagnino & Briggs-Cowan, 2014) was completed by mothers, and a family risk index was created based on nine indicators: teenage pregnancy, single parenthood, low parental education, household overcrowding, parental unemployment, economic social assistance, parental chronic health conditions, absence of social support, and unstable or violent marital relationship. Each indicator was scored '1' for presence and '0' for absence. Descriptions of each indicator and percentage are presented in Table 2.1. The items were summed, with higher scores reflecting higher family risk (Min = 0, Max = 9).

Table 2.1. Risk indicators, descriptions, and percentage meeting criteria.

Indicator	Description of criteria	%
Teen parent	Under 18 years of age at first child's birth	4.9%
Low education	At least one parent did not complete 9 years of school	43.1%
Single motherhood	Mother as a single adult in the home	18.5%
Overcrowding	Fewer rooms than people	14.1%
Parental unemployment	At least one parent does not have a job	36.1%
Economic social assistance	Family receive an economic assistance from Portuguese government (RSI)	9.8%
No social support	Parents does not have support from family and friends	8.8%
Parental chronic health conditions	At least one parent suffered from a chronic disease	42.6%
Unstable/violent marital relationship	Mother characterized the marital relationship as unstable or with episodes of violence	13.2%

Maternal interactive behaviours

The Sensitivity-Insensitivity and Cooperation-Intrusiveness subscales of the Maternal Care Scales (Ainsworth et al., 1974) adapted to the preschool years, were used to assess mother's sensitive and intrusive behaviours in interaction with the child, during a 15-min videotaped task, divided into three episodes: (1) child plays with a challenging toy with the mother's guidance (5 min); (2) researcher provides child with uninteresting toy while placing more interesting ones out of reach, but in view, with mother directed to complete a (sham) questionnaire while preventing him/her from contacting the interesting toys (5 min); (3) child–mother play with previous out-of-reach toys (2.5 min), followed by a clean-up task for the child (2.5 min). The majority of the dyads carried out this task

at the preschool setting, and the others (23.6%) at the family's home. No group differences were found across the two settings (home vs. preschool) regarding sensitivity ($t_{(203)}$ = .287, p = .775) or intrusiveness ($t_{(203}$ = .404, p = .687). The scales were rated by highly trained coders who were blind to the data included in this inquiry. Intraclass correlation for intercoder reliability was .87 for sensitivity and .77 for intrusiveness. The two scales were highly correlated (r = .73, p < .001).

Mental development

Mental development was assessed using the Griffith's Mental Development Scales (Griffiths, 1984), evaluating six dimensions: motor, personal and social, hearing/language, eye and hand coordination, performance and practical reasoning. A global development score was calculated by averaging the six dimensions, with all children presenting a mental developmental within the mean (i.e., ≥ 80; M = 106.92, SD = 10.91).

Length of teacher-child relationship

The length of teacher-child relationship, in terms of how many months the teacher knew the child, was considered as a control variable.

Data Analytic strategy

Firstly, descriptive statistics and bivariate correlations among study variables were examined. Then, two separate regression analysis were run to predict internalizing and externalizing problems, focusing on the contribution of family risk and maternal interactive behaviour: one based on insensitivity, the other on intrusiveness.

To examine the mediation hypothesis, the causal-steps method (Baron & Kenny, 1986) was used separately for maternal insensitivity and intrusiveness. The causal-steps approach tests (i) whether the effect of the predictor (family risk) on the dependent variable (internalizing and externalizing problems) is significant (path c); (ii) whether the effect of the predictor on the mediator (maternal insensitivity and intrusiveness) is significant (path α); (iii) whether the effect of the mediator on the dependent variable is significant (path β); and (iv) whether the effect of the predictor on the dependent variable, while controlling for the mediator, is smaller (path c′) than the total effect of the predictor on the dependent variable (path c) (Fig. 2.1).

III. RESULTS

DESCRIPTIVE RESULTS

Descriptive statistics and bivariate correlations among study variables can be found in Tables 2.2 and 2.3, respectively. The mean for internalizing problems was 6.30 (*SD* = 6.61), with 6.8% (*n* = 14) of the children scoring within the clinical range (*S*core of ≥17; Achenbach et al., 2014). The mean for externalizing problems was 7.43 (*SD* = 8.31), with 2.9% (*n* = 6) of the children scoring within the clinical range (*S*core of ≥29; Achenbach et al., 2014). A *t*-test revealed a significantly higher score for boys in externalizing problems (*M* = 9.49, *SD* = 9.60), in comparison to girls (*M* = 5.69, *SD* = 6.60), (*t* (203) = -3.34, *p* = .001). Children who demonstrated higher levels of internalizing and externalizing problems had lower levels of mental development. No significant associations were found between internalizing and externalizing problems and child age or length of the teacher-child relationship. All correlations between the predictors and internalizing and externalizing problems were significant.

Table 2.2. Descriptive statistics

	M	*SD*	Min.	Max.
Child age in months	58.01	7.62	40	77
Child mental development	106.92	10.91	80.05	138.89
Length of the child-teacher relationship[a]	19.79	12.40	2	64
Family risk	1.92	1.49	0	6
Maternal interactive behaviours				
Insensitivity	5.52	1.69	2	9
Intrusiveness	5.29	1.61	1	9
Child problems				
Internalizing problems	6.30	6.31	0	43
Externalizing problems	7.43	8.31	0	49

[a] Data available only for 146 participants;

Table 2.3. Bivariate correlations between variables

	1.	2.	3.	4.	5.	6.	7.
1. Child age in months							
2. Child mental development	-.18*						
3. Length of the child-teacher relationship[a]	.14	.01					
4. Family risk	-.17*	-.26***	-.01				
5. Maternal insensitivity	-.01	-.36***	-.06	.31***			
6. Maternal intrusiveness	.00	-.27***	-.06	.28***	.73***		
7. Child internalizing problems	-.05	-.30***	-.06	.28***	.18**	.25***	
8. Child externalizing problems	-.02	-.37***	.01	.32***	.22**	.26***	.53***

[a] Data available only for 146 participants;

* $p < .05$.

**$p < .01$.

***$p < .001$.

PREDICTING INTERNALIZING PROBLEMS

In order to estimate the contribution of family risk and maternal interactive behaviours separately for insensitivity and intrusiveness, two hierarchical linear regressions were performed (Table 2.4). Given the significant associations with internalizing problems, child mental development was included in the first step of the

analysis as a control variable. Family risk was then entered in the second block of the model and proved to be a significant predictor (β = .22, t = 3.20, p = .002), explaining additional 4% of the variance. Finally, maternal interactive behaviours were entered in the final step of the analysis in separate models (Table 2.4, section *a* and *b*). Only maternal intrusiveness emerged as a significant predictor of child internalizing problems (β = .15, t = 2.17, p = .031), explaining additional 2% of the variance.

Table 2.4. Hierarchical regression for predicting Internalizing problems based on family risk and maternal insensitivity (a) and intrusiveness (b)

	B	**SE**	***β***	***t***
Block 1 R^2 = .09 (ΔR^2 = .08) F(1,201) = 18.79***				
Mental development	-.17	.40	-.29	-4.33***
Block 2 R^2 = .13 (ΔR^2 = .12) F(1,200) = 10.23**				
Mental development	-.14	.04	-.24	-3.52**
Family risk	.97	.30	.22	3.20**
a				
Block 3 R^2 = .13 (ΔR^2 = .12) F(1,199) = .46				
Mental development	-.13	-04	-.22	-3.13**
Family risk	.92	.31	.21	2.94**
Maternal insensitivity	.19	.29	.05	.68
b				
Block 3 R^2 = .15 (ΔR^2 = .14) F(1,199) = 4.70*				
Mental development	-.12	.04	-.21	-3.00**
Family risk	.82	.31	.18	2.65**
Maternal intrusiveness	.62	.29	.15	2.17*

* p < .05.
**p < .01.
***p < .001.

PREDICTING EXTERNALIZING PROBLEMS

Aiming to estimate the contribution of family risk and maternal interactive behaviours separately for insensitivity and intrusiveness, two hierarchical linear

regressions were performed (Table 2.5). Child sex and mental development were included in the first step of the analysis as covariates given the significant associations with externalizing problems. Family risk was then entered in the second block of the model and proved to be a significant predictor (β = .24, t = 3.62, p < .001), explaining additional 5% of the variance. Lastly, maternal interactive behaviours were entered in the final step of the analysis in separate models (Table 2.5, section *a* and *b*). Neither of the maternal interactive behaviours proved to be significant a predictor of externalizing problems according to the teacher's reports.

Table 2.5. Hierarchical regression for predicting Externalizing problems based on family risk and maternal insensitivity (a) and intrusiveness (b)

	B	**SE**	***β***	***t***
Block 1 R^2 = .16 (ΔR^2 =.15) F(2,200) = 19.30***				
Sex	3.08	1.09	.19	2.82**
Mental development	-.24	.05	-.33	-5.06***
Block 2 R^2 = .21 (ΔR^2 =.20) F(1,199) = 11.11***				
Sex	2.91	1.06	.17	2.74**
Mental development	-.20	.05	-2.28	-4.21***
Family risk	1.32	.36	.24	3.62***
a				
Block 3 R^2 = .21 (ΔR^2 =.20) F(1,198) = .18				
Sex	2.85	1.07	.17	2.66**
Mental development	-.20	.05	-.27	-389***
Family risk	1.28	.38	.23	3.41**
Maternal insensitivity	.15	.35	.03	.43
b				
Block 3 R^2 = .22 (ΔR^2 =.21) F(1,198) = 2.47				
Sex	2.66	1.07	.16	2.48*
Mental development	-.19	.05	-.26	-3.83***
Family risk	1.19	.37	.21	3.19**
Maternal intrusiveness	.55	.35	.11	1.57

* p < .05.
**p < .01.
***p < .001.

MEDIATION MODEL

The first three conditions required for a mediation were met, both for internalizing and externalizing problems as shown in Table 2.3. However, the fourth condition was only met in the case of maternal intrusiveness and child's internalizing problems. Therefore this was the only mediational model ran. Regression analysis showed that the effect of family risk on internalizing problems was reduced when controlled for the effect of maternal intrusiveness, Path c'; $\beta = .14$, $t = 2.17$, $p = .031$ (Table 2.4, Block 3b). Thus, as Figure 1 illustrates, maternal intrusiveness was identified as a mediator of the effect of family risk on child's internalizing problems, according to the teacher's reports.

Because the effect of family risk on internalizing problems remained significant, a formal significance test of the indirect effect was carried out, using Preacher and Hayes' (2004) bootstrapping methodology on 1,000 bootstrap resamples. The true indirect effect was estimated to lie between .40 and 1.52 (CI = 95%). As the confidence interval excluded zero, the indirect effect is significantly different from zero at a significance of.05 (two-tailed), meaning that maternal intrusiveness is indeed a mediator of the link between family risk and child's internalizing problems.

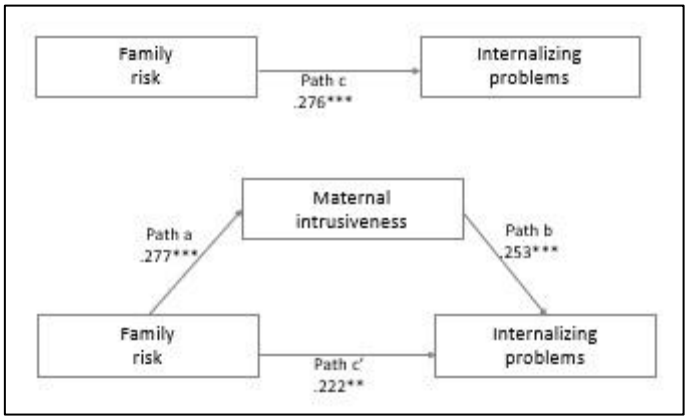

Figure 2.1. Maternal intrusiveness as a mediator of the relationship between family risk and internalizing problems. Values are standardized regression coefficients (β). R^2 change when maternal intrusiveness is added the model is 3,6%, p=,005.

IV. DISCUSSION

The present study aimed to extend the current understanding of the mechanisms by which family risk relate to internalizing and externalizing problems in the preschool context, by examining whether such relationship is differentially mediated by maternal insensitivity and intrusiveness. Firstly, the results showed that family risk and maternal insensitivity and intrusiveness were positively related to both children's internalizing and externalizing problems reported by preschool teachers. Thus, children who lived in a more adverse contexts, experiencing an accumulation of risk factors and, children who received more insensitive and intrusive care from their mothers displayed higher levels of internalizing and externalizing problems in preschool.

Regarding externalizing problems, our results revealed a unique contribution of family risk, meaning that, independently of maternal insensitive or intrusive behaviour, when family risk was higher, children tended to exhibit more externalizing behaviours. Conversely, internalizing problems were better explained by taking into account, not only family risk, but also maternal interactive behaviours. Specifically, our results show that maternal intrusiveness mediated the relation between family risk and children's internalizing behaviours.

Interestingly, maternal behaviour does not seem to be as relevant to children's externalizing problems as it is to internalizing problems. As previously mentioned, we found the effect of family risk on children's internalizing problems to be mediated by maternal intrusiveness; result which is in line with our hypothesis on the effect of maternal intrusive behaviour on children's internalizing problems. As evidenced by research, intrusive parenting, wherein parents excessively restrict children's engagement with situations or behaviours, has been associated most strongly with children's anxious-withdrawal symptoms (Rapee, Schniering, & Hudson, 2009). Some researchers attribute

the risk of these parenting behaviours to their direct implications on the autonomy of children (Hawes, 2017). The Vygotsky concept of "zone of proximal development" also helps us understand the preferential effect of the intrusiveness on children's internalizing problems. According to Vygostsky (1978), this zone represents an internal developmental process that represents the distance between a child's independent ability and a higher level of performance achieved with adult guidance, which is an essential feature of a child's socialization. Thus, children who encounter fewer opportunities to internalize regulatory strategies from mothers, likely develop smaller gains in self-regulation, which further compromises their ability to explore the environment (Kopp, 2009).

As we expected, there was a differential effect of maternal interactive behaviours, with a greater relevance of intrusiveness compared to sensitivity. A developmental framework allows us to hypothesize that maternal sensitivity is a structuring dimension in the first years of life, where the construction of the bonding takes place; however its effect decreases as children's age increases (see recent meta-analysis by Madignan et al., 2016).For example, the preschool context requires the acquisition of others competences, such as autonomy, emotional regulation abilities, and more advanced problem-solving strategies. That is, as other developmental tasks emerge, other parental dimensions, such as cooperative behaviour become more relevant.

In addition to reinforce the idea that internalizing and externalizing problems have different relational influences, our findings make evident that the interactive pattern in the mother-child dyad affects the child's functioning in preschool context, and highlight the differential role of the different maternal interactive behaviours. Such evidence suggests that interventions for internalizing symptoms that ignore specific maternal interactive behaviour may place children at risk of reinstating relationship dynamics that contribute to the maintenance of their symptoms. At present, evidence-based treatments

for internalizing problems place considerably less emphasis on parenting targets than those for externalizing problems (Hawes & Allen, 2016). Our study suggests the clinical relevance of parental training focused on cooperative interactive behaviours, giving the child opportunities for autonomy. A greater emphasis on such parenting behaviour before school entry may promote socioemotional competences, which may in turn improve school readiness and future adaptation (e.g., Landry, Smith, Swank, & Guttentag, 2008).

Limitations and future directions

There are some limitations to this report that should be addressed in future research. First, this study used a cross-sectional design, which limits the interpretation of the results, and does not allow for clear conclusions about directionality. Despite the advantage of considering teacher's perceptions of preschoolers' socioemotional adjustment, it did not consider alternative measures. Incorporating, for instance, observational measures could provide a more robust perspective of children's adaptation to the preschool setting. Additionally, it is important to note that there might be other factors contributing to children's internalizing and externalizing problems in preschool context. The quality of teacher-child relationship may be of particular relevance, as previous studies have found that a more positive relationship between teacher and student during the preschool period is linked to socioemotional adjustment in those and in the following years (Dobbs & Arnold, 2009; O'Connor, Dearing & Collins, 2011). In line with this, future studies should incorporate the putative contribution of the quality of preschool social environment when exploring the mechanisms underlying the links between family risk and children's socioemotional adjustment in the preschool setting.

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CHAPTER 3

GENE-ENVIRONMENT INTERACTIONS IN PSYCHOPATHOLOGY THROUGHOUT EARLY CHILDHOOD: A SYSTEMATIC REVIEW

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CHAPTER 3

GENE-ENVIRONMENT INTERACTIONS IN PSYCHOPATHOLOGY THROUGHOUT EARLY CHILDHOOD: A SYSTEMATIC REVIEW

I. INTRODUCTION

Globally, up to 20% of children and adolescents suffer from a mental health problem (World Health Organization, 2000). Epidemiological studies have shown that some psychopathological symptoms are present at an early age (Egger and Angold, 2006; Lavigne et al., 2009; Skovgaard, 2010) and could last throughout development into adulthood. In addition, longitudinal studies have suggested that there is continuity between preschool symptoms and problems later in life (Luby et al., 2009) and that the same cluster of symptoms already present in preschoolers is observed in older children and adolescents (Strickland et al., 2011). The early emergence and persistence of the symptoms of mental health problems have an obviously dramatic individual and social impact and clearly underscore the relevance of studying children's psychopathology. Understanding the process by which psychopathological disturbances emerge and develop over time has important implications for treatment and prevention.

In the past decades, the study of psychopathology has focused on its multifactorial nature, accounting for the individual variability in the expression of different traits. A multidisciplinary perspective that considers the interaction between environmental factors and individual characteristics (i.e. genetic characteristics) has been adopted to better understand the etiological mechanisms of psychopathology.

Twin studies are among the most traditional approaches and constitute the first step for establishing the genetic influence on a child's psychopathology (Rutter, 2011). However, more recently, rather than estimate heritability (overall genetic contribution), researchers have been focusing on the study of specific variation in DNA sequences that, in the presence of specific environmental factors, would contribute toward the development of psychopathology (Rutter et al., 2006). This gene-environment interaction (G× E) occurs when the relationship between an environmental experience (e.g. stressful experience; enriched environment) and the emergence of a phenotype (e.g. psychopathology, better immune function) is contingent with individual differences in genetic makeup, or, conversely, when the effect of an individual genotype on a phenotype is conditional on an environmental experience (Moffitt et al., 2006).

According to this paradigm, empirical evidence with replication (e.g. Caspi et al., 2002, 2003; Eley et al., 2004; Foley et al., 2004; Kaufman et al., 2004; Caspi et al., 2005; Grabe et al., 2005; Kendler et al., 2005; Wilhelm et al., 2006) has shown that individual genetic variations can lead to a distinct impact of environmental experiences in the development of psychopathology. These findings have promoted the robustness of the G×E hypothesis in the study of psychopathological determinants, leading to an exponential increase in the number of studies in this area. However, more than one decade has passed since the first reports, and given the inconsistencies in the findings, some questions remain on the specific genetic markers that are involved in the emergence of psychopathology. Are they acting individually or synergistically? What is the best-fitting model (diathesis-stress vs. differential susceptibility) for explaining G×E for children's psychopathology?

Considering all this empirical background, the present literature review aims to systematize the information on G×E studies focused on psychopathology in early

childhood. The aim of this review is to survey the published literature with respect to studies' research designs to provide a critical overview of the etiological factors.

II. METHODS

The search for articles was performed through the PubMed and PsycArticles databases from their inception through 2 April 2015, with the keywords 'child' and 'psychopathology', 'psychopathology symptoms', 'internalizing problems', 'externalizing problems', 'behavioural problems', 'affective problems', 'emotional problems', 'behavioural disorders', 'emotional disorders' or 'affective disorders' and 'gene', 'interaction' and 'environment' or 'parenting' or 'maternal behaviours' in the title or abstract.

We also searched for articles by examining the references of review articles, meta-analyses, and other empirical studies using the same index terms located in the title. The search was limited to scientific articles published in English. Figure 3.1 shows the details of the search (PRISMA flow diagram; see Liberati et al., 2009). Through this search, we located 1168 studies. To identify the articles for our review, we applied the following criteria: (a) psychopathological symptoms as an outcome; (b) participants younger than 6 years old; and (c) studies with candidate genes. Some studies assessed children's psychopathology after 6 years of age, and considering our cut-off point, we ignored the data for those ages. We excluded studies focused on genetic neurodevelopmental disorders, such as autism spectrum disorders, given the influence of genetic factors in its etiology. Two authors performed the search process independently. After applying these criteria, 14 studies remained, which were published between 2006 and 2015. For the purpose of describing the current state of the art of G×E research, we summarize the

research designs and samples, measurements of psychopathology, genotype and environment variables used in the models, and the main findings. Table 3.1 presents a detailed description of the studies included in this review.

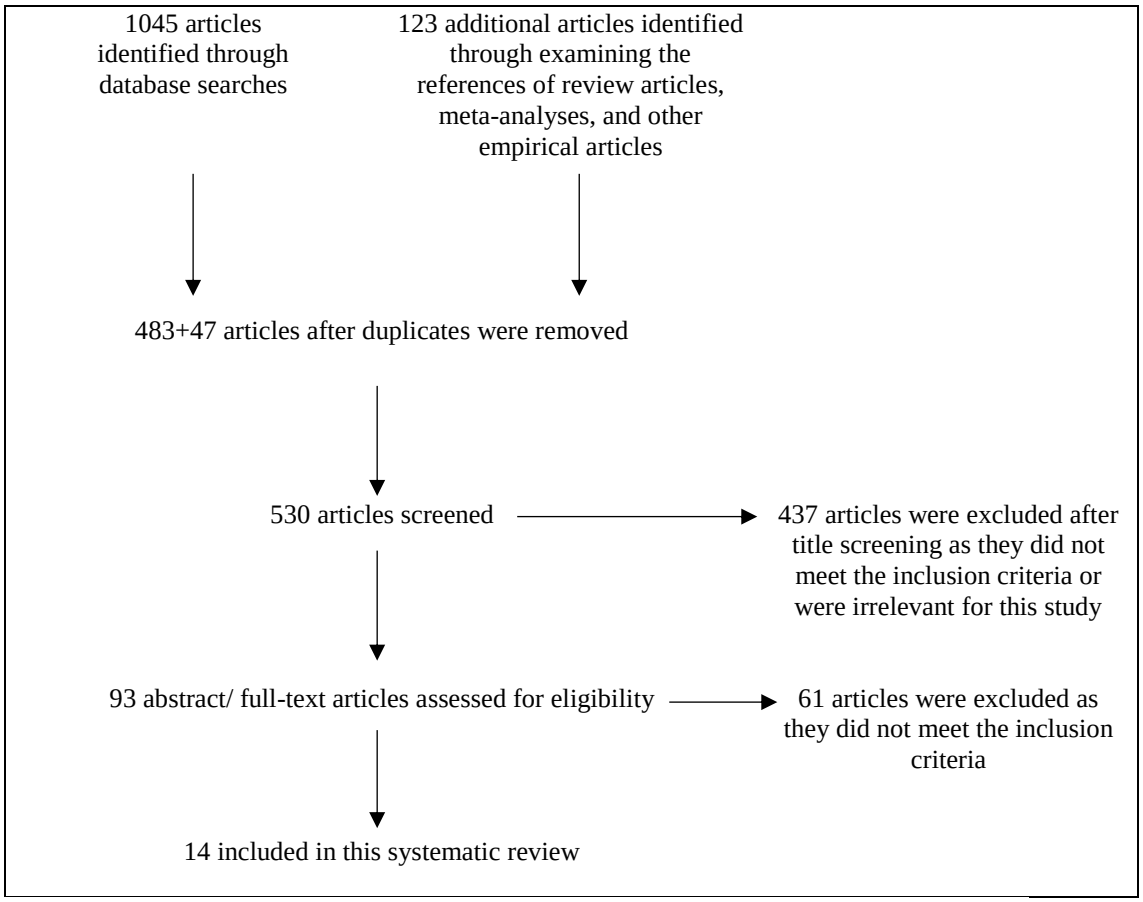

Figure 3.1. PRISMA flow diagram of the literature review and selection process

TABLE 3.1 Description of gene–environment interaction studies

REFERENCES	SAMPLE (N)	RACIAL CHARACTERISTICS	GENE	ENVIRONMENTAL CONDITIONS	PSYCHOLOGICAL SYMPTOMS	MAIN FINDINGS
Bakermans-Kranenburg & van IJzendoorn (2006)	47	No information	DRD4	Parental interactive behaviours	Internalizing problems Externalizing problems	GxE effect
Belsky & Pluess (2013)	508	WHITE	5-HTTLPR DRD4	Child care quantity, quality, and type	Externalizing problems	GxE effect E effect
Berry et al. (2013)	711	MIXED	DRD4	Parental interactive behaviours	Externalizing problems	E effect
DiLalla et al. (2009)	62	MIXED	DRD4	Parental interactive behaviours	Externalizing problems	GxE effect
Enoch et al. (2010)	6129	WHITE	MAO-A	Family adversity Family stressful life events	Externalizing problems	GxE effect E effect G effect
Hayden et al. (2010)	473	MIXED	DRD2	Parental interactive behaviours	Internalizing problems	GxE effect
Lavigne et al. (2013)	175	NON-HISPANIC WHITE	DRD4 5-HTTLPR MAO-A	Socioeconomic risk Familial stress Familial conflict Maternal psychopathology Parental interactive behaviours	Internalizing problems Externalizing problems	GxE effect
Mills-Koonce et al. (2007)	172	MIXED	DRD2	Parental interactive behaviours	Internalizing and externalizing problems	GxE effect
Propper et al. (2007)	169	MIXED	DRD4	Parental interactive behaviours	Internalizing behaviours Externalizing behaviours	GxE effect E effect
Sulik et al. (2012)	138	MIXED	LPR STIN2 HAPLOTYPE	Parental interactive behaviours	Externalizing behaviours	GxE effect E effect G effect
Tiemeier et al. (2012)	2136	MIXED	5-HTTLPR	Life difficulties Parental anxiety	Internalizing behaviours	GxE effect E effect
Velders et al. (2012)	1727	WHITE	GR FKBP5	Parental psychopathology	Internalizing and externalizing problems	GxE effect E effect
Willoughby et al. (2013)	171	MIXED	BDNF	Parental interactive behaviours	Externalizing behaviours	GxE effect G effect
Windhorst et al. (2015)	548	WHITE	DRD4	Parental interactive behaviours	Externalizing behaviours	GxE effect E effect

III. RESULTS

Research designs and studies samples

The research designs used to test G×E varied across the studies. Most studies (n =12) used a longitudinal design, where the associations between the environmental exposure and outcome were prospective. All studies used home-reared samples, with sample sizes that varied from 47 participants to 6129 participants. All studies included mixed samples with respect to sex; however, one study carried out an independent analysis by sex (Enoch et al., 2010). The studies varied in racial and ethnic diversity, with most studies (n =8) including mixed samples. Children's psychopathology assessed through phenotypes also varied in the measures that were used and the age ranges varied from 18 months to 5 years old. Six studies may have shared the sample because they belong to the two main longitudinal research datasets in the field.

Assessment of psychopathology

Children's psychopathology was assessed according to a dimensional approach as opposed to a categorical approach. On the basis of a dimensional perspective, the majority used the Achenbach System of Empirically Based Assessment scored through the Child Behaviour Checklist and the Caregiver-Teacher Report Form. The externalizing problems were more often the focus of inquiry than the internalizing problems, and most studies (n= 12) examined psychopathology by considering the internalizing and externalizing problems separately. The instruments to assess psychopathology included scales, clinical standardized interviews, and observation. All studies used questionnaires; nevertheless, some studies (n= 3) combined questionnaires with observation measures (DiLalla et al., 2009) or clinical interviews (Lavigne et al., 2013). The majority of the studies (n= 11) used only one instrument to assess psychopathology. With the exception of clinical

interviews (Diagnostic Interview Schedule for Children – Young Child) and observational measures, which were conducted and coded by the researchers, all of the other assessments were based on self-report questionnaires administered to the child's caregiver (i.e. parent, nonparent, or preschool teacher) and only one study assessed used two informants (Velders et al., 2012).

Assessment of genotype

In all of the studies, the child's genotype was assessed on the basis of a selection of specific polymorphisms, with the exception of Sulik et al.'s (2012) study, which carried out a haplotype analysis, combining two different polymorphisms of the SLC6A4 gene (5-HTTLPR and STin2 polymorphisms). Among the genotypes, there was a prevalence of candidate genes integrated in dopaminergic and serotonergic systems. Furthermore, two studies focused on polymorphisms in the BDNF gene (Willoughby et al., 2013) as well as in genes implicated in the regulation of the hypothalamic–pituitary–adrenal axis, such as the glucocorticoid receptor (GR) and FKBP5 genes (Velders et al., 2012). In addition, it should be noted that most of the studies failed to properly report the genetic data, namely, by providing information on the Hardy–Weinberg equilibrium of the allelic frequencies. According to Little et al. (2009), this lack of information can lead to significant bias in results.

Assessment of environment

The environmental variables varied across the studies, namely, with respect to the type of experiences, time point assessments, and quality of the measures used. All studies focused on environmental postnatal experiences; however, three studies extended the analysis to the prenatal period (Enoch et al., 2010; Tiemeier et al., 2012; Velders et al.,

2012). The environmental experiences also ranged from transitory exposure, such as maternal depressive symptoms or stressful life events, to more potentially long exposures, such as family socioeconomic status or quality of parental interactive behaviours. The measurement of those experiences included the administration of scales and observation procedures. Despite this, most studies (n =10) used only one measure to assess environmental experience. Across the studies, we can distinguish different levels of environment experiences, operating from a more proximal to a more distal level: (a) parental interactive behaviours, (b) parental psychopathological symptoms, (c) familial stress and adversity, (d) familial socioeconomic context, and (e) nonfamilial childcare. Most environmental experiences analyzed across the studies were related to parental influence (n=13), namely, through parental interactive behaviours and parental psychopathology symptoms. Parental interactive behaviours were assessed by 10 studies; however, the conceptualization of this experience varied among the studies, considering different specificities of interactive behaviour, such as sensitivity (Bakermans-Kranenburg & van IJzendoorn, 2006; Mills-Koonce et al., 2007; DiLalla et al., 2009; Berry et al., 2013; Willoughby et al., 2013; Windhorst et al., 2015), supportive presence (Hayden et al., 2010), warm-responsive behaviours (Propper et al., 2007), hostility (Lavigne et al., 2013), and harsh and intrusive behaviours (Propper et al., 2007; Willoughby et al., 2013). In contrast, parental psychopathological symptoms were assessed by three studies that were focused on depressive (Lavigne et al., 2013) and anxiety symptoms (Tiemeier et al., 2012) and in terms of a broad spectrum of psychological symptoms (Velders et al., 2012). Familial stress and adversity were considered by three studies (Enoch et al., 2010; Tiemeier et al., 2012; Lavigne et al., 2013) using composite measures of stress experience: familial conflict, chronic difficulties, and life events. One study analyzed familial socioeconomic status (Lavigne

et al., 2013), and it integrated information on parental education and employment. The last level of analysis is related to nonfamilial childcare, which was also examined by one study (Belsky and Pluess, 2013), specifically in terms of type, quantity, and quality.

Main findings

We will summarize the main findings in terms of the interaction effect (G×E), main effect of genotype, and main effect of environment.

Interaction effect (G×E)

The majority of studies (n =12) found at least one significant G×E effect ($p \leq 0.05$) in the prediction of children's psychopathology.

Dopamine receptors

Across the studies that we reviewed, the dopamine receptor 4 gene (DRD4) was found to predict externalizing problems during early ages. This predicting effect occurred only in interactions with some environmental experiences because no studies identified any main effects of the DRD4 gene. DRD4 has been described as a highly polymorphic gene, because of the 48-bp VNTR in the third exon, a region responsible for encoding a third intracellular loop of the protein receptor. This VNTR can vary from 2 to 11 repeats (2R–11R), leading to different functional activities of the D4 receptor (van Tol et al., 1992). Although the exact number of copies that are critical for adequate function of the protein is unknown, some research has suggested that it is the 7R isoform that has an inferior ability to reduce cyclic AMP because of an impaired activation of adenylyl cyclase, even when compared with shorter forms (Oak et al., 2000). Thus, studies included in the present review examined the influence of polymorphisms in the DRD4

gene using different approaches. Specifically, five studies addressed the role of DRD4 polymorphisms contrasting the presence versus the absence of the seven-repeat (7R) allele (Bakermans-Kranenburg and van IJzendoorn, 2006; Belsky and Pluess, 2013; Berry et al., 2013; Lavigne et al., 2013; Windhorst et al., 2015), whereas others examined the short versus long alleles. Specifically, DiLalla et al. (2009) considered two, three, or four repeats as the short allele and six to eight repeats as the long allele; Propper et al. (2007) considered the short allele as having two to six repeats and the long allele as having seven or more repeats. Thus, externalizing problems at different developmental time-points (i.e. 18, 36, 39, 54, and 5 years old) were predicted by the interaction between the DRD4 gene and parental sensitivity (Bakermans-Kranenburg & van IJzendoorn, 2006; DiLalla et al., 2009; Windhorst et al., 2015) or nonfamilial childcare quality (Belsky and Pluess, 2013). In addition, one study also reported a marginally significant interaction between the DRD4 gene and parenting on externalizing problems (Propper et al., 2007). Namely, the DRD4 genotype did interact with parental warm-responsive behaviour, but not with parental negative-intrusive behaviour, and this interaction was further moderated by race, where high warm-responsive parenting was associated with decreased externalizing behaviour only for African American children (vs. European American children) who were carriers of the short polymorphism of DRD4. In contrast to the demonstrated relevance in externalizing problems, the DRD4 gene was not a significant predictor of internalizing problems (Bakermans-Kranenburg & van IJzendoorn, 2006; Propper et al., 2007). The results outlined above highlight the potential role of DRD4 polymorphisms, namely, the ones including longer repeats, in externalizing problems. Interestingly, only the presence of the 7R seemed to be consistent with a differential-susceptibility hypothesis. Specifically, in an unfavorable environment, such as one with insensitive parenting, children carrying the 7R allele showed more externalizing problems. However,

children with the same genotype exposed to a favorable environment, namely, higher quality care, tend to show fewer externalizing problems. This differential-susceptibility nature of the interaction, specifically in terms of the DRD4 gene, is not surprising because it has already been shown as a 'plasticity gene' (Belsky et al., 2009; Belsky and Beaver, 2011). The other dopamine receptor gene is DRD2. The most studied polymorphism in DRD2 is located at the TaqI A site and consists of a single nucleotide polymorphism with two possible alleles: major A2 and minor A1. The DRD2 TaqI A site is 9.4 kb downstream from the coding region for the dopamine D2 receptor gene, and although the A1 allele is associated with a decrease in dopamine D2-binding capacity and glucose metabolic rates in many brain regions, its mechanism for influencing DRD2 expression is unknown. Two studies analyzed this dopamine receptor gene, and they showed its relevance in children's psychopathology. Mills-Koonce et al. (2007) observed that infants with the A1 allele reared by high-sensitive and low-sensitive mothers had fewer and more affective problems, respectively, at 3 years of age than children who were homozygous for the A2 allele. In contrast, Hayden et al. (2010) reported that the DRD2 A1 allele was associated with depressive and anxious symptoms depending on the extent of a parent's intrusiveness, with children showing fewer symptoms when parenting was intrusive. The results reported by Mills-Koonce et al. (2007) are also consistent with the differential-susceptibility hypothesis, indicating the plastic role of the DRD2 gene, as described previously. Further, in Hayden's study, depressive and anxious symptoms were associated positively with parental intrusiveness in children lacking the A1 allele. Children with at least one copy of the A1 allele appeared to have lower levels of symptoms when parenting was intrusive. The authors did not discuss this result in terms of the nature of the interaction; however, they explained this counterintuitive finding, that is, the notion that genetic risk might be attenuated by seemingly undesirable parenting

practices such as maternal intrusion – considering that such parents are less tolerant to children's symptoms, and therefore compel their children to modify the anxious and depressive behaviours that they are genetically predisposed to show. Although somewhat contradictory, these two studies reported the important role of DRD2 gene in the exhibition of child psychopathology. A better understanding of how this gene operates in interaction with environmental conditions, namely parenting, is needed.

Serotonin transporter

Across the studies dedicated to the analysis of the serotonergic system, all of them considered polymorphisms in the serotonin transporter gene (SLC6A4). This gene, located on chromosome 17, has several polymorphic loci that affect its expression and ultimately its function. The most studied is the serotonin-transporter-linked polymorphic region (5-HTTLPR), situated in the promoter of the gene where a deletion/insertion of 44 bp generates two different alleles that differently affect the transcriptional activity of the promoter (D'Souza and Craig, 2006). The short (s) allelic variant with 14 repeats [in contrast to the long variant (l) with 16 repeats] has been shown to be linked to a lower transcriptional rate of the gene and decreased functional capacity of serotonin transporter proteins, resulting in reduced serotonin reuptake in the synaptic cleft (Heils et al., 1996). Another polymorphism studied commonly in this gene is the STin2, located in intron 2 of the gene, where a VNTR of 17 bp generates 10 repeat (10R) or 12 repeat (12R) alleles. Animal studies have reported greater transcriptional activity of the SLC6A4 gene for the 12R compared with the 10R (MacKenzie & Quinn, 1999). Across studies, significant interactions between the 5-HTTLPR and environmental experiences emerged to explain both internalizing and externalizing problems. Noncompliance behaviour across childhood (from 18 to 54 months) was predicted by the interaction between an SLC6A4

haplotype (5-HTTLPR and STin2 polymorphisms) and parenting, such that the quality of parenting was related negatively to the slope of noncompliance only for children with the 5-HTTLPR s allele/STin2-10R haplotype. In addition, quality of parenting was negatively related to noncompliance at 18 months, but only for children with haplotypes that did not include the s allele (Sulik et al., 2012). Furthermore, and according to the differential susceptibility model, this haplotype was sensitive to both supportive and unsupportive environments in the expression of noncompliance behaviours. Lavigne et al.'s (2013) study, using only the 5-HTTLPR polymorphism, also found a G×E effect. Specifically, the 5-HTTLPR gene did interact with family variables (i.e. family SES, family conflict, life stress, and caretaker depression) in predicting depressive and anxiety symptoms as well as oppositional defiant behaviours at 42 months. Therefore, homozygous children for the l allele showed the most psychopathological symptoms, which is consistent with a diathesis-stress model. Tiemeier et al. (2012) also found a G×E effect for internalizing problems at 5 years of age. Specifically, the 5-HTTLPR genotype did interact with maternal prenatal and postnatal anxiety (and not with paternal prenatal or postnatal anxiety), suggesting that during fetal life and early childhood, the effect of maternal anxiety is moderated by the child's 5-HTTLPR genotype, with s allele carriers being at an increased risk for developing internalizing problems. It should be noted that this result was only explored within the diathesis-stress framework. Although there is some evidence for the plasticity of the 5-HTTLPR polymorphism, two of the three studies did report the 5-HTTLPR genotype as a vulnerability risk factor (Tiemeier et al., 2012; Lavigne et al., 2013). Further, some inconsistencies are present, specifically in terms of the genotype conferring more risk. In Lavigne et al.'s (2013) study, homozygotic children for the l allele showed more symptoms than children carrying the s allele, whereas in

Tiemeier et al.'s (2012) study, increased social emotional problems were observed in children carrying the s allele.

Monoamine oxidase

For the monoamine oxidase A (MAO-A) gene, different studies showed an interaction effect with environmental variables for the expression of both internalizing and externalizing behaviours. The MAO-A gene located in the short arm of the X chromosome encodes an enzyme, located in a neuron's nerve terminals, that plays an important role in the degradation of monoamines (such as dopamine, serotonin, and norepinephrine) in the central nervous system. The MAO-A LPR is a functional polymorphism in the promoter region of the gene consisting of a 30-bp repeat sequence that affects its transcriptional efficiency. This sequence exists naturally in 3, 3.5, 4, or 5 copies, although allelic frequencies have marked variability across ethnic groups. Functional studies have shown that alleles with 3.5 or 4 copies of the repeat sequence are transcribed two to 10 times more efficiently, giving rise to a higher activity variant of the enzyme than those with three or five copies of the repeat, suggesting an optimal length for the activity of the enzyme (Sabol et al., 1998). In terms of the studies included in this review, internalizing problems, and specifically depressive symptoms, were predicted by the interaction between an unfavorable environment (such as family conflict, life stress, and caretaker depression) and a low-MAO-A activity variant. This genotype also interacted with life stress in predicting anxious symptoms (Lavigne et al., 2013). These effects were only significant in boys. However, externalizing behaviours, in particular hyperactivity, were predicted by the interaction between stressful life events (experienced between 6 months and 3.5 years) and the MAO-A genotype, such that in children who had been exposed to the greatest stress, the low-activity allele was associated with

increased hyperactivity. In contrast, in children who had been exposed to little or no stress, the low-activity allele was associated with lower hyperactivity. This effect was only significant in girls. In contrast to Lavigne's findings, where the carriers of the low-MAO-A activity variant were at an increased risk of developing internalizing symptoms, Enoch et al.'s (2010) findings were consistent with the differential susceptibility model.

Brain-derived neurotrophic factor

The role of brain-derived neurotrophic factor (BDNF) polymorphisms was analyzed in one study (Willoughby et al., 2013) on externalizing problems, specifically in oppositional defiant disorder and callous–unemotional behaviours at age 3. This neurotrophic factor is an important intervenient in proper synaptic establishment and neural plasticity mechanisms, playing a significant role in insuring the maintenance of neuron survival and activity. BDNF has also been shown to impact the function of the key neurotransmitter systems such as the dopaminergic (Guillin et al., 2001) and serotonergic (Mossner et al., 2000) systems. Disruption of neurotrophic action may lead to severe impairments in cognitive processes and has been shown to be associated with numerous neuropsychiatric disorders (Gong et al., 2009; Verhagen et al., 2010; Bialecka et al., 2014). One particular genetic variation that has gained more attention in these last few years is the G196A polymorphism (rs6265) in the proregion of BDNF that results in a substitution of a valine (Val) for methionine (Met) residue at position 66 (Val66Met). A significant number of in-vitro and in-vivo studies have reported the impact of the Val66Met polymorphism in neuronal survival and function. The Met allele has been referenced as the susceptibility allele, resulting in decreased activity-dependent release of BDNF with implications for learning processes (Casey et al., 2009) and the depression phenotype (Hosang et al., 2014). In Willoughby et al.'s (2013) study, children's BDNF

genotype did interact with harsh and intrusive parenting, but not with sensitive parenting, to predict both oppositional defiant disorder and callous–unemotional behaviours. Thus, children carrying Val/Met or Met/Met variants, in the presence of early (6 and 12 months old) and late (2 and 3 years old) harsh parenting, showed more oppositional defiant behaviours, whereas carriers of the same genotype, in less harsh parenting environments (early and late), showed fewer oppositional defiant behaviours. Similarly, children carrying the Met allele, in the presence of early harsh parenting, showed more callous–unemotional behaviours, whereas carriers of the same genotype, in less early harsh parenting environments, showed fewer callous–unemotional behaviours. This pattern of results is consistent with the differential susceptibility model and highlights the potential plastic role of the BDNF genotype with a differential susceptibility to the environment.

Glucocorticoid receptor

The GR gene was analyzed in only one study (Velders et al., 2012) in relation to children's affective problems. The GR is the low-affinity receptor of cortisol and is present at several peripheral tissues as well as within the central nervous system. These receptors are key players in the hypothalamic–pituitary–adrenal axis function, which is the main neuroendocrine system that is activated in response to stress. This axis involves hypothalamic and pituitary hormones, and it ultimately leads to the production of cortisol at the adrenal glands. In the brain, GR mediates the negative feedback response of cortisol and thus has an effect on behavioural adaptation. A dysregulation of GR function will alter stress regulation and adaptation, with possible adverse implications. The identification of a G/C transversion in intron B of the GR gene (BclI polymorphism) has been shown to have a functional impact on this axis, leading to increased corticosteroid sensitivity (DeRijk and Kloet, 2005). Velders et al.'s (2012) study highlights the potential

role of the GR gene, specifically showing that prenatal maternal psychological symptoms have an effect on child affective problems later in life. It is noteworthy that Velders et al. (2012) analyzed five different polymorphisms at the GR gene; however, only one polymorphism (rs41423247) was found to play a significant role in the prediction of children's affective problems.

Main effect of genotype

Only three studies reported significant main effect of genes in the prediction of children's psychopathological symptoms. A low-MAO-A-activity variant was a main effect in predicting hyperactivity behaviours in 4-year-old girls (Enoch et al., 2010). The BDNF Val66Met polymorphism (presence of the Met allele) also showed a main effect on the occurrence of oppositional defiant disorder and callous–unemotional behaviours at age 3 (Willoughby et al., 2013). Finally, the STin2 polymorphism significantly predicted early noncompliance and aggressive behaviours (Sulik et al., 2012).

Main effect of environment

Slightly more than half (n =8) of the studies found significant main effects for at least one of the environmental variables. The other studies did not report or even test for this effect.

Covariates

Some variables, such as sex, ethnicity, or developmental period, are important factors for understanding the etiology of psychopathology and may relate to differences in genotype frequency. It is important to note that there are different prevalences of some psychopathological symptoms between boys and girls. Without treating sex as a

covariate, it is unknown whether a G×E effect may manifest differently for boys and for girls, biasing the results. Although it is important, not all studies (Propper et al., 2007; Enoch et al., 2010; Tiemeier et al., 2012; Velders et al., 2012; Belsky and Pluess, 2013; Berry et al., 2013; Willoughby et al., 2013; Windhorst et al., 2015) controlled for sex in their statistical analyses. Similar to sex, it is crucial to control for race/ethnicity in the analysis, namely, in mixed-ethnicity samples because the population stratification may imply the presence of different allele frequencies among different sub-populations (Price et al., 2010). This proper control prevents bias in G×E findings, especially when investigating ethnicity × environment and gene × environment (Dick et al., 2015). The results outlined in this review suggest that the G×E effect in early psychopathology should take into account sex (Enoch et al., 2010; Lavigne et al., 2013) and racial composition (Propper et al., 2007). Nevertheless, not all studies controlled for these variables in their models.

IV. DISCUSSION

Since the first studies by Caspi et al. (2002), there has been a surge of interest of candidate genes that can interact with particular environmental variables in the emergence of psychopathology. We carried out a systematic review based on studies that investigated this interaction effect in relation to children's psychopathology at the preschool age. One of the main issues that emerged in this review is the heterogeneity across the studies, namely, in terms of the methods and measures used as well as the polymorphisms involved in different neurobiological systems.

Overview of G×E research on psychopathology in early childhood

The biological pathways preferentially addressed by G×E research are consistent with the child psychiatry and psychopathology literature. Across the reviewed studies, we verified a prevalence of 'usual suspect' candidate genes, mostly integrated in the dopaminergic and serotonergic systems. However, our review also shows that most of the studies discussed considered the effect of individual genes as opposed to combining different genes in a cumulative genetic approach. Following Beaver et al. (2010), despite the fact that G×E research has provided a great insight into particular genes interacting with particular environments to produce different behaviours, G×E studies focused on a single gene fail to explain polygenic phenotypes, such as psychopathology. Indeed, with increasing knowledge of the functional network of genes, it is necessary to integrate into G×E tests a set of genes that interact biologically (Dick et al., 2015). Among the reviewed studies, only one (Sulik et al., 2012) addressed the role of a haplotype combining two different polymorphisms of the same gene. Interestingly, the haplotype measure did interact with parenting quality in predicting the child's psychological symptoms more powerfully than the two polymorphisms in independent models. The present review also highlights the absence of relevant information on the methodology used to conduct the studies. Specifically in terms of genetic data, transparency in reporting information is essential for interpretation of results and reproducibility (see STREGA guidelines; Little et al., 2009). The fact that not all reviewed studies reported the Hardy–Weinberg equilibrium, which can lead to significant bias in the results, is noteworthy. Another important topic that is not always reported is the gene–environment correlations (rGE) analysis. rGE refer to genetic differences that result in differential exposure to certain environments (Jaffee and Price, 2007). For example, the study by Willoughby et al. (2013) found that children with the Met allele of the BDNF gene received more sensitive caregiving during the infancy and toddler period. When the authors analyzed the G×E

effect of this gene with parenting quality in the prediction of externalizing behaviours, they took this information into account, namely because the G×E effect could be biased by such an evocative rGE, increasing the apparent G×E effect (Lau et al., 2008). The absence of rGE reduces some potential confounding effects in a G×E analysis (Dunn et al., 2011), preventing biased findings (Dick et al., 2015). Although some studies did carry out and report this type of analysis, it was not widespread among the reviewed studies. Finally, another consideration of G×E research in early psychopathology relates to the fact that measurements of children's psychopathology favored parental reports, mostly using only one informant. Despite the psychometric robustness of the most used instruments, G×E research might benefit from combined observational measures and the use of more than one informant.

Etiological insights into development of early psychopathology

According to the findings of the reviewed studies, early psychopathology is explained better using G×E effects rather than environment or genetic main effects. Most studies examined the findings by following a differential susceptibility approach instead of a diathesis-stress model. The differential susceptibility model informs the nature of the G×E effect by considering that 'plastic genotypes' (instead of vulnerability genotypes) make individuals more sensitive to positive and negative environments 'for better and for worse'. Among the studies that analyzed G×E effects using this approach, the results show that in an unfavorable environment, the plastic genotype will be associated with an increased risk of developing psychopathological symptoms; however, in positive environments, the plastic genotype will respond positively, placing the children at lower risk. This pattern indicates the plastic nature of some G×E effects where the particular

genotype seems to be affected by a variety of environmental factors in a 'for-better-and-for-worse' manner (Belsky et al., 2007).

Another important etiological insight is that candidate genes did not seem to be sufficient by themselves for eliciting children's psychopathology because the moderating role of the environmental variables was required. Parental interactive behaviours and parental psychopathology emerged as relevant variables when studying early psychopathology using a G×E model. In most studies, these variables interacted with the child's genotype in predicting their psychopathology (Bakermans-Kranenburg & van IJzendoorn, 2006; Mills-Koonce et al., 2007; Propper et al., 2007; DiLalla et al., 2009; Lavigne et al., 2013; Sulik et al., 2012; Tiemeier et al., 2012; Velders et al., 2012; Hayden et al., 2010; Willoughby et al., 2013; Windhorst et al., 2015), showing that parenting has a huge influence on children's development. Despite the power of these proximal experiences, it also seems to be relevant in the study of early psychopathology using a G×E model to consider more distal conditions, such as socioeconomic status (Lavigne et al., 2013). Although proximal experiences confer an increased risk for the development of psychopathology compared with distal circumstances, the relative risk of macro-social environmental variables suggests that their role in determining psychopathology outcomes may be substantial (Dunn et al., 2011). Specifically in childhood, the more distal social contexts can influence parents' performance in raising their children (Tendulkar et al., 2010).

Another important issue in the study of early psychopathology using the G×E model is the consideration of covariates. Our review showed that the G×E effect can be moderated by child variables, such as sex and ethnicity. For example, Propper et al. (2007) showed that high warm-responsive parenting is associated with decreased externalizing behaviour only for African American children with the short polymorphism

of DRD4. Further, the low-activity MAO-A gene was associated with increased child anxiety and depression in interaction with caretaker depression, hostility, family conflict, and family stress. However, these results were only significant for boys (Lavigne et al., 2013). The same gene did interact with stressful life events experienced from 6 months to 3.5 years to influence hyperactivity in girls at the age of 4 (Enoch et al., 2010). The longitudinal design of most of the studies also showed that the presence of a G×E effect is not stable over time (e.g. Windhorst et al., 2015). This draws our attention to the importance of examining G×E effects from a developmental perspective; however, supplementary research is necessary to understand the reasons for fluctuating G×E effects during early development. Finally, we also note some inconsistencies in the findings. For instance, Belsky and Pluess (2013) found a G×E effect for the DRD4 gene and not for the 5-HTTLPR gene, whereas Lavigne et al. (2013) found a G×E effect for the 5-HTTLPR gene, but not for the DRD4 gene. These inconsistencies could be explained by the heterogeneity in the conceptual and methodological approaches used. According to some authors (e.g. Rutter and Dodge, 2011; Dick et al., 2015), some findings appear to be simultaneously overreaching and misunderstood, promoting uncertainty in terms of the results of using a G×E approach, which highlights the relevance of extending the G×E paradigm to the field of neurobiology, to gain a more comprehensive understanding of the processes underlying the etiology of children's psychopathology.

Neurobiological processes associated with genetics

Although behaviour is determined by both genetic and environmental experiences, a missing gap between G×E interactions and observable phenotypes still needs further elucidation. Neurobiological processes play an important role in translating genetic predispositions into behavioural outcomes and modulating behaviour under a

variety of environmental conditions. Identifying links between G×E and brain function is important for not only establishing a mechanistic foundation for individual differences in behaviour but also understanding the pathways through which G×E shape risk for psychopathology (Hariri, 2011). The integration of genetics, brain, and behaviour variables will allow us to understand that a common functional polymorphism in a specific gene can bias the processing of environmental experience within a neural circuitry supporting some psychopathological symptoms. A good example of the relevance of this multilevel approach is the 5-HTTLPR gene. Functional MRI has shown a clear neural systems-level signature of the 5-HTTLPR gene. Most specifically, the presence of the 5-HTTLPR s allele is associated with increased amygdala volume, which in turn biases the brain circuits in response to environmental stress activation (Hariri et al., 2002; Munafò et al., 2008). These findings showed that addressing the neurobiological correlates provides a functional approach to the study of psychopathology and highlights the plausible mechanisms through which genes, in interaction with the environment, affect behaviour (Hyde et al., 2011).

This review of G×E research in children's psychopathology is intended to summarize the main contributors in terms of environmental and genetic factors to the emergence of externalizing and internalizing problems. The heterogeneity across the studies in terms of the predictors analyzed and the instruments for assessing psychopathology is noteworthy. However, the prevalence of G×E effects in predicting early psychopathology is also salient, with parental variables, namely, parental psychopathology and parental interactive behaviours, as important moderators. This general finding has practical and clinical implications, specifically for the importance of offering support to families with young children to promote parents' mental health and improve parental interactive behaviours with children. Finally, this systematic review also

clarifies that G×E effects on psychopathology in early age are complex and multifaceted. This scientific field can be improved by considering a polygenic approach and addressing the neurofunctional relevance of G×E interactions to predict behaviour.

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CHAPTER 4

MOTHERS' DISTRESS EXPOSURE AND CHILDREN'S WITHDRAWN BEHAVIOUR – A MODERATING ROLE OF THE *IFNG* GENE

A version of this Chapter is currently submitted to Developmental Psychobiology

CHAPTER 4

MOTHERS' DISTRESS EXPOSURE AND CHILDREN'S WITHDRAWN BEHAVIOUR – A MODERATING ROLE OF THE *IFNG* GENE

I. INTRODUCTION

While some young children are ever ready to approach and engage novel experiences, like playing a new game or meeting new people, others are inclined in the opposite direction. In fact, some even actively withdraw from novel stimuli, including other people. Here we consider environmental and genetic factors that could contribute to the emergence of WB in very young children. Indeed, we test a gene-environment – interaction (GXE) hypothesis that the emergence of WB in young children is a function of the interactive effect of mother distress and the gene encoding the IFN-γ cytokine (*IFNG*), using a sample of 198 Portuguese preschool children. We predict that children exposed to high levels of maternal distress *and* who carry the T allele of *IFNG* will manifest the most WB.

Withdrawn behaviour

Withdrawn behaviour (WB) emerge early in life and is a core neuropsychiatric phenomenon in developmental psychopathology (Rubin, Coplan, & Bowker, 2009). In preschool age, WB is a significative obstacle to learning (Coplan et al., 2001; Ladd, 2006; Lloyd & Howe, 2003), peer relationships (Chen et al., 2006; Gazelle & Ladd, 2003; Hart et al., 2000; Ladd, 2006; Nelson, Rubin, & Fox, 2005; Oh et al., 2008), and teacher-child relationships (Hamre & Pianta, 2006; Ladd & Burgess, 1999; Rudasill et al., 2006). WB in childhood predicts psychopathology later in development, including depression (Caspi

et al., 1996; Goodwin, Fergusson, & Horwood, 2004), anxiety (Aschenbrand, Angelosante, & Kendall, 2005; Kasius et al., 1997), psychosis (Miller et al., 2002), and suicide (Ferdinand & Verhulst, 1995). There is increasing evidence that the transition to school is particularly problematic for withdrawn children (Coplan & Arbeau, 2008, Rimm-Kaufman & Kagan, 2005).

Environmental Influences on Withdrawn Behaviour

Parenting has been consistently associated with the early emergence of WB, with most evidence addressing mothers' negativity and insensitive parenting strategies that are considered detrimental to children's social efficacy (Degnan et al., 2008; Hane et al., 2008). For example, research indicates that maternal depression is related to child WB and social reticence (Kochanska, 1991; Rubin et al., 1991) and that maternal emotional instability and negativity are related to social withdrawal and internalizing problems (Ellenbogen & Hodgins, 2004; Hettema et al., 2006).

Maternal psychological distress may influence children's WB in multiple ways. Children may simply model their mother's negative affect and highly vigilant oreientation toward the environment (Kochanska, 1991). Then there is the possibility that mothers experiencing psychological distress may respond to their children's emotions in qualitatively different ways relative to non-distressed parents. In fact, mothers with higher levels of emotional instability react with greater negative affect and greater vigilance to their children's distress than do other mothers (Scalzo, Williams, & Holmbeck, 2005). For instance, those who are depressed attempt to limit their children's negative experiences in an effort to protect them, decreasing the child's likelihood of developing skills to self-regulate their distress in novel or stressful social situations (Hastings & Rubin, 1999; Moehler et al., 2007; Rubin, Burgess, & Hastings, 2002). In addition,

anxious mothers are more likely to display negative affect and to engage in overcontrolling parenting, while being less likely to display positive affect and to promote autonomy during parent-child interactions (Ginsburg, Grover, & Ialongo, 2004; Moore, Whaley, & Sigman, 2004).

Genetic Influence on Withdrawn Behaviour

Despite such evidence suggestive of environmental influence on WB, its biological basis has long been recognized. Twin studies indicate that WB is 45-70% heritable (Derks et al., 2004; Eley et al., 2003; Hoekstra et al., 2008; Lamb et al., 2010). Despite the clinical relevance of WB and the evidence just cited of its genetic basis, few studies have actually identified molecular-genetic correlates of WB. Nevertheless and despite some inconsistencies, associations have emerged with polymorphisms in the serotonin transporter (*5-HTT*) and the dopamine receptors, specifically homozygosity for the short allele variant of the *5-HTTLPR* in the case of children with ADHD (Zhao et al., 2005), and the 7-repeat allele of the dopamine receptor, *DRD4*/48bp-repeat polymorphism (Marino et al., 2004). More recently, Rubin and collaborators (2013) found that *HTR2A* and *ADRA2A* genes are associated with WB, which underscores the role of monoaminergic system in the etiology of WB.

There are two main reasons why genetic research has been limited. First, most studies have focused on neurotransmission-related genes, disregarding other putatively relevant systems (e.g. immune system). Second, absence of GXE studies based on the fact that effects of some allelic variants may only become evident in the presence of specific contextual conditions (e.g. maternal distress), as has emerged for other psychopathological traits (e.g., Lavigne et al., 2013).

The immune system emerges as a promising candidate when it comes to selecting genes that might affect WB. Evidence indicates that the dysregulation of the inflammatory response, including the cytokine network, is associated with the etiology and pathophysiology of depression-like behaviour, including WB (for review see Capuron & Miller, 2011; Felger & Lotrich, 2013). Several studies indicate that peripheral administration of pro-inflammatory molecules or their inducers (e.g., Lipopolysaccharide – LPS) produces neuropsychiatric symptoms and behavioural alterations, including withdrawal from the physical and social environment, decreased motor activity, reduced food and water intake and altered cognition. Notably, this is true in both animals (Bay-Richter et al., 2011) and humans (Bonaccorso et al., 2002; Capuron et al., 2002; Harrison et al., 2009; Reichenberg et al., 2001). In fact, the link between pro-inflammatory activity and depression-like behaviour emerges in multiple meta-analyses showing elevated peripheral levels of pro-inflammatory cytokines in depressed patients (Dowlati et al., 2010; Howren, Lamkin, & Suls, 2009; Liu, Ho, & Mak, 2012).

The link between immune function and human behaviour appears to derive largely from the ability of some cytokines to cross the blood–brain-barrier and modulate central nervous system activity. One of the pro-inflammatory cytokines that has been studied is the IFN-γ. Interestingly, this cytokine appears to be involved in the pathophysiological mechanisms that mediate the link between inflammatory activity and psychopathological symptoms. Research indicates that IFN-γ up-regulates indoleamine 2,3-dioxygenase (IDO), the enzyme involved in tryptophan degradation, decreasing the availability of tryptophan for serotonin synthesis (for review see Oxenkrug, 2011). As a result, IFN-γ may influence social inhibitory behaviour by virtue of its ability to modulate 5-HT activity. Indeed, there is evidence chronicling a link between 5-HT activity and anxiety-related traits (Melke et al., 2001). In this context, it is remarkable that a functional SNP

located at position +874 in the first intron of the human IFN-γ gene (*IFNG* +874 T > A, rs2430561) influences both the mRNA expression and secretion of IFN-γ, with the AA genotype being associated with reduced IFN-γ levels, and the TT genotype being associated with high IFN-γ levels (Matos et al., 2007).

Although no studies have addressed WB, there is evidence of an association linking the A allele with increased harm avoidance and decreased extraversion and exploratory excitability traits (MacMurray, Comings, & Napolioni, 2014). All these phenotypes are considered as early-appearing manifestations of behavioural inhibition (Cloninger, 1987). On the other hand, another study chronicled a higher frequency of T carriers among depressed individuals than those not depressed (Oxenkrug, 2011). However, none of this research addressed child psychopathological functioning, nor took into account the interaction of these genetic factors and environmental experiences in an effort to explain psychiatric symptoms.

In summary, the *IFNG* +874 T > A SNP would seem to be a pertinent candidate gene variant for study WB in the context of a GxE approach. The T allele is related to social-inhibitory behaviour, such as low extraversion, exploratory excitability and high harm avoidance (MacMurray et al., 2014). Further, it is known to be a functional SNP, regulating expression of the gene product and modifies central nervous system activity. Despite the plausibility of these models, the interaction between genetic variants of immune system and environmental factors has not been investigated with respect to WB. Thus, the goal of the research reported herein is to evaluate whether the functional *IFNG* +874 T > A SNP moderates the impact of maternal psychological distress on child WB at preschool age. It is predicted that children carrying the T allele who experience high levels of maternal distress will be most likely to dispay high levels of WB, contrary to AA children who will be less vulnerable to maternal distress variations. These predictions

are consistent with diathesis-stress thinking that particular diatheses or vulnerability factors--in this case the T allele--will amplify the adverse effect of a stressor, in the current case, high levels of maternal distress

II. METHODS

Participants

Participants included 198 children (109 girls, 55.1%) and their biological mothers drawn from 34 preschool classrooms. The study's purposes procedures were explained to parents. Written informed consents were then obtained. Additionally, if mothers had participated with multiple children (n = 7), one child was randomly selected for inclusion in the analyses (because data from two or more children of the same mother is no longer independent data). The mean age of the children was 58 months (M = 57.89, SD = 7.56) at time of WB assessment. Criteria for exclusion of child participants were the presence of moderate to severe mental or physical impairments, genetic syndromes or autism spectrum disorders. Only children of Caucasian ethnicity were included in the present study to avoid confounding effects of ethnic differences in gene frequency. Mothers' age ranged from 20 to 48 years (M = 33.36, SD = 5.76). The majority of mothers (53.0%) had less than high school education; 26.3% of the mothers were unemployed; and 18.2% were single mothers.

Procedures

Genetic and environmental predictors, as well as covariates, were all gathered during a single occasional.

Measures

Child WB

To assess children's WB, a validated Portuguese version of the Caregiver Teacher Report Form for ages 1.5-5 (CTRF; Achenbach & Rescorla, 2000; Achenbach et al., 2014) was completed by the preschool teacher. The CTRF 1.5-5 is a widely used questionnaire with 99 items, each of which is coded 0 ("not true"), 1 ("sometimes or somewhat true"), or 2 ("very/frequently true"), designed to assess emotional and behaviour problems of young children. The instrument has good psychometric properties and has been extensively used to assess child mental health (Achenbach et al., 2014). For the purposes of the present study, the 10-item Withdrawn syndrome scale was used (e.g., Item 71, "*Shows little interest* in *things around* him/her"); it has an internal consistency of .79 in our sample, with higher scores reflecting the presence of more WB.

Genotyping of IFNG (+874; T/A) polymorphism

For reasons stipulated above, we selected the IFN-γ SNP rs2430561. Saliva samples were collected with Oragene DNA collection kits (DNA Genotek, Canada) and genomic DNA was isolated as instructed by the manufactures, using the standard protocol from PrepIT L2P (DNA Genotek). Samples concentration was accessed using Nanodrop technology. Thus, samples of 5ng of DNA were used, along with the corresponding KASPar assay (LGC Genomics, UK), for a final volume of 8μL. The thermal profile was performed as instructed be the manufacturers, in a 7500 Fast Real-Time PCR System (Applied Biosystems by Life Technology, USA). Allelic frequencies were in Hardy–Weinberg equilibrium, $\chi^2(1) = .04$, $p = .83$. Genotype distribution was AA (n = 42; 21.2%), AT (n = 100; 50.5%) and TT carriers (n = 56; 28.3%), with 78.8% of the sample carrying T allele.

Maternal psychological distress

Mothers completed the highly reliable and well validated Brief Symptom Inventory (BSI; Derogatis, 1982; Canavarro, 1999), a self-report measure of nine primary dimensions of psychological distress. Mothers rated 53 items pertaining to their levels of distress in the preceding week using a 5-point scale ranging from 0 (*not at all distressful*) to 4 (*extremely distressful*). The measure is scored to generate profiles spanning nine symptom dimensions and three global distress markers. In the present study the Positive Symptom Distress Index was used, because it was considered the best discriminant of individuals with and without psychopathological symptoms in the Portuguese population (Canavarro, 2007).

Covariates

Child factors that could influence child behavioural problems were selected as potential confounders. Therefore, sex, age and child's mental development were included in this study as covariates. Child mental development was assessed by the Griffith's Mental Development Scales (Griffiths, 1984), evaluating six developmental dimensions: motor, personal and social, hearing/language, eye and hand coordination, performance and practical reasoning. A total score was calculated by averaging the six dimensions to capture general developmental level.

Statistical analysis

First, descriptive statistics on the measurements included in this report were carried out. After this preliminary analysis, hierarchical analysis was used to predict WB.

The hierarchical regression model included covariates in the first step, maternal psychological distress and child *IFNG* (+874; T/A) polymorphism as main effects in the second step and, in the third step, the 2-way interaction involving these two factors. To illuminate any detected interaction, regions of significance were determined. Statistical analysis was performed with SPSS Version 20.0 statistic software package.

III. RESULTS

Descriptive statistics

Means and standard deviations of all variables are shown in Table 4.1. Independent-samples *T-test* comparing the two genotype groups revealed no significant differences in child or mother measurements.

Table 4.1. Descriptive statistics of study variables between genotype groups and total sample

			***IFNG* (+874; T/A)**		
	Total sample		**AA**	**AT/TT**	***T-test***
	N=198		**N=42**	**N=156**	
	M (SD)	***Range***	***M (SD)***	***M (SD)***	***t (196)***
Children					
Age (mo)	57.89 (7.56)	40-76	58.52 (8.80)	57.72 (7.42)	-.608
Mental development	106.64 (11.10)	64-130	108.36 (9.81)	106.18 (11.41)	-1.134
Withdrawn Behaviour	1.81 (2.54)	0-12	1.86 (2.71)	1.79 (2.50)	-.141
Mothers					
Psychological distress	.66 (.51)	0-3.06	.61 (.40)	.67 (.54)	.698

Predicting WB

Table 4.2 displays results of the multiple regression analysis predicting child WB. Child gender, age and mental development were found to be significant predictors of WB, such that boys (B = .179, p = .006), younger children (B = -.279, p < .001), and those with a lower developmental quotient (B = -.386, p < .001) evinced more WB. No main effects emerged for maternal psychological distress (B = .087, p = .168) or child genotype (B = .072, p = .256), however. Notably, though, the interaction of maternal psychological distress and child *IFNG* genotype proved significant (B = .280, p = .013), explaining approximately 25% of the variance of children's WB. Specifically, greater maternal distress predicted more WB for children carrying AA genotype (r = .477, p = .001), but not for those carrying the T allele (r = .018, p = .824). However, we also found that in the presence of low maternal distress, AA carriers exhibited lower levels of WB than did T carriers. Therefore, we conducted a "regions of significance" analysis, following Kochanska and associates (2011), to illuminate the nature of this GxE.

Visual inspection of Figure 4.1 reveals a cross-over interaction consistent with the differential-susceptibility hypothesis (Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2007; Belsky & Pluess, 2009, 2013; Ellis, Boyce, Belsky, Bakermans-Kranenburg & Van Ijzendoorn, 2011). That is, AA children had the most withdrawn behaviour when exposed to high maternal psychological distress yet the least when maternal psychological distress was low. Analysis of Regions of Significance, using the Johnson-Neyman technique, revealed both a lower and a higher bound of significance within the observed range of maternal psychological distress. More specifically, the slope between *IFNG* polymorphism and withdrawn behaviour proved significant when maternal psychological distress was lower than -0.32, representing 30.08% of the sample, and higher than 0.43, representing 17.17% of the sample (i.e., shaded areas in Figure 4.1).

Table 4.2. Summary of hierarchical linear regression analysis predicting child WB

	R^2 (R^2 Aj)	*B*
Step 1	.236 (.225)	
Child gender		.179**
Child age		-.279***
Child development		-.386***
Step 2	.248 (.229)	
Maternal psychological distress		.087
IFNG +874 polymorphism		.072
Step 3	.272 (.249)	
M psychological distress**IFNG +874*		.280*

*$p < .05$; **$p < .01$; ***$p < .001$;

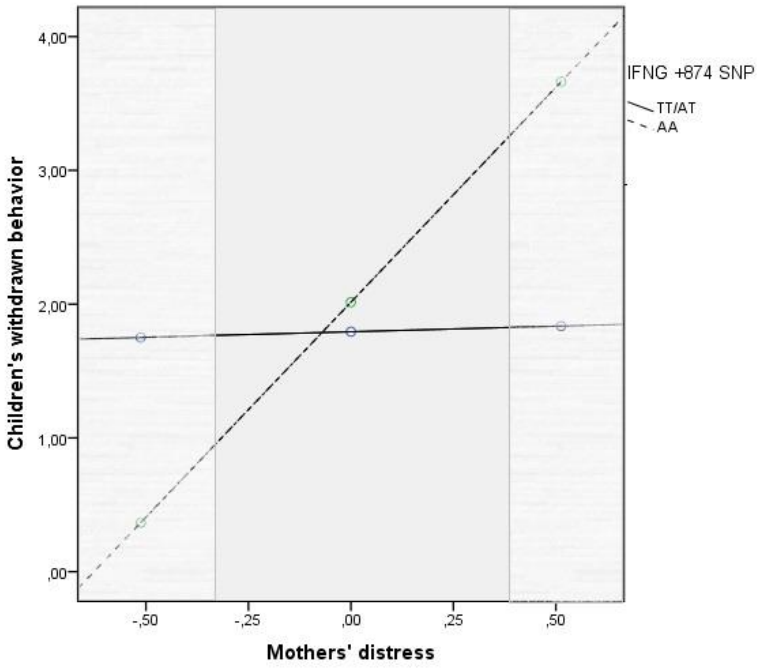

Figure 4.1. Plot of the interaction between the IFNG genotype (AA vs. AT/TT) and mothers' distress on the explanation of children's withdrawn behavior.

IV. DISCUSSION

The aim of the present study was to evaluate the interaction of early experience of maternal distress with an immune-related gene as precipitant of WB in early childhood. As anticipated, a GXE interaction involving *IFNG* +874 T > A SNP and maternal distress accounted for significant differences in WB during childhood, over and above the contributions of child's gender, age, and mental development. Notably, however, the form of the interaction proved inconsistent with our diathesis-stress-based expectations. Rather than it being children carrying the T allele—which has been associated with a pro-inflammatory profile and thus was conceptualized as a "vulnerability gene"—who manifest higher levels of WB in the face of high levels of maternal distress, this proved true only for those homozygous for the A allele. Considering the well-established relationship between high levels of inflammatory activity – which in the particular case of *IFNG* +874 SNP is associated with the T allele – and depression-like behaviour (Bonaccorso et al., 2002; Capuron et al., 2002; Harrison et al., 2009; Reichenberg et al., 2001), we had expected it to be the T allele that interacted with maternal distress in predicting WB.

Reflection on these surprising findings calls attention to the "behavioural immune system" hypothesis (Schaller, 2011; Schaller, Murray, & Bangerter, 2015) -- which stipulates that the immune system and social-inhibitory behavioural repertoires are complementary, each of which functions to reduce risk of infection. The 'behavioural immune system' is comprised of processes that evolved as a mean of facilitating behaviour that minimized infection risk and enhanced fitness and life quality. Empirical research on human populations suggests that in regions that have suffered from high levels of infectious diseases, people report higher mean levels of social-inhibitory behavioural traits (Schaller & Murray, 2008); this suggests that a social-inhibitory behavioural repertoire may protect individuals from pathogenic agents, by perhaps

reducing their contact with infected persons. It is as if WB works as an adaptive response when the individual is highly susceptible to infection.

The interaction between *IFNG* +874 AA genotype and maternal distress vis-à-vis WB that we discerned may, then, be explained by the fact that low levels of inflammatory activity typically associated with the AA genotype increase susceptibility to infection, thereby activating the behavioural inhibitory system. WB may thus be a primitive bio-behavioural posture that proves adaptive when the environment is aversive (or distressing), increasing proneness to infection. Thus, although, contrary to the diathesis-stress-related, pro-inflammatory hypothesis of psychopathological symptoms on which we based our own predictions, the present results would seem to be in line with previous evidence linking the A allele of IFNG +874 SNP and social-inhibitory behavioural traits in adults, such as harm avoidance, introversion and low exploratory excitability (MacMurray et al., 2014). Clearly, our GXE results need to be replicated before our post-hoc interpretation of findings can be confidently embraced.

Another possible explanation of our results is that WB is better sustained by the pathophysiological mechanisms of anxiety-like behaviour than of depression-like behaviour. Anxiety is associated with increased, rather than decreased serotonergic activity (Frick et al., 2015; Hansenne & Ansseau, 1999). Although IFN-γ provokes a significant reduction in serotonergic activity, the *IFNG* +874 AA genotype produces less IFN-γ protein, and therefore less 5-HTT reduction when compared to the TA and TT genotypes (Oxenkrug, 2011). Raitala and colleagues (2005) found an association between T allele and increased IDO activity, but only in females. Unfortunately, few studies have been conducted so far to evaluate blood tryptophan/serotonin levels in *IFNG*-genotyped subjects—which could clarify this issue. Here, then, is another direction for future research.

Interestingly, we also found that in the presence of low maternal distress, AA carriers exhibited lower levels of WB than did T carriers. This effect could be interpreted in light of the differential susceptibility model person-X-environment interaction proposed by Belsky and colleagues (2007, 2009, 2013; Ellis et al., 2011), with this specific SNP operating as a susceptibility factor. The differential susceptibility model posits that, in addition to being disproportionately affected adversely by negative environmental conditions, individuals with certain susceptibility factors also benefit more from positive environmental conditions. That is, they are highly susceptible to environmental influences "for better and for worse". Testing this hypothesis ideally requires a full range of environmental experiences, from very adverse to very favorable contexts, to be measured (Belsky & Pluess, 2009; Dick, 2011). Because in the present study the environmental experience ranged from very adverse (high maternal distress) to absence of risk (absence of maternal distress), we were not well positioned to test this hypothesis with regard to *IFNG* +874. Perhaps these ideas and our results will stimulate future work in this direction.

The present study had a number of strengths, including hypotheses based in theory, concurrent assessment of the relationship between exposure to maternal distress and withdrawn symptoms, and independently-rated measurement of maternal distress and children's WB, given that mothers reported their own distress while child withdrawn behaviour was based on teacher reports of behaviour in a preschool context. Alternative explanations for WB were ruled out, including gender, age and mental development. Of course, our findings must be interpreted in light of some limitations, including the sample size that was relatively small for a candidate gene study, which reduced statistical power. We readily acknowledge that other effects, such as epistasis could also be relevant to a

deeply comprehension of the interaction effect we found on childhood WB. Therefore, replication in a larger sample is desirable in future studies.

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CHAPTER 5

GENERAL DISCUSSION

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I. SUMMARY OF RESEARCH FINDINGS

Research has focused abundantly on emotional and behavioural disorders during early childhood, with accumulated evidence documenting the contribution of environmental experiences to the emergence and maintenance of internalizing and externalizing problems. Family background and quality of parenting have been described as crucial influencers, serving as regulatory experiences through which children develop emotion regulation patterns and problem-solving skills (Morris, Silk, Steinberg, Myers, & Robinson, 2007).

In this thesis we aimed at understanding the effect of the quality of parenting in contexts beyond the familial realm, such as preschool. Preschool is indeed one of the first extra-familial contexts to which children need to adapt and, therefore, understanding the quality of its functioning in this context could be an interesting marker of their social-emotional development. On the other hand, the effect of specific parental competencies has not been fully addressed, not entirely understanding the differential effect of parental behaviours that are usually integrated into more global parenting constructs. For example, little is known about the differential effect of maternal sensitivity and cooperation on a child's emotional and behavioural functioning. Both have been considered valuable contributions to the field of parenting and child development (Cassidy, 2016), however, there has been a tendency for research to focus exclusively on sensitivity (Bernard, Meade, & Dozier, 2013) or to create a composite measure in which both behaviours are considered (e.g., Luijik et al., 2011). Therefore, one of the main goals of the present thesis

was to explore the differential role of maternal sensitivity and maternal nonintrusive interactive behaviour in relation to a child's internalizing and externalizing behavioural problems in the preschool context. Additionally, and apart from the progress that has been made in illuminating contextual influences on these behavioural and emotional problems, the mechanisms by which they arise remain to be fully elucidated. During the last decade, extensive research on developmental psychopathology has highlighted the contribution of both genetic and environmental variables, enlightening the determinants of typical and atypical development. In fact, GxE research demonstrates a well-established relationship between polymorphisms in genes implicated in the dopaminergic (MAOA, DRD2, DRD4) and the serotoninergic (5-HTT) transmission and emotional and behavioural problems. Nonetheless, the contribution of other systems has been underappreciated, such is the case of the immune system that emerges as a promising candidate when it comes to genes that can affect emotional and behavioural functioning (O'Connor, Moynihan, & Caserta, 2014). This is particularly relevant because there is evidence indicating that the dysregulation of the inflammatory response, including the cytokine network, is associated with the aetiology and pathophysiology of some neuropsychiatric symptoms. However, no study has yet explored the role of polymorphisms in immune-related genes, and that was one of the goals of the present thesis. In addition to the systematization of the GxE literature in this developmental period, we aimed to explore the role of an immune-related gene regarding emotional and behavioural functioning during childhood.

Conceptually sustained in Developmental Psychopathology, three studies were carried out so as to address the gaps identified in the current literature. The first experimental chapter (Chapter 2) focused on the familial influences, aimed at extending the current understanding of the mechanisms by which family risk relates to internalizing and externalizing problems in the preschool context, by examining whether such

relationship is differentially mediated by maternal insensitivity and intrusiveness. Results obtained from a sample of 205 children, their mothers and their preschool teachers, revealed that only maternal intrusiveness mediated the relationship between family risk and the child's internalizing behaviours. This study highlighted the need for further research focused on the differential effect of specific maternal behaviours underlying the link between family risk and a child's adjustment in the preschool context, in order to achieve more conclusive results on this matter.

The second study (Chapter 3) reviewed the GxE literature focused on internalizing and/or externalizing problems in early childhood, aiming to provide a critical overview of the interaction between putative target genes and environmental influences on the explanation of emotional and behavioural problems in this developmental age. The results of this systematic review showed that the biological pathways, preferentially addressed by GxE research, are consistent with the child psychiatry and psychopathology literature. Across the reviewed studies, we verified a prevalence of 'usual suspect' candidate genes, mostly integrated in the dopaminergic and serotonergic systems. Another important aetiological insight was that candidate genes, alone, did not seem to be sufficient to elicit a child's psychopathology because the moderating role of the environmental variables was also required. Parental interactive behaviours and parental psychopathology emerged as relevant variables when studying early psychopathology using a GxE model. In most studies, these variables interacted with the children's genotype in predicting their psychopathology, showing that parenting has a huge influence on child's emotional and behavioural development.

Building on previous literature, which established that immunological hypotheses offer important and compelling alternative explanations for the aetiology of behavioural and neurodevelopmental disorders within psychology and psychiatry, Chapter 4 aimed to

expand on the GxE literature to other putative genes, by focusing on the gene encoding interferon-gamma (*IFNG*), a pro-inflammatory cytokine. In a sample of 198 Caucasian children, results revealed a GxE interaction, such that AA homozygous children proved to be more susceptible to maternal distress than T allele carriers (i.e., AT/TT), displaying the highest levels of withdrawn behaviour. Findings in this chapter provided preliminary support for the importance of considering immune-related genes in interaction with environmental variables when investigating the determinants of a child's emotional and behavioural functioning.

In the following sections, an integrative perspective of our studies' main findings and clinical implications will be presented and the methodological limitations and contributions for future research will be pointed out.

II. CHILD'S EMOTIONAL AND BEHAVIOURAL PROBLEMS

Following the limitations evidenced by the literature, which tends to use more general constructs of parenting, giving little attention to the specific effect of distinct parenting behaviours, in Chapter 2 we examined the differential mediating effect of maternal interactive behaviour in predicting child's internalizing and externalizing problems as reported by their preschool teachers. We found a differential effect of maternal interactive behaviours, with a greater relevance of intrusiveness when compared to sensitivity. Specifically, our results showed that maternal intrusiveness mediated the relationship between family risk and child's internalizing behaviours. One of the added values of this study was the differential analysis of maternal sensitivity and intrusiveness in the link between family risk and the child's internalizing and externalizing problems,

showing that this ‘unpacking’ approach of parenting behaviours has developmental relevance. Indeed, our findings suggested that dyadic interactions marked by nonintrusive, cooperative, and autonomy-related behaviours are influential in shaping child’s emotional and behavioural functioning in the preschool context, as evaluated by teachers. The quality of mother-child interactions plays a key role working as a buffer to the negative effect of family adversity on internalizing problems.

Overall, extensive research on developmental psychopathology currently emphasizes the notion that to better understand the effects of environmental experiences on the development of child’s emotional and behavioural symptoms, it is important to consider the contribution of biological markers. Probably one of the most recent of these is immune function. Experimental animal and adult human data suggest that stress is associated with alterations in the immune system that may underlie increased susceptibility to emotional and behavioural disorders (O’Connor et al., 2014). Although the implications of this data for child psychiatry are not yet clear, in the next section we will discuss the immune system as a candidate to a deeper understanding of the aetiological underpinning of emotional and behavioural disorders in childhood.

III. GXE APPROACH – THE IMMUNE SYSTEM AS A CANDIDATE

One of the merits of this dissertation is to extend the repertoire of genes usually studied in GxE research concerning child’s emotional and behavioural functioning to immune-related genes, as is the case of *IFNG*. Following the recent interest in developmental literature concerning GxE research, in Chapter 3 we carried out a systematic review of GxE research on the emergence of externalizing and internalizing

problems in childhood. Through this review we found an over-representation of 'usual suspect' candidate genes, mostly focused on neurotransmission-related genes, disregarding other putative relevant systems, such as the immune system.

In fact, recent studies have shown that the dysregulation of the inflammatory response, including the cytokine network, is associated with the aetiology and pathophysiology of some emotional and behavioural symptoms (for review see Capuron & Miller, 2011; Felger & Lotrich, 2013). Further evidence has identified a relationship between functional allelic variants and single-nucleotide polymorphisms (SNPs) of genes encoding inflammatory molecules and some psychopathological phenotypes, such as depressive-like behaviour (Bufalino, Hepgul, Aguglia & Pariante, 2012).

The knowledge on this topic raises the need to better understand the role of the immune system in the emergence of emotional and behavioural problems. Following this framework, in the study presented in Chapter 4, we focused on a SNP of an immune-related gene – *IFNG* (rs2430561), that has not been previously studied in relation to child emotional and behavioural functioning. In this study we focused specifically on withdrawn behaviour, firstly because it is a core neuropsychiatric phenomenon in developmental psychopathology, being described as a significant predictor of later psychopathology, including depression (Goodwin, Fergusson, & Horwood, 2004), anxiety (Aschenbrand, Angelosante, & Kendall, 2005), psychosis (Miller, Byrne, Hodges, Lawrie, & Johnstone, 2002), and suicide (Ferdinand & Verhulst, 1995); but also because this behaviour has been related to inflammatory activity.

In fact, associations between inflammatory activity and behavioural changes compatible with an inhibitory functioning are well established (Bonaccorso et al., 2002; Capuron et al., 2002; Reichenberg et al., 2001), adding biological plausibility to the model. At this point our study has two major innovations: i) the withdrawn behaviour has

not yet been studied as regards inflammatory activity, despite recent hypotheses on social-inhibitory behavioural traits and its anthropologically adaptive role (see Behavioural Immune System Hypothesis; Schaller, 2011; Schaller, Murray, & Bangerter, 2015); and ii) in GxE literature, the *IFNG* has never been studied in interaction with environmental experiences. Indeed, we did find an interaction involving *IFNG* and early experience of maternal distress as a precipitant of withdrawn behaviour. Our results suggest that GxE models considering immune-related genes may offer a deeper understanding of withdrawn behaviour.

Despite the innovation and strengths of the study presented in Chapter 4, future research should pay attention to the mechanisms by which the *IFNG* gene, in interaction with stressful experiences, can translate into behavioural alterations. A well-recognized pathway by which cytokines may mediate behavioural effects is through the activation of indoleamine 2,3-dioxygenase (IDO) (Fujigaki et al., 2006), an enzyme expressed in multiple cell types, including macrophages, dendritic cells, microglia, astrocytes, and neurons (Guillemin, Smythe, Takikawa, & Brew, 2005, Huang, Baban, Johnson, & Mellor, 2010). IDO breaks down tryptophan (TRP), the primary amino acid of serotonin synthesis, into kynurenine (KYN). The breakdown of TRP is believed to contribute to reduced serotonin availability and increased KYN levels. Indeed, correlations between increased KYN/TRP ratio and depressive-like behaviour have been reported in human studies (Bonaccorso et al., 2002; Oxenkrug, Turski, Zgrajka, Weinstock, Ruthazer & Summergrad, 2014), suggesting that the up-regulation of the TRP-KYN pathway is one of the putative mechanisms for the onset of depression-like behaviours. Therefore, it would be interesting to analyze whether the strength of this mechanism would be under genetic control. Unfortunately, until now only a few studies have been conducted to evaluate blood tryptophan/serotonin levels in *IFNG*-genotyped subjects. Raitala and

colleagues (2005) found an association between T allele and increased IDO activity, but only in females. Nonetheless, the replication of this type of study in paediatric populations is very important to data robustness, but also because the development of the immune response varies with age (O'Connor et al., 2014).

Another direction for future research has to do with glutamate neurotransmission. KYN, formed from the IDO catabolization of tryptophan and further catabolized into the neuroactive metabolites kynurenic acid (KA) and Quinolinic acid (QUIN), both affecting glutamate neurotransmission (Schwarcz & Pellicciari, 2002; Stone, 2000; Tavares, Tasca, Santos, Alves, Porciuncula, Emanuelli, & Souza, 2002), may contribute to glutamate dysfunction in depression (Dantzer, O'Connor, Lawson, & Kelley, 2011; Haroon, Raison, & Miller, 2012). In fact, increased glutamate has been found in the frontal cortex of patients with mood disorders (Matute, 2011).

IV. STRENGTHS AND LIMITATIONS

Some of the strengths and limitations of this thesis need to be addressed. In the two empirical studies, we applied a well-validated measure to assess the child's internalizing and externalizing problems (Achenbach et al., 2014). The assessment of the child's outcomes in the preschool context, through the teacher's report, in both empirical studies, enables one to overcome a limitation identified in the literature, by examining how parenting behaviours are linked to a child's emotional behavioural functioning in contexts outside the family, such as preschool. In fact, preschool is one of the first extra-familial contexts to which the child needs to adapt, and the quality of this adaptation, measured by their functioning, is an interesting marker of socioemotional development.

Research suggests that teachers' perception of a child's emotional/behavioural functioning may be of particular interest for understanding preschool experience and subsequent school adjustment (Baker, Tichovolsky, Kupersmidt, Voegler-Lee, & Arnold, 2014). Indeed, in addition to the privilege of observing the child in the classroom and in the playground, practizing emotional and behavioural skills, teachers also have the ability to compare several children of the same age and developmental level.

In the study presented in Chapter 4, based on teachers' reports to measure child's behavioural and emotional problems, the independence of the assessments was guaranteed, since the mother reported her own psychological well-being, thus preventing the potential association between maternal psychological well-being and their report on the emotional and behavioural difficulties of their child.

Regarding the conceptualization and assessment of environmental experiences throughout the two empirical studies of this dissertation, special attention was given to the mother's contributions - maternal interactive behaviours (Chapter 2) and maternal psychological distress (Chapter 4) - as well as to family risk. To study maternal interactive behaviours, we used Ainsworth's Maternal Sensitivity Scales (Ainsworth et al., 1978), a well-established observational measure proven highly reliable and valid in a wide range of studies, including in the preschool period (Baptista, Belsky, Mesquita, & Soares, 2017; Oliveira, Fearon, Belsky, Fachada, & Soares, 2005). We used a structured task where the choice of the episodes was based on relevant previous research in the field (Bakermans-Krunenburg & Ijzendoorn, 2008), thus guaranteeing that the behaviours of interest would be elicited. The fact that maternal interactive behaviour scores have a large range suggests that a wide variety of behavioural performances were captured. One of the advantages of using observational measures is related to the operationalization of

constructs, namely by the non-reliance on the parental ability to describe or quantify complex behavioural and relational constructs (Lindahl & Malik, 2001).

Regarding sample size, the studies reported herein relied on a global sample of 220 Portuguese dyads. Specifically, in relation to the GxE study presented in Chapter 4, it is important to make some considerations regarding sample size. Several critical reviews of GxE research in psychiatry were published during the course of the development of the studies included in this thesis (Dick et al., 2015; Munafò, Zammit, & Flint, 2014), indicating that most studies were underpowered and preclude the drawing of any firm conclusions from existing GxE research. In fact, studies based on the candidate gene approach generally need larger sample sizes to have adequate power to test for interactions as compared to main effects. However, McGue and Carey (2017) draw our attention to the trade-off between achieving a large sample *versus* having an in-depth assessment. On the other hand, Wong and colleagues (2003), based on their simulations, concluded that the sensitivity of tests for GxE could in certain situations benefit more from an improved measurement than from an enlarged sample size. Despite being restricted in sample size, the work presented in Chapter 4 addressed, for the first time, an immune-related gene in the paediatric population and its possible interaction with environmental experiences. Thus, the findings must be interpreted as an exploratory assay that should merit attention and replication.

It should also be noted that in the study presented in Chapter 2, the cumulative risk index was developed taking into account nine binary risk variables based on prior research and on the established cumulative risk literature (Sameroff & Fiese, 2000), which draws attention to both the quantity and quality of familial and social risk factors experienced by children. It is also important to consider the sample used in the studies carried out in this dissertation. Although presenting risk variability, there are a limited

number of families with a very high risk. For instance, according to the multi-dimensional composite of the family context used in the study presented in Chapter 2 (which included: teenage pregnancy; single parenthood; (low) parental educational level (i.e., one of the parents with under nine years of schooling); parental unemployment (i.e., one of the parents was unemployed at the time of the study); household overcrowding (i.e., less rooms than the number of residents); economic difficulties; absence of social support; unstable or violent marital relationship; and chronic parental health conditions), none of the cases presented all the risk factors, and only 9.8% of the sample presented 5 or 6 risk factors. Therefore, it would be interesting to replicate the studies presented herein using a high-risk sample. First, in the study presented in chapter 2, it would be interesting to understand if, in a sample of higher psychosocial risk, the nonintrusiveness would still work as a buffer in the linkage between family risk and internalizing problems in the preschool context. Second, it would also be very interesting to examine the GxE model presented in the study of Chapter 4, using a high-risk sample, since the literature is clear on the relationship between psychosocial stress and inflammatory functioning (O'Connor et al., 2014). Moreover, from the perspective of its biological plausibility it would be a very pertinent GxE model.

As far as the GxE study is concerned, although it is valuable and clinically relevant, the approach has been criticized in some aspects. A major criticism relates to its reductionism as most developmental outcomes are genetically complex and are likely to be determined by variations at multiple – not just one – loci, with each exerting a small effect, and possibly doing so in interaction with multiple genetic variants (Robinson, Wray, & Visscher, 2014). Therefore, it would be valuable to analyze other immunological markers using a polygenetic approach, such as the balance between pro- and anti-inflammatory cytokine genes.

Additionally, the nature of the GxE results found in Chapter 4 seems to be compatible with the differential-susceptibility hypothesis. In fact, literature focused on adult psychopathology had already shown that genotype of another pro-inflammatory cytokine (IL-1β) moderates the impact of interpersonal stress exposure on depressive symptoms (Tartter, Hammen, Bower, Brennan, & Cole, 2015). However, this evidence is supported by studies in adults where the nature of GxE interaction has never been explored. Taking into consideration the state of the art in developmental psychopathology, the results obtained in our study are very auspicious. Although replication is required, our findings suggest, for the first time, that the *IFNG* gene can be plastic as it has been shown for genes involved in the dopaminergic and serotinergic pathways (Belsky, Jonassaint, Pluess, Stanton, Brummett, & Williams, 2009). In fact, we found that greater maternal distress predicted more withdrawn behaviour for children carrying AA genotype, but not for those carrying the T allele. However, we also found that in the presence of low maternal distress, AA carriers exhibited lower levels of withdrawn behaviour than did T carriers. The mechanisms that might mediate this apparent relationship between stress exposure, inflammation, and emotional/behavioural functioning are not yet clear. Nevertheless, a primary candidate region that may play a central role here is the amygdala. Besides the amygdala being a neurobiological substrate of stress, is a hub for emotion processing circuits (Davis and Whalen, 2001). Accordingly, imaging genetics studies utilizing functional magnetic resonance imaging (fMRI) have supported the relationship between some genes, such as the serotonin transporter (SLC6A4) and amygdala reactivity to emotional stimuli (Stuhrmann et al., 2013). Despite immune-related genes have not been widely studied within the context of amygdala reactivity, there is evidence revealing a significant association between *IL1* genotype and amygdala responsiveness during emotion processing (Baune et al., 2010). Furthermore, a recent study also found, in a sample of healthy adults, a significant GxE interaction

between *IFNG* genotype (rs1861494) and maltreatment in relation to amygdala reactivity to negative emotional stimuli (Redlich et al., 2015). Additional analysis specifically regarding to the SNP used in the study presented in Chapter 4 yielded a main effect of rs2430561 on the middle cingulate gyrus and the supplementary motor cortex. At the time, these interactions were not studied in light of the differential-susceptibility model. Here, another direction for future research.

V. CLINICAL IMPLICATIONS

In general, our findings are consistent and reinforce empirical evidence from previous studies, showing that emotional and behavioural problems are displayed as early as the preschool age, and revealing a set of identified risk factors, both biological and environmental, related to a child's emotional and behavioural functioning.

Indeed, children's mental health has received increased attention in recent years. International organizations (World Psychiatry Association, World Health Organization, & International Association for Child and Adolescent Psychiatry and Allied Professions, 2005) have been calling for attention to the need to act on child mental health, through more science-based mental health services. Specifically by providing a comprehensive, integrated, and responsive mental health and social interventions in community-based settings for early detection and evidence-based management of childhood mental health disorders. Interestingly, and based on the evidence boosted by a large body of research on the relevance of parenting on child development, the World Health Organization, in its guidelines, emphasises parenting as a fundamental dimension when addressing child mental health (WHO, 2016). In fact, particularly in Chapter 2, our findings highlight the role of maternal interactive behaviours in the relationship between family risk and

emotional and behavioural problems, calling attention to the influence of quality interactive behaviour in shaping a child's emotional and behavioural functioning in contexts outside the family, such as preschool. Finally, our results shed some light on important components of mother-child interaction that should be considered in parenting programmes for families with a psychosocial risk. Indeed, several studies have demonstrated the effectiveness of interventions aimed at improving parental behaviours and socio-emotional competences in preschool children (Landry, Smith, Swank, & Guttentag, 2008; Lees, Fergusson, Frampton, & Merry, 2014). Stimulatingly, some evidence has shown that parenting programmes are cost-effective (Bonin, Stevens, Beecham, Byford, & Parsonage, 2011; Sampaio et al., 2018). Accordingly, findings from independent research in economics, neuroscience and developmental psychology have concluded that the financial return from early interventions is high, and the return from later interventions is low (Heckman, 2006). Although adaptation continues throughout life, human abilities are formed in a predictable sequence of sensitive periods, during which the development of specific neural circuits and the behaviours they mediate are most plastic and therefore optimally receptive to environmental influences (Knudsen, Heckman, Cameron, & Shonkoff, 2006).

Beyond this focus on environmental experiences, and as revealed by our GxE approach, to discuss clinical implications requires another level of analysis. If there is no doubt that family factors play an essential role in the aetiology and maintenance of emotional and behavioural problems in childhood, it is also clear that genetic factors can moderate the impact of environmental experiences on child functioning. Two arguments are often put forward to demonstrate why GxE studies may be helpful. First, they increase our understanding of underlying pathophysiological mechanisms of a particular disorder. Second, they may inform clinical practice by identifying high-risk groups that might

benefit from targeted interventions (Munafò et al., 2014). In fact, GxE studies offer a wider and deeper understanding of the underlying pathophysiology, revealing the role of different molecular pathways and neural circuits and allowing for the development of more targeted prevention and treatment strategies (Halldorsdottir & Binder, 2017).

How do GxE models help to explain the phenomenon that the present dissertation addresses? They focus on person-environment interactions, advancing the role of organismic characteristics in moderating the effects of both stressful and supportive environmental conditions on human development. The most recent GxE perspective on developmental psychopathology assumed that there are individual differences in the sensitivity to environmental influences. Moreover, GxE studies have also begun to further elucidate the neurobiological processes involved in translating genetic predispositions into behavioural outcomes, something that was not explored in the present dissertation. Here, a limitation of study presented in Chapter 4. Identifying links between GxE and brain function is, indeed, important, not only to establish a mechanistic foundation for individual differences in behaviour but also to understand the pathways through which GxEs shape the risk for emotional and behavioural functioning. The integration of genetics, brain and behavioural variables will allow us to understand that common functional polymorphisms in a specific gene can bias the processing of environmental experiences within a neural circuitry, underlying the emergence of some emotional/behavioural disorders. A good example of the relevance of this multilevel approach is the 5-HTTLPR gene. Recent empirical evidence has focused on some possible differential-susceptibility related mechanisms at different levels of analysis. For instance, positive and negative attentional biases (Pergamin-Hight, Bakermans-Kranenburg, van Ijzendoorn, & Bar-Haim, 2012), reward sensitivity (Roiser, Rogers, Cook, & Sahakian, 2006), accurate processing of emotional faces (Jacobs et al., 2011),

and amygdala reactivity to fearful faces (Alexander et al., 2012) have been found to be moderated by *5-HTTLPR* in a for better or for worse manner, depending on the environmental context. With specific regard to withdrawn behaviour, as introduced in Chapter 4, there are no studies addressing this phenotype using the GxE approach. In the future, it would be interesting to replicate the plastic properties found here for *IFNG* SNP, and also to explore neurobiological correlates so as to better understand this behaviour at such an early age.

VI. CONCLUSIONS

The studies presented in this dissertation were designed to analyze the effects of family risks and quality of maternal care and their respective action mechanisms on child's emotional and behavioural problems during the preschool age, whilst, on the other hand, aiming to go beyond the environmental influence and examine the GxE interaction, exploring the contribution of an immune-related gene on the aetiology of emotional and behavioural problems.

Both environmental influence and GxE interaction were described in this study as aetiological factors, taking into account the impact of family context, maternal functioning in terms of interactive behaviours and psychological distress, as well as the genetic make-up of the child with specific regard to an immune-related gene, among the Portuguese population. Together, the data presented herein can give new insight into the development of emotional and behavioural disorders at this early age, in which mechanisms of environmental mediation and genetic moderation are of relevance.

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