Genes, environment and their interplay in the development of psychopathological characteristics and their neuroimaging correlates in general population

Genes, ambiente y su interacción en el desarrollo de características psicopatológicas y sus correlatos de neuroimagen en poblaicón general

Silvia Alemany Sierra

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in solicitation of the degree of
Doctor by the University of Barcelona

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This Predoctoral Research has been performed thanks to several subsidized projects and entitites:

National:

- Ministerio de Ciencia e Innovación (SAF2008-05674-C03-00 y PSI2008-05988)
- Plan Nacional sobre Drogas. Ministerio de Sanidad y Consumo (2008/090) (2009/2011)
- Generalitat de Catalunya. Grup de Recerca Consolidat (SGR-827-2009)
- Ministerio de Ciencia e Innovación, Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Salud Mental, CIBER de Salud Mental (CB07/09/0037)
- Ministerio de Sanidad y Consumo, Ayuda Predoctoral de Formación en Investigación en Salud (FI07/00272) (Enero 2008-Diciembre 2011)

International:

- European Twin Study Network on Schizophrenia – 6th Framework Programme on Research, Technological Development and Demonstration, European Commission (MRTN-CT-2006-035987)

ACKNOWLEDGEMENTS

Neither this work nor the manuscripts of the thesis would have been possible without the help and support of colleagues, participants, friends and family. I would like to thank you all for accompanying me in the development of my thesis, probably one of the most relevant experiences of my life.

A mi directora de tesis, la Dra. Lourdes Fañanás, le agradezco que depositara su confianza en mí y en mis posibilidades desde el primer momento. Lourdes, aprecio mucho que me hayas dado libertad y espacio para desarrollar mi iniciativa. Siempre has escuchado mis propuestas y mis dudas con paciencia. Me has guiado aún y cuando yo no era consciente, porque, como sabes, tu visión suele estar bastantes más pasos por delante de lo que uno es capaz de concebir. He aprendido muchísimo junto a ti y lo que he aprendido me acompañará toda la vida, tanto a nivel profesional como personal.

A mis compañeros/as de equipo. Bárbara, Mar, gracias por vuestro apoyo y ayuda en todos los pasos que uno va dando cuando entra en un equipo: el primer *abstract*, la primera beca que pedir, el primer congreso, los *brainstormings*, las primeras risas nerviosas cuando se acerca el *deadline*, etc. He aprendido mucho con vosotras, sois un valioso ejemplo para mí y aprecio todas las discusiones científicas que he tenido la oportunidad de compartir con vosotras. A Bárbara le quiero agradecer especialmente toda su ayuda y apoyo durante la redacción de esta tesis. A parte del trabajo, estáis presentes en muchos de los buenos recuerdos que tengo de esta etapa. A Sergi, porque aunque compartí poco tiempo con él, formó parte de mi bienvenida al equipo y puedo decir que es un excelente compañero y persona. A Araceli por introducirme en el maravilloso mundo de los estudios de gemelos y ayudarme siempre. A Ximena, compañera de tantos viajes y muestreos. He aprendido muchísimo de ti y de tu experiencia clínica en las evaluaciones. Además, ha sido un placer compartir contigo la aventura de buscar gemelos y acudir a reuniones, congresos y *workshops* diversos. Me siento muy afortunada de haber tenido no sólo una persona profesional, inteligente y sensible a mi lado, sino por tener también una excelente compañía. A Mariajo, la alegría personificada con una paciencia infinita suficiente para ayudarnos y cuidarnos a todos y además hacer su trabajo eficientemente; gracias por estar siempre ahí para dar o recibir un achuchón, echarse unas risas con una infusión entre las manos y sobre

todo, por tu enorme comprensión y apoyo incondicional. A Marina, mi compañera infatigable de congresos y presentaciones orales en los últimos años. Adoro tu personalidad, la energía y la vitalidad que desprendes. Gracias por ayudarme con una sonrisa las cien veces al día que te interrumpo. Cada día aprendo más de ti y contigo, ¡eres un sol!

A mis queridas Mari, Nadia, Gemma, Helen y Virginia. Con todas vosotras también he tenido la gran suerte de trabajar y compartir viajes y muestreos. He aprendido tanto de vosotras y vuestro apoyo ha sido tan importante que –sorprendentemente- me quedo sin palabras. Mari, una noche de viernes allá por el 2006 vi de cerca la muerte pero allí estuviste tu para pedir unas pizzas y me conquistaste para siempre! Gracias por ayudarme tantas veces durante este largo viaje, eres una compañera y amiga excelente, adoro tu alegría, tu inteligencia y tu generosidad, te debo mucho. Nadia, guardo recuerdos inolvidables de nuestras aventuras en *terres de bessons*! Recuerdo perfectamente dónde nos conocimos y conectar contigo enseguida, poco a poco ha ido creciendo un vínculo muy especial entre nosotras y te siento siempre cerca. He aprendido muchísimo de ti, de tu desparpajo y tus habilidades sociales excepcionales, eres valiente, generosa, divertida y un espíritu libre. Sin ti esta tesis no hubiese sido posible. Gemma, me encanta tu forma de ser y tu actitud ante la vida, he sentido tu apoyo en momentos difíciles, me haces ver las cosas desde otra perspectiva y eso es algo que simplemente adoro. Virginia, Londres no hubiera sido lo mismo sin ti… ¿te acuerdas de las carreras para coger el 45? De unos spaghetti carbornara en el Zizzi o unos helados en el Scoop? Quizá no recuerdes algunos detalles porque ¡siempre estamos hablando! Todas esas charlas me han ayudado a crecer y siempre siento tu apoyo (¡y además eres melliza!). Helen, I know you are always ready for a nerd session but for some bravas too! I learnt a lot with you and you have shown me that I can keep asking you for your wise advices, you are incredibly kind, sweet and generous (and you are a twin too!). Gracias por ser como sois, os admiro, os adoro y os quiero.

A Anna Valldeperas, moltes gràcies per la teva espontaneïtat, el teu suport i les teves inesgotables ganes d'ajudar. A Claudia y Aldo gracias por estar dispuestos a ayudar y por vuestro apoyo, os deseo lo mejor en el camino que estáis iniciando.

A l'Àlex, no només t'haig d'agrair el teu ajut imprescindible en tirar endavant els primers anàlisis de neuroimatge en bessons, t'haig d'agrair la teva paciència i el teu interés escoltant tots els meus dubtes, idees i reflexions; tot i que al final les nostres converses han anat molt més enllà de la part científica... Ha estat un plaer compartir amb tu una part molt important d'aquest treball. Eloy, significa mucho para mí llegar a un laboratorio nuevo y encontrarme con alguien tan amable como tú, siempre dispuesto a ayudar. Gracias por tu generosidad y apoyo. Gracias a ti también Elena, por escucharme, animarme y simplemente estar ahí con tu sonrisa, ternura y buen humor.

A Carles Falcón, gràcies pel teu temps i generositat. A la Nuria Bargallo, gràcies pel teu ajut en tot moment. També agraïr a César Garrido el seu ajut en el protocol de neuroimatge i a ell i a Santi Sotés els agraeixo moltíssim la seva amabilitat amb els participants de l'estudi.

To Fruhling Rijsdijk, I spent a great time with you in London and I learnt a lot from you both at a professional and at a personal level. You not only taught me how to model twin data (a big challenge for me) but also that it is possible to do research the way you think it should be done, I love that and I admire you. To Claire Haworth and especially to Robert Plomin, I am deeply grateful to you for giving me the chance and the honour of working with you and be involved in the amazing project, the TEDS study.

To Igor Nenadic, thank you very much for all your support and help. I learn a lot and I meet very nice colleagues at the workshops organized by the EU-TwinsS project.

A Víctor Peralta y Salvador Miret, muchas gracias por vuestra acogida en las estancias que he realizado junto a Ximena en vuestros hospitales. Ha sido un honor veros "en acción".

A Pedro por estar siempre dispuesto a ayudarme y escuchar pacientemente mis dudas. Muchas gracias por tu apoyo.

Als meus estimats bessons, participants d'algunes mostres utilitzades en aquesta tesi, especialment agraïr la paciència i generositat (i la valentia) dels que han estat avaluats més d'un cop, especialment aquells que s'han fet la ressonància magnètica. Gràcies pel vostre temps, la vostra bona disposició i actitud col·laborativa totalment desinteressada. Sense gent com vosaltres la recerca simplement no seria possible. M'heu ensenyat que hi ha molta gent maca i bona en aquest món, disposada a ajudar sense esperar res a canvi. Sou simplement gent extraordinària. En especial, vull agraïr l'ajuda rebuda dels bessons Jaume i Ricard Rafecas amb els quals a més a més, tinc l'honor de mantenir una bonica amistat juntament amb la meva estimada Nadia (No oblidaré mai l'himne dels bessons!). Sou excepcionals, tant de bo hi haguès més persones com vosaltres al món i també vinguessin per duplicat!

A todos mis amigos y familia, especialmente a Sara, Noemi, Júlia, Ferran, Demian, Aniol, Isaac y mi querida familia adoptiva, Marga, Paco y Karen. Gracias por escuchar pacientemente, incluso con interés (:P), mis aventuras y desventuras y mis entusiasmadas explicaciones sobre mis temas favoritos como los tipos de gemelos, enfermedad mental y un largo etcétera. Dais sentido a mi vida. Os quiero.

A mis padres, Salva y Paquita, mis "entrenadores". Algo que he estudiado en esta tesis (quién me lo iba a decir…) ha sido cuán importante es tener "buenos" padres y yo he tenido la gran suerte de ser vuestra hija. Muchas gracias por ayudarme y apoyarme incondicionalmente siempre y por vuestros sabios consejos (por algo sois "el consejo de sabios"), aún sabiendo que al final haré lo que quiera… me habéis enseñado a pensar y a ser crítica, a que todo se puede hablar, a que muchas cosas se pueden cambiar y a valorar cada día todo lo positivo que uno tiene y tratar de hacer las cosas lo mejor posible. He crecido y sigo creciendo junto a vosotros, sois mi ejemplo, mi guía y mi fuente de apoyo y motivación. Us estimo molt *plumis*!

A mi Héctor, el "Sam" de esta historia, animándome en los momentos bajos… cuando el anillo pesaba demasiado siempre has estado ahí para decirme que juntos, podemos con todo. Gracias por ser como eres, por tu honestidad y autenticidad, por estar a mi lado, por hacer que se me ilumine la cara y se me olvide el mundo cuando estoy contigo. Te adoro, te quiero y quiero seguir trazando el camino de nuestras vidas juntos, nos lleve dónde nos lleve porque si estás tú, lo tengo todo.

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1. General Introduction

1.1. Brain development and the role of the environment

Brain development is largely guided by genetic factors, but the final form is sculpted by environmental factors and early experience.

(Tomoda, 2011)

From an evolutionary perspective, it has been suggested that brains evolved as devices to avoid hazards and threats to the organism's survival, as well as to evaluate the environment for extractable resources (food, shelter and mates). At some point during evolution, brain became an organ that not only prepares the organism to *react* to environmental fluctuations but also allows *active* exploration of the environment (Brüne, 2010).

In a sense, the evolution of the brain is the history of maximizing the capacity to exploit and manipulate the environment, including social environment which is particularly important for social animals including human beings. However, the evolution of brains has always been constrained by the brain's energy consumption because neurons are highly expensive in energetic terms. They have to maintain the ionic balance between themselves and their environment, and also need a lot of energy to synthesize transmitters for communication between nerve cells. The human brain contains an estimated 100 billion neurons (Brüne, 2010). It has been estimated that $1mm^3$ of human cortex contains about 44,000,000 neurons comprising 150m of dendrites, an additional 100 m of axonal connections and 50 million of synapses (Brüne, 2010).

A big brain (such as the human brain compared to other species) develops slowly, because the wiring process requires a lot of environmental input over extended periods of time, and thus constrains the reproductive potential of its bearer. In order to reach

adulthood, an immature organism (such as human children) has to be protected from environmental hazards such as predation or starvation. From an evolutionary perspective, this immature organism is protected by adult individuals in whose genetic interest it must be that the offspring reproduces, therefore, the parental effort in raising offspring increases proportionally with the immaturity of descendants.

Biologically speaking, the most crucial task for any newborn is to manage to survive childhood in order to reach sexual maturity. Human babies face this challenge with the problem of being extremely immature at birth. If human babies were at birth as mature as chimpanzee babies, the human gestation would last approximately 22 months. Human preterm parturition represents an evolved design compromise, which is ultimately linked to the evolution of bipedalism. Briefly, upright walking was accompanied by a change of the human pelvis anatomy which leads to a narrowing of the birth canal. Narrow birth canals were not a problem for ancestral hominoid species who have smaller brain sizes of about 450 cm^3 (*australopithecus afarensis*) and hence small skulls too. However, the successive increases in brain size (*Homo sapiens sapiens* have a brain size of about 1350 cm^3) needed an antedating parturition, which came at the expense of greater immaturity of offspring at birth.

Due to preterm birth, human newborns are less precocial. Precociality refers to the ability of offspring to move on their own legs soon after birth. Altriciality by contrast, embraces signs of immaturity such as sealed eyes and ears at birth, immobility and nakedness. These features indicate that the baby needs a period of development in a nest, burrow or den. The inability to move on their own and to actively seek comfort and shelter are altricial features of human newborns. Human babies are actively carried by their own mothers. This can only be possible in conjunction with bipedalism, which enables the mother to carry the baby and others things such as food (Brüne, 2010).

This special situation implies that the formation of the dyad between mother and offspring became one of the most crucial psychological adaptations in early humans. Successful attachment on the baby's side and bonding on the mother's side have remained vital for psychological well-being throughout human evolution to the present day in every known culture. In addition, the dependence of the mother on helpers increased proportionally, such that close cooperation between women, between mother and father of the child and kinship alliances were positively selected (Brüne, 2010).

1.1.1. Brain development

It is widely accepted that development should be full of successful achievements of each and every milestone.

The human foetus possesses a primitive brain by 3 weeks after conception (fertilization of the egg). This primitive brain mainly consists of a sheet of cells at one end of the embryo which rolls up to form the neural tube (Fig 1). The body and the nervous system change rapidly in the next 3 weeks of development (Stiles, 2011). By 7 weeks, the embryo begins to resemble a miniature person and by about 100 days after conception, the brain looks distinctly human. By

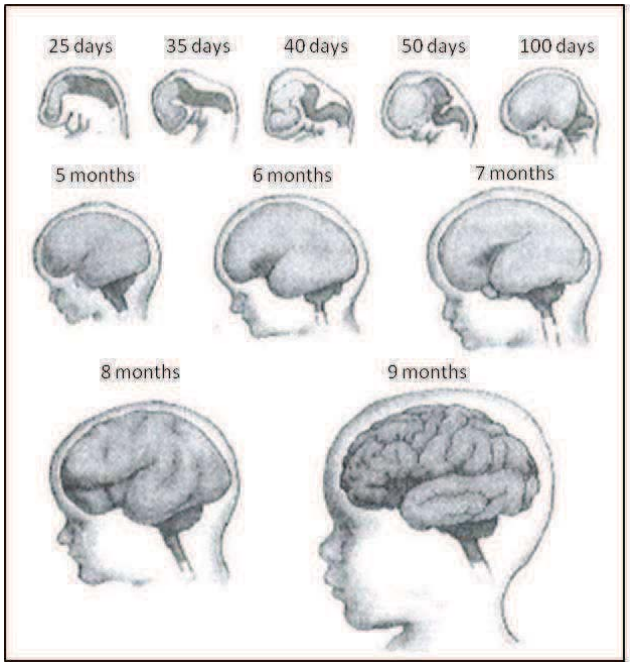

Figure 1. Embryonic and fetal stages of development of the human brain (Kolb and Wishaw, 2003).

the end of 9th month, the brain has the gross appearance of the adult human organ, even though its cellular structure is different (Fig 1).

The program of development has two features. First, subcomponents of the nervous system are formed from cells whose destination and function are largely predetermined before they migrate from the ventricular wall where they originate. Second, development is marked by initial abundance of cells, branches and connections with an important part of subsequent maturation consisting of cell death or pruning back of the initial surfeit (Kolb and Wishaw, 2003, Stiles, 2011).

The stages of brain development include: cell birth, cell migration, cell maturation, formation of synapses and formation of myelin (Kolb and Wishaw, 2003). In the first stage of cell birth (neurogenesis; gliogenesis), cells lining neural tube are known as neural stem cells. Stem cells, cells with an extensive capacity for self-renewal, give rise to progenitor (precursor) cells which, eventually, produce neuroblasts and glioblasts which mature into neurons and glia. The second stage of cell migration and cell differentiation is characterized by the production of neuroblasts destined to form the cerebral cortex is largely complete by the middle of gestation ($4^{1/2}$ months), whereas the migration of cells to various regions continues for a number of months even postnatally. Neuronal migration in the cerebral cortex develops from the inside out, like layers being added to a ball. If migration stops prematurely, a group cells that belong to an outer layer might be scattered among inner layers of cells. Cell differentiation is essentially complete at birth, although neuron maturation, which includes the growth of dendrites, axons and synapses, continues for years (Abrous et al., 2005, Blakemore, 2012, Lenroot and Giedd, 2006). Dendritic development begins prenatally in humans and it continues for a long time after birth. The third stage of cell maturation (dendrite and axon growth) takes place after neurons have migrated to their final destinations and differentiated into specific neurons types. They must begin

the process of growing dendrites to provide the surface area for synapses with other cells. Compared to development of axons which grow at the rate of a millimetre per day, dendritic growth proceeds at a relatively slow rate (micrometers per day). This difference in development allows faster-growing axon to contact its target cell before the dendrites of that cell are completely formed and enabling the axon to play a role in dendritic differentiation. Axons have specific targets that they must reach so that the neuron can survive and become functional. In the fourth stage of formation of synapses (synaptogenesis) and cell death (synaptic pruning), there are numerous synapses in the human cerebral cortex, on the order of 10^{14}. Formation of synapses starts early in embryonic life with low-density synapses which are generated independently of experience. After birth, the number of synapses grows rapidly. The rate in the macaque peaks at about 40,000 synapses per second. In humans, this phase continues until nearly 2 years of age. Then, an initial stop in synapse number is followed by a rapid elimination of synapses that continues through puberty. The rate of synapse loss may be maximal during puberty. The reduction in synapses may be 50% of the number present at age 2. Just as synapses can be formed very rapidly furing development, they may be lost at a rate of as many as 100,000 per second in adolescence. Is not surprising that teenagers are moody when their brains are undergoing such rapid changes in organization. After birth, synapses are formed both by *experience-expectant* and by *experience-dependent* mechanisms (Greenough et al., 2001, Kleim and Jones, 2008, Withers et al., 2011). Experience-expectant means that the synaptic development depends on the presence of certain sensory experiences for the organization of cortical circuits. Experience-dependent refers to the generation of synapses that are unique to an individual organism, because they are produced in response to experiences that are unique and personal. Adulthood is characterized by a plateau in synapse number through middle age, followed by a slow, steady decline in the density of synapses with

advancing age and a final rapid drop during senescence before death. This phase is experience dependent. Finally, in the last stage of formation of myelin (myelogenesis), the birth of glial cells (astrocytes and oligodendrocytes) begins after most neurons are born and continues throughout life (Andersen, 2003). Although axons can function before they are encased by myelin, normal adult function is reached only after myelination is complete. Consequently, myelination is useful as a rough index of cerebral maturation.

The timing of these stages described by Kolb and Wishaw (2003) is variable, some of them should be completed at time of birth but others extend into the postnatal period, even into adulthood (Fig 2) (Marsh et al., 2008). Development is biologically probabilistic rather than biologically predetermined. Brain development involves predictable neuronal migration but this leads only to an approximation to the optimal

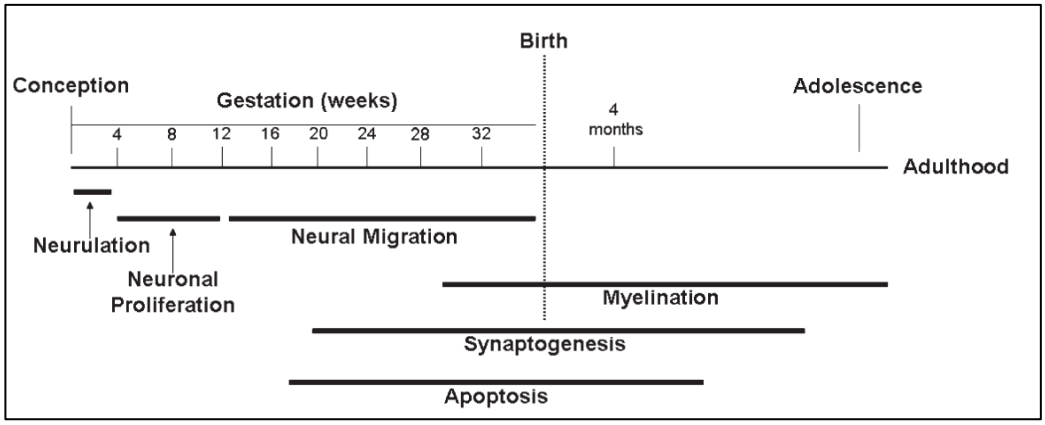

Figure 2. Major events during brain development. Brain development proceeds in a sequence that begins with neurulation, followed by neuronal proliferation, neural migration, and apoptosis. The sequence ends with synaptogenesis and myelination, which continue into adulthood (Marsh et al., 2008).

end product (Rutter, 2003). Deficits in the genetic program, intrauterine insults, the influence of toxic agents among other factors may lead to peculiarities or errors in development which may lead to a wide range of undesired outcomes. Less-pronounced deficits may lead to learning disabilities or subtle changes in behaviour

(Kolb and Wishaw, 2003). In this regard, information about brain development, complete with its points of vulnerability or windows of opportunity, provides a starting point to understanding emergence, course and severity of psychopathology in general.

Regarding to behaviour and cognition, it is assumed that certain behaviours cannot emerge until the neural machinery for them has developed; however, when the machinery is in place, related behaviours develop quickly and are shaped significantly by experience. The first person to try to identify stages of cognitive development was the Swiss psychologist Jean Piaget (Piaget, 1947). By observing the behaviour of children, he made inferences about their understanding of the world. Four major stages of cognitive development were identified by Piaget (Table 1).

Table 1. Piaget's stages of cognitive development (Kolb and Wishaw, 2003).

Typical age range	Description of the stage	Developmental phenomena
Birth to 18-24 months	*Stage 1: Sensorimotor* Experiences the world through senses and actions (looking, touching, mouthing). Babies learn to distinguish between themselves and the external world, they come to realize that objects exist even when out of sight, and they gain some understanding of cause-and-effect relations.	- Stranger anxiety - Object permanence
About 2-6 years	*Stage 2: Preoperational* Represents things with words and images but lacks logical reasoning. Able to form mental representations of things in their world and to represent them in words and drawings.	-Pretend play -Egocentrism -Language development
About 7-11 years	*Stage 3: Concrete operational* Thinks logically about concrete events; grasps concrete analogies and performs arithmetical operations. Able to mentally manipulate concrete ideas such as volumes of liquid and dimensions of objects.	-Conservation -Mathematical transformations
About 12 + years	*Stage 4: Formal operational* Reasons abstractly.	-Abstract logic -Potential for mature moral reasoning

Brain changes that produce or accompany the progress in child cognition and behaviour are still a matter of research. One place to look for brain changes is in the relative rate of brain growth. After birth, brain does not grow uniformly but instead tends to increase in mass during irregularly occurring periods commonly called growth spurts. Consistent spurts in brain growth had been found from 3 to 10 months, as well as between ages 2 and 4, 6 and 8, 10 and 12, and 14 and 16+ years (Epstein, 1978). Brain weight is estimated to increase by about 5% and 10% in each of these 2-year periods. Brain growth takes place without a concurrent increase in the number of neurons; so it is most likely due to the growth of glial cells and synapses. Although synapses themselves would be unlikely to add much weight to the brain, the growth of synapses is accompanied by increased metabolic demands, which causes neurons to become larger, new blood vessels to form, and new astrocytes to be produced.

As articulated by several investigators, structure of the brain at any time is a product of interactions between genetic, epigenetic and environmental factors (Brüne, 2010, Lenroot and Giedd, 2011, Stiles, 2011). Exposures to stress during the development of the individual produce a mismatch between his or her capacities and the demands placed by the environment. This can result in compensatory physiological responses and behaviours that in time may affect brain structures and function. This can be part of a normal learning process, or, if the mismatch is too severe, can result in pathology (Lenroot and Giedd, 2006, Tomalski and Johnson, 2010).

1.1.2. Brain plasticity

Most human psychological mechanisms are "open programmes" that highly depend on appropriate environmental stimulation to develop properly. Accordingly, deficient environmental input may cause dysfunction (Brüne, 2010).

Environmental conditions affect nervous system functioning and development because the brain is pliable, like plastic, as suggested by the term brain *plasticity*. The capacity to change is a fundamental characteristic of nervous systems and can be seen in even the simplest of organisms, such as the tiny worm *C. elegans*, whose nervous system has only 302 cells. When the nervous system changes, there is often a correlated change in behaviour or psychological function. This behavioural change is known by names such as learning, memory, addiction, maturation, and recovery.

As the brain develops and neural systems mature, they become receptive and interact with particular aspects of environment, helping the organism build its behavioural repertoire. Such developmental sequences and behavioural patterns can be observed shortly after birth. The existence of highly plastic brief and defined periods of development, the outcome of which has long-term functional consequences, make time-points particularly vulnerable for disruption by environmental influences. Similarly, this concept suggests that exposures to the same environmental influence at different points in time may result in dramatically different outcomes (Leonardo and Hen, 2008). It is generally assumed that experiences early in life have different effects on behaviour than similar experiences later in life. The reason for this difference is not understood, however. To investigate this question, Kolb and colleagues placed animals in complex environments either as juveniles, in adulthood, or in senescence (Kolb et al., 2003). The authors expected that there would be quantitative differences in the effects of experience on synaptic organization, but they also found qualitative differences. Specifically, the length of dendrites and the density of synapses were found to be increased in neurons in the motor and sensory cortical regions in adult and aged animals housed in a complex environment (relative to a standard lab cage). In contrast, animals placed in the same environment as juveniles showed an increase in dendritic length but a decrease in spine density. In other words, the same environmental

manipulation had qualitatively different effects on the organization of neuronal circuitry in juveniles than in adults. A possible implication of such findings is that an animal whose brain is stimulated in development may more easily change its brain in response to experience later in life (Kolb et al., 2003). An example might be children who are exposed to different languages in development and who then learn additional languages in life more quickly than do peers whose early experience was unilingual (Kolb and Wishaw, 2003).

The fact that brain is plastic to environmental influences enables the individual to learn and adapt to the environment. At the same time though, some environmental influences may involve negative consequences such as stressful conditions, especially when stress occurs early in life.

1.1.3. Brain response to stress

Threats to well-being, whether physical or psychological, are components of life experience. Studies in animals and humans have shown that during both early childhood and old age the brain is particularly sensitive to stress, as abovementioned, probably because it undergoes such important neurobiological changes during these periods (Lupien et al., 2009).

Stress can be defined as a psychological condition in which the individual perceives or experiences challenges to physical or emotional well-being as overwhelming their ability and resources for coping (Gunnar and Quevedo, 2007). However, certain levels of stress may be *tolerable* or *positive*. This is in agreement with the conceptually guided taxonomy proposed by the National Scientific Council on the Developing Child (National Scientific Council on the Developing Child, 2007) based on three categories of stress experience: positive, tolerable and toxic. The aim of this taxonomy was to

differentiate normative life challenges that are growth promoting from significant threats to long-term health. Positive stress is characterized by moderate, short-lived increases in heart rate, blood pressure, serum glucose and circulating levels of stress hormones such as cortisol and inflammatory cytokines. The essential characteristic of positive stress is that it is an important aspect of healthy development that is experienced in the context of stable and supportive relationships that facilitate adaptive responses that restore the stress response system to baseline. Situations that may imply positive stress include falling in love, having a baby or get a job promotion. Tolerable stress refers to a physiological state that could potentially disrupt brain architecture (e.g. through cortisol-induced damage to neural circuits in the hippocampus) but is buffered by supportive relationships that facilitate adaptive coping. The defining characteristic of tolerable stress is the support provided by invested adults that helps restore the body's stress-response systems to baseline, thereby preventing neuronal disruptions that could lead to long-term consequences. Exam situations might constitute an example of tolerable stress. Finally, toxic stress refers to strong, frequent and/or prolonged activation of the body's stress-response systems in the absence of the buffering protection of stable adult support. Childhood maltreatment, which is discussed below, can be classified in this category. The defining characteristic of toxic stress is that it disrupts brain architecture, adversely affects other organs, and leads to stress management systems that establish relatively lower thresholds for responsiveness that persist throughout life, thereby increasing the risk of stress-related diseases as well as cognitive impairment well into the adult years (Gunnar and Quevedo, 2007, National Scientific Council on the Developing Child, 2007).

From a biological perspective, stress triggers the activation of a key system in the stress response, the hypothalamus-pituitary-adrenal (HPA) axis, culminating in the

production of glucocorticoids (Fig 3) (Lupien et al., 2009). When the brain detects a threat, a coordinated physiological response involving autonomic, neuroendocrine, metabolic and immune system components is activated. The hypothalamus region release corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP). This triggers the subsequent secretion of adrenocorticotropic hormone (ACTH) from the pituitary gland, leading to the production of glucocorticoids by the adrenal cortex. Receptors for these steroids are expressed through the brain; moreover, they can act as transcription factors and so regulate gene expression. Thus, glucocorticoids can have potentially long-lasting effects on the functioning of the brain regions that regulate their release. Furthermore, glucocorticoids are important for normal brain maturation: they initiate terminal maturation, remodel axons and dendrites and affect cell survival: both suppressed and elevated glucocorticoid levels impair brain development and

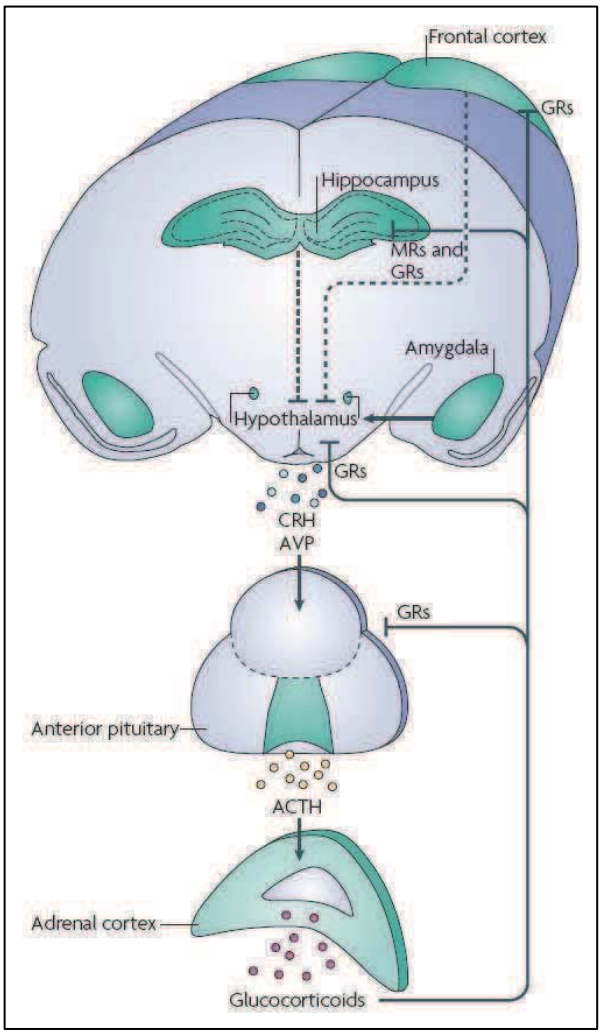

Figure 3. A key system in stress response is the hypothalamus-pituitary-adrenal (HPA) axis. When brain detects a threat, after a cascade of neurochemical events, glucocorticoids are produced and released by the adrenal cortex. Following HPA axis activation and once the stressor has been subsided, feedback loops are triggered in order to shut down the system and return to a set homeostatic point (Lupien et al., 2009). *Abbreviations: GRs, glucocorticoid receptors; CRH, corticotropin-releasing hormone; AVP, arginine vasopressin; ACTH, adrenocorticotropic hormone.*

functioning. Following the activation of the HPA axis, and once the perceived stressor has subsided, feedback loops are triggered at various levels of the system (that is, from the adrenal gland to the hypothalamus and other brain regions such as the hippocampus and the frontal cortex) in order to shut down and return to a set homeostatic point (Lupien et al., 2009).

By contrast, the amygdala, which is involved in fear processing, activates the HPA axis in order to set in motion the stress response that is needed to deal with the challenge.

There are other major systems and factors that respond to stress and affect and are affected by the HPA axis activity such as the autonomic nervous system, the inflammatory cytokines and the metabolic hormones. Individuals differ markedly, however, in the frequency with which they experience stressful life events and their vulnerability or resilience to stressful challenges. How genetic variants may account for interindividual variation in stress response will be discussed later.

Abnormal response to stress, including the development of depression and stress syndromes such as posttraumatic stress disorders, are attributed to failures in central nervous system (CNS) plasticity, more so in predisposed persons (Gottesman and Hanson, 2005). Chronic stress is implicated in CNS signal transduction cascades that normally allow neuronal plasticity. Chronic stress damages a wide variety of plasticity modulators and, at the biochemical level, causes a reduction in expression of genes associated with synaptic plasticity, resulting in diminished frontal cortical activity (Kuipers et al., 2003).

1.1.4. Exploring the brain: Neuroimaging techniques

Over the last three decades, a number of unbiased and objective techniques have been developed to characterize neuroanatomical differences *in vivo* using structural and functional Magnetic Resonance Imaging (MRI) (Mechelli et al., 2005). Neuroimaging techniques are being applied to psychiatry research since the 1970s when it was shown that patients with schizophrenia had enlarged cerebral ventricles (Johnstone et al., 1976, Linden, 2012). In the last decades, different neuroimaging modalities have been used in psychiatry including Structural Magnetic Resonance Imaging (MRI), Functional MRI (fMRI), Magnetic Resonance Spectroscopy (MRS), Positron Emission Tomography (PET), Single Photon Emission Computed Tomography (SPECT) or Diffusion Tensor Imaging (DTI). The focus of this section would be MRI and fMRI because these modalities were applied in studies included in the present dissertation. For many years, MRI has been a useful diagnostic tool for focal brain diseases such as tumour and stroke, but its utility for psychiatric diagnoses remains as a matter of research. Nevertheless, with the advances in MRI technology and quantitative measures of brain physiology, MRI is becoming a key component in psychiatric studies (Lu and Yang, 2009).

It is important to mention that MRI methods present a great advantage since they are minimally invasive and do not use radioactive materials. In MRI studies the source of the signal is the nuclei of biological molecules. The organism is made up of atoms, a large proportion of which is hydrogen. The nuclei of hydrogen behave like little magnets. These little magnets, when placed in a magnetic field align with it and rotate around the axis of the field in a movement called precession, similar to spinning tops on a table. This precession or turning movement is faster the higher the magnetic field is. If electromagnetic radiation, like radio waves, at exactly the same frequency of the

processing nuclei is emitted near them they can absorb this radiation, which is said to be at resonance, and they flip, becoming aligned in the opposite direction of the field. When the radiation is switched off the nuclei get rid of the energy they absorbed by emitting back the radiation. Each tissue of the body, because of its different chemical composition and physical state, re-emits radiation at a different rate, known as the tissue relaxation time. This radiation is picked up by an antenna, transforming it into electrical current, which is then used to construct the image we want. Because nuclei are used in a magnetic field and absorb radiation at resonance, the method is called Nuclear Magnetic Resonance Imaging. However because of the bad connotations of the word "nuclear" it has been dropped from the name and the method is usually know as Magnetic Resonance Imaging (MRI).

Whole brain images with a resolution of 1 x 1 x 1 mm^3 are now routinely acquired with scan duration of 4 to 8 minutes. This renders to MRI the capability of evaluating regional volumetric changes for various tissue types, such as gray matter, white matter and cerebrospinal fluid (CSF); this is especially useful for studies of psychiatric disorders because the involved brain regions may be relatively diffused and changes can be subtle. The typical magnetic resonance pulse sequence used is called magnetization-prepared rapid acquisitions of gradient-echo (MPRAGE) (Mugler and Brookeman, 1991) and the resulting images are T1-weighted, with clear contrast between gray and white matters (Fig 4) (Lu and Yang, 2009).

A widely used method to analyse MRI data is voxel-based morphometry (VBM). This processing strategy first uses spatial co-registration to normalize individual brains into the coordinates of a brain template (Ashburner and Friston, 2000), so that equivalent structures in different brains are roughly in the same location. Image segmentation is then performed to partition the normalized brain image intro gray matter, white matter and CSF. After segmentation, the signal intensity in the original

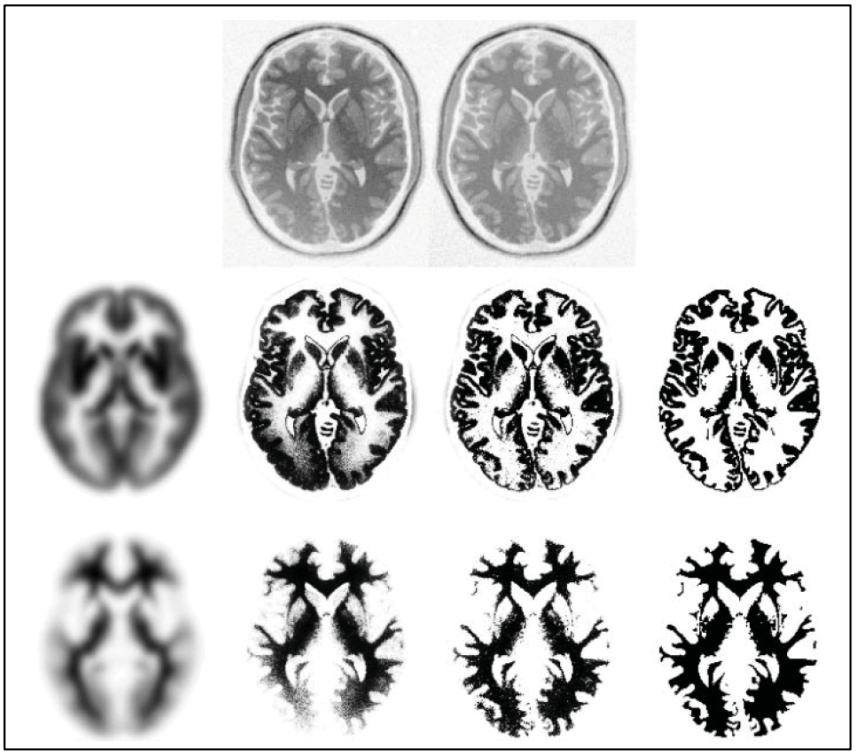

Figure 4. The top row shows the original simulated T1-weighted MR image with 100% nonuniformity and the nonuniformity corrected version. From left to right, the middle row shows the *a priori spatial distribution of gray* matter used for the segmentation, gray matter segmented without nonuniformity correction, gray matter segmented with nonuniformity correction, and the "true" distribution of gray matter (from which the simulated images were derived). The bottom row is the same as the middle, except that it shows white matter rather than gray. Without nonuniformity correction, the intensity variation causes some of the white matter in posterior areas to be classified as gray (Ashburner and Friston, 2000).

image is replaced by a value between 0 and 1, indicating the probability that the voxel belongs to gray matter, white matter or CSF. The next step is smoothing these mask images. Spatial smoothing allows the signal in a single voxel to reflect the concentration of the tissue in its surrounding areas. Following smoothing, statistical comparison is performed on a voxel-by-voxel basis to detect the regions that show significant changes in tissue concentration. VBM has been useful in characterizing subtle changes in brain structure in a variety of diseases associated with neurological and psychiatric dysfunction (Mechelli et al., 2005). Magnetic resonance techniques not only permit the study of brain structure but also the study of brain function. These

studies are known as functional MRI (fMRI) studies. The basis for fMRI concerns the fact that neuronal activity in the brain is accompanied by an increased consumption of glucose and oxygen. In addition, there are pronounced changes in blood supply to the activated regions, characterized by increased cerebral blood flow (CBF) and cerebral blood volume (CBV) (Lu and Yang, 2009). Increase in blood supply overcompensates for the increase in oxygen metabolism. As a result, the blood oxygenation in the draining veins and the capillaries is actually more oxygenated during the stimulation period compared to the resting state. This forms the basis of blood oxygenation level dependent (BOLD) fMRI signal (Ogawa et al., 1993). The haemoglobin in erythrocytes has different MR properties during the oxygenated and deoxygenated states. Deoxygenated blood is paramagnetic, which reduces the transverse relaxation times (T2 and T2*) of the water signal, whereas oxygenated blood is not paramagnetic. As a result, the MR signal is directly correlated with the amount of deoxyhemoglobin in the voxel (Ogawa et al., 1993). The BOLD effect on T2* is more pronounced than that on T2. As a result, the T2* weighted gradient-echo echo-planar-imaging (EPI) sequence is the most widely used pulse sequence. Of note, the BOLD signal is an indirect assessment of underlying neuronal activity, and its spatial and temporal characteristics will not completely match those of neuronal activity (Logothetis et al., 2001).

1.2. Psychopathological characteristics in general population

Abnormal behaviour and mental phenomena (i.e. disorders of experience and expression) constitute the object of psychiatry. At the phenotypic level, the term used is usually *phenomenology*. It is at this level that delineation of phenotypes is used for classification or as variables in empirical research. *Psychopathology* refers, in a general sense, to the empirical and theoretical study of anomalous experience, expression and action. Its goal is to offer a description, typology and general comprehension of anomalous mental states and associated forms of behaviour (Parnas et al., 2013). Psychopathology borders on an array of natural sciences, including genetics, epidemiology, neurobiology, neuroscience, and neuropsychology, as well as experimental and developmental psychology. Its history is, however, also marked by affinities to the humanities and social sciences, including philosophy and sociology (Parnas et al., 2013). A psychopathological description involves converting the patient's experiences (lived in the first-person perspective), or translating certain aspects of his/her expression and behaviour, into specific categories of symptoms and signs that are defined in third-person terms, thus providing "objective," sharable information for diagnosis, treatment, and research (Parnas et al., 2013).

By definition, abnormal behaviour and associated psychopathological signs and symptoms -which we broadly refer as mental disorders-, are maladaptive because they cause harm to the individual and are dysfunctional by their abnormal intensity (hypo-functioning, hyper-functioning or dysregulation), appearance in inappropriate context and/or abnormal duration (Brüne, 2010).

In every year over a third (38.2%) of the total European Union (EU) population suffers from mental disorders. Adjusted for age and comorbidity, this corresponds to 168.8 million persons affected. Among the most frequent we can find anxiety disorders

(14.0%) and major depression (6.9%). Disorders of the brain and mental disorders in particular, contribute 26.6% of the total all cause burden (Wittchen et al., 2011). Despite its frequency and associated economic and social cost, mental disorders are still not understood, recognized or even accepted as disorders in the community and the society.

In order to understand the stigmatisation of mental disorders and the difficulties inherent to the definition and comprehension of the causes of mental disorders we need to consider the history of psychiatry and psychopathology.

1.2.1. Brief overview of the history of the study of mental disorders and their classification

For a long time mental illness was seen as the result of personal, spiritual or moral failure or punishment by God, rather than caused by brain dysfunction or adverse experiences. Many mentally ill people were therefore incarcerated and exposed to cruelty (Brüne, 2010). The term "psychiatry" was coined by Johann Christian Reil (1759-1813) in 1808. In that time, in France, Philippe Pinel (1745-17826) and his pupil Jean Etienne Dominique Esquirol (1772-1840) were the first to challenge the common view that mental illness could not be cured and that mentally ill people had to be confined for their unpredictable behaviour and the protection of society. They introduced the *traitement morale*, characterized by empathy and compassion, and developed the first scientifically grounded psychiatric nosology. Also, Wilhelm Griesinger (1817-1868) published in 1845 one of the first scientific textbooks of psychiatry *Die Pathologie und Therapie der psychischen Krankheiten*, in which he emphasized the necessity to adopt a naturalistic perspective in psychiatry and to characterize mental illness as "disorders of the brain". In methodological terms, Karl

Ludwig Kalbhaum (1828-1899) developed the "clinical method" comprising unprejudiced behavioural observation, and an exhaustive recording and description of all psychic and somatic (physical) signs and symptoms. Kahlbaum's intention was to link the empirically acquired clinical material with neuropathological correlates, an aim, which remained unsuccessful in his time. Kahlbaum's most famous publications on *catatonia* and *hebephrenia* were later adopted by Emil Kraepelin (1856-1926) who coined the term *dementia praecox* which was later replaced by *schizophrenia* by Eugen Bleuer (1857-1939). Bleuler aimed to highlight the fact that not all patients had a poor prognosis associated with inevitable cognitive deterioration (Brüne, 2010). In this context, the publication of *Allgemeine Psychopathologie* by Jaspers in 1913 (Jaspers, 1997) provided a first systematic description of anomalous mental phenomena. Jaspers' vision of psychopathology places a decisive emphasis on phenomenology, in the sense of a systematic exploration of the patient's subjective experience and point of view. The object of psychopathology is the "conscious psychic event," and psychopathology consequently involves and requires an in-depth study of experience and subjectivity. Jaspers certainly acknowledges that "psychological phenomena" or "psychic events" must also be studied using methods of behavioural description and measures of performance, and in causal relationship with neural structures and processes (Parnas et al., 2013).

Late 19th and early 20th century witnessed a first wave of biologizing mental disorders, based on false biological premises and an almost complete lack of acknowledging social factors as causative for mental disorders. The pendulum swung back in the 1950s, when psychoanalytic theory became the dominating framework in psychiatry. Later, the advent of new diagnostic tools during the 1980s, brought a growing interest in the genetic underpinnings of psychiatric disorders, also in anatomical brain abnormalities, abnormal neurotransmission and correlates of

abnormal cognition, emotions and behaviour. Still, a strong movement sought to reformulate insights from psychoanalysis and behaviourism into a new concept of the understanding of psychiatric disorders being caused by adverse early experiences. These two conceptual perspective, the biological (genetics and neurotransmission) and the psychological (adverse interpersonal factors) have been long treated as opposite theoretical frameworks and have led quite solitary lives (Brüne, 2010). This is also known as the debate nature vs. nurture which will be discussed below.

Nowadays, both normal and abnormal emotional and behavioural phenomena are seen as the consequence of neural activity in the central nervous system. Similarly, it is widely accepted that both genetic and environmental factors and their interplay have to be considered in order to understand the aetiology of any complex characteristics such as psychopathological symptoms.

1.2.2. Categorical and dimensional approaches

Whether mental disorders should be classified and conceptualised in *categorical* or *dimensional* terms is still a matter of debate among clinicians and researchers. This debate has been recently energized by the preparation and revision of the next version of the *Diagnostic and Statistical Manual of Mental Disorder Fifth Edition* (DSM-V) by the American Psychiatric Association and the consequent renewed interest in nosology and diagnostics (Coghill and Sonuga-Barke, 2012).

On the one hand, those who propose a categorical approach regard mental disorders as qualitatively different from variation across the normal range of expression in the population, and as having their own pattern of rather distinct causes – *disorder differs from normality in both degree and kind*. On the other hand, the dimensional approach embraces those who regard disorder as an extreme expression

of normal variation in the population emphasise continuity in underlying causes – *disorder and normality differ only in degree but not kind* (Coghill and Sonuga-Barke, 2012).

The publication of the *International Classification of Disease* by the World Health Organization (ICD-9 (World Health Organization, 1993)) and the *Diagnostic and Statistical Manual of Mental Disorder Third Edition* by the American Psychiatric Association (DSM-III) (American Psychiatric Association, 1980)), represented the start of the modern era of classification. The way of identifying a disorder changed with the introduction of this category-based approach with clear and explicit criteria for making a diagnosis. Partially, the categorical view represented also an attempt to bring psychiatry closer to other medical specialities, as governmental and private insurance had cut reimbursements to psychiatry due to the confusion and lack of reliability of mental disorder diagnosis (Wilson and Mathew, 1993). Fundamentally, category-based approach has been widely adopted for its clinical utility (i.e. ease of use, ability to improve communication and inform treatment planning) and also served to guide scientific research. At the practical clinical reality, it is a clinician's job to make decisions about whether and individual should or should not receive specialist health interventions and which interventions they should receive. By definition, these are categorical decisions (Coghill and Sonuga-Barke, 2012).

However, psychopathological heterogeneity and comorbidity among other issues have undermined the categorical approach to classification (Sonuga-Barke, 1998). Comorbidity in psychiatry is common and appears to work against the concept of disorders as discrete entities with clear boundaries (Clark et al., 1995). Heterogeneity, which refers to the fact that not everybody with a disorder has the same pattern of symptoms or even defining features, together with the fact that some mixed symptom patterns fall between categories or just below diagnostic thresholds, are likely to impact heavily on the clinical utility of category-based diagnostic systems. The

problem is that if one seeks to reduce comorbidity by reducing categories, one is faced with increasing levels of heterogeneity and vice versa (Sonuga-Barke, 1998). The strict application of categorical diagnostic rules can also result in individuals with significant symptoms and impairments, but who fall just short of the diagnostic criteria, being denied support and treatment. Whilst many of the current criteria include definitions for subtypes, the true meaning of these groupings is often unclear and within a disorder the evidence for stability within these subgroups is poor, with individuals also moving between different subgroups over time (Lahey et al., 2005). These criticisms have been accompanied by calls to abandon current category-based approaches (Cuesta et al., 2009, Peralta and Cuesta, 2008).

In this regard, dimensional approaches that characterise disorders on a linear continuum of graded severity offer an alternative. Dimensions assume continuity between normality and psychopathology, presuppose linear quantification, and use internal empirical data to quantify and separate dimensions (Kendell, 1975). Advocates of dimensional approaches point out that they avoid waste of potentially important information associated with categorical approaches. Dimensions have been found to some times have greater predictive validity than do their diagnostic counterparts (Fergusson and Horwood, 1995). Other have simply argued that dimensional approaches are preferable as they provide a better fit with the data than the categorical ones (Coghill and Sonuga-Barke, 2012). Indeed, several researchers have shown that in some cases when categorical latent structure is identified, dimensional measurement approaches can still have superior psychometric properties and predictive validity (Peralta and Cuesta, 2008, Peralta, 2003). However, when these types of comparisons are expressed in this sort of way, it is tempting to suppose that empirical research should help deciding which approach is correct or valid. This is based on the misconception that there can be a universally "right" answer whereas this

is not the case. Rather than that, how we classify may vary according to the purposed that the classification is to be put (Rutter, 2007). Thus, for example, intelligence quotient (IQ) works best as a dimension if the interest is in predicting scholastic attainment or even social functioning in adult life. On the other hand, it works better as a category if the interest is in biological causes because the causes of severe mental retardation are likely to be different from those than concern individual differences within the normal range of IQ (Rutter, 2003).

In the field of psychopathology, epidemiological findings have been consistent in showing that most forms of common mental disorder show continuous distributions with no discernible point of demarcation between normality and psychopathology (Rutter, 2003). Indeed, it has been argued, on the basis of emprirical analyses, that the underlying liability distributions show very little evidence of nonnormality (van den Oord et al., 2003). Most people readily accept this argument for features such as depression or antisocial behaviour, but, until recently, severe disorders such as autism or schizophrenia have been thought of as entirely separate from variations within the normal range (Rutter, 2003).

1.2.3. Childhood and adolescence behaviour problems

Multivariate approaches to child psychopathology often distinguish between externalizing and internalizing problems and disorders (Achenbach, 1991). In this regard, childhood and adolescence behaviour problems often refers to externalizing or conduct problems but this concept can also include internalizing problems. Internalizing and externalizing problems may develop as early as early childhood and can place children on a developmental pathway to peer problems, negative interactions with parents, delinquency, and other negative social and behavioural outcomes (Coie

and Dodge, 1998, Kovacs and Devlin, 1998). Also, early emotional and behavioural problems have been found to precede child, adolescent and adult antisocial and depressive psychopathological problems (Moffitt, 1993).

Externalizing behaviour problems in childhood include hostile and aggressive physical behaviour toward others, impulsivity and hyperactivity and non-compliance with adult and peer limit setting (McMahon, 1994). These behaviours are often used by toddlers to solve conflicts with peers or playmates and with the development of cognitive abilities and the skills to regulate emotions, these externalizing behaviours tend to decrease over the preschool and school age period (Coie and Dodge, 1998). Indeed, the majority of children exhibit low levels of externalizing behaviour over time and most of the children with moderate to high levels of externalizing behaviour early in development exhibit decreases in behavioural problems after the preschool years. However, a small number of antisocial children, representing 5% to 7% of the population, do not outgrow the temper tantrums and the defiant and irritable behaviour that characterizes toddlers and follow a life-course persistent trajectory of externalizing problems (Moffitt, 1993). Among the risk factors and predictors of child externalizing behaviours, a wide range of family characteristics such as parental criminality and psychiatric disorder, parental discord, and critical, hostile or coercive parenting have been associated with conduct problems (Hill, 2002).

The clinical expression of externalizing behaviours from The *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV-TR) (American Psychiatric Association, 2000) perspective includes two patterns of behaviour problems which constitute two different disorders. On one hand, the Oppositional Defiant Disorder (ODD) is defined as a recurrent pattern of negativistic, defiant, disobedient and hostile behaviours leading to impairment of day-to-day activities. On the other hand, Conduct Disorder (CD) is defined as the repetitive and persistent violation of the basic rights of others

and societal norms. It is not clear yet how valid is the distinction between ODD and CD because the items in each diagnosis are age related (Hill, 2002, Angold and Costello, 2001). Furthermore, ODD and CD cannot be assumed to be distinctively different from other child emotional and behavioural problems. Numerous studies of general population and clinical samples have shown strong associations of childhood antisocial behaviours with attention-deficit and hyperactivity symptoms and symptoms of anxiety and depression (Angold et al., 1999).

Internalizing problems include somatic complaints, anxiety, depression, and social withdrawal. In contrast to externalizing problems, internalizing problems on average tend to increase gradually from infancy to early childhood with girls showing a higher increase in internalizing problems across time (Bongers et al., 2003). Symptoms of anxiety are quite common in childhood and adolescence, but their type and content vary with age. Anxiety problems change from separation anxiety in early childhood to social phobia or generalized anxiety in adolescence. Specific phobia has been described in children of all ages. For the total number of anxiety symptoms, no consistent gender or age differences have emerged (Bernstein et al., 1996, Bongers et al., 2003). For symptoms of depression, consistent age and gender differences have been found (Angold and Rutter, 1992). Prepubertal boys and girls show equal levels of depressive problems, but around midpuberty girls begin to exhibit more depressive problems, a trend that continues into adulthood (Angold et al., 1998). Children and adolescents do not differ in level of self-reported somatic complaints. In regard to gender differences, girls report more somatic complaints than do boys, and this difference continues into adulthood (Taylor et al., 1996). As with externalizing problems, exposure to a negative familial context has been associated with high levels of internalizing problems across development (Duggal et al., 2001).

Internalizing and externalizing problems are not separate entities, but, rather, are likely to co-occur (Angold and Costello, 1993, Beyers and Loeber, 2003, Wiesner and Kim, 2006). For instance, comorbidity rates of oppositional defiant disorder and conduct disorder in children and adolescents with major depressive disorder range from 21% to 83% in clinical and community samples (Angold and Costello, 1993). Furthermore, both sets of behavioural problems share common risk factors such as negative home environment, maternal depression or cognitive deficiencies (Angold and Costello, 1993, Rutter, 1997).

1.2.4. Anxiety and depression

Anxiety disorders

Anxiety disorders share psychological symptoms of subjectively highly distressing and excessive worry and anticipation of impending danger with the feeling of little chance to escape. At the physiological level, these symptoms are accompanied by tachycardia, hyperventilation, dizziness and nausea, and sweating. Unlike the relative mild, brief anxiety caused by a stressful event (such as speaking in public or a first date), anxiety disorders last at least 6 months and can get worse if they are not treated. Anxiety disorders commonly occur along with other mental or physical illnesses, including alcohol or substance abuse, which may mask anxiety symptoms or make them worse (Brüne, 2010).

As a group, anxiety disorders have a lifetime prevalence of up to 30% and a 12-months prevalence of about 15%, however, with considerable cultural variation (Brüne, 2010). A recent study in USA indicates that lifetime prevalence for Specific Phobia is 13.8%, for Social Phobia is 13%, for Panic Disorder is 5.2%; for Obsessive-Compulsive

Disorder is 2.7% and for Agoraphobia with or without panic disorder is 2.6% (Kessler et al., 2012).

Anxiety disorders are twice as likely to affect women as men. Onset of anxiety disorders is difficult to determine, because many individuals who later seek treatment for anxiety disorder has "precursor" symptoms as children, including "inhibited" temperament and avoidance behaviour. The average age at clinical manifestation is around adolescence or early adulthood and peaks towards the end of the third decade. Post-traumatic stress disorder is, by definition, highly dependent on environmental factors.

Family and twin studies have revealed mixed results regarding the involvement of genetic factors in the aetiology of anxiety disorders. First-degree relatives of index subjects have a three to five times higher risk of developing a disorder compared to controls. In all anxiety disorders, both shared and individual-specific environmental conditions play a major role. Concordance rates for PD in MZ twins have been reported between 40% and 70%, as compared to 0% to 20% in DZ twins (Brüne, 2010).

Anxiety disorders include panic disorder, obsessive-compulsive disorder (OCD), post-traumatic stress disorder (PTSD), social phobia or social anxiety disorder, specific phobias and generalized anxiety disorder (GAD). Main characteristics of some of these disorders are summarized below.

Panic disorder is characterized by sudden attacks of terror, usually accompanied by a pounding heart, sweatiness, weakness, faintness or dizziness. During these attacks, people with panic disorder may flush or feel chilled; their hands may tingle or feel numb and they may experience nausea, chest pain or smothering sensations. A part from these somatic symptoms, panic attacks usually produce a fear of losing control, sense of unreality, or a fear of impending doom. People having panic attacks sometimes believe they are having heart attacks, losing their minds, or on the verge of

death. Not everyone who experiences panic attacks will develop panic disorder. Panic disorder appears when the worry about having another episode interferes with the daily life of the person (American Psychiatric Association, 2000).

People with obsessive-compulsive disorder (OCD) usually present persistent, upsetting thoughts, impulses or images (obsessions) and use rituals (compulsions) to control the anxiety these thoughts and the urge to perform the rituals produce. The rituals may end up controlling the person because they are time consuming (more than 1 hour a day) or significantly interfere with the person's normal routine. Of note, the diagnostic requires the presence of obsessions and/or compulsions (sometimes patients only present one of these two symptoms). Obsessions are not simply excessive worries about real-life problems and they are experienced as intrusive and inappropriate, and cause marked anxiety or distress. Compulsions can be repetitive behaviours (e.g. hand washing, ordering, checking) or mental acts (e.g. praying, counting). To be considered compulsions, the aim of these behaviours or mental acts is to prevent or reduce distress. However, performing the rituals is not pleasurable and at best, they may produce temporary relief.

A specific phobia is an intense, irrational fear of something that actually poses little or not threat. Some of the more common specific phobias are heights, closed-in places, flying, dogs, spiders, and injuries involving blood. While adults with phobias realize that these fears are irrational, they often find that facing, or even thinking about facing the feared object or situation brings on a panic attack or severe anxiety. In children, the anxiety may be expressed by crying, tantrums, freezing or clinging. If the feared situation or object is easy to avoid, the individual may not seek help; but if avoidance interferes with their careers or personal lives, it can become disabling.

People with social phobia, also called social anxiety disorder, have an intense and persistent fear of one or more social situations in which they are being watched and

judged by others and of doing things that will be humiliating or embarrassing. Individuals with social phobia become overwhelmingly anxious and excessively self-conscious in everyday situations. This fear becomes so severe that it interferes with work, school and ordinary activities and can make it hard to keep social relationships.

Agoraphobia refers to the experience of anxiety in places or situations where escape might be difficult (or embarrassing) or in which help may not be available. The individual is afraid of having an unexpected PC or panic-like symptoms in the feared place or situation. Of note, in DSM-IV-TR agoraphobia is coded as Panic Disorder with Agoraphobia or Agoraphobia without History of Panic Disorder. Typically agoraphobic fears involve being outside the home alone, being in a crowd or standing in line, travelling in bus, train or automobile. In agoraphobia, the feared situations are avoided or are endured with marked distress or with anxiety about having a PD or panic-like symptoms or require the presence of a companion.

Early trauma including emotional and sexual abuse, parental neglect and heightened anxiety in parents comprise the most significant environmental risk factors for anxiety disorders. In addition, accidents, violence, and chronic exposure to life-threatening events also constitute important risk factors. Insufficiently developed coping strategies for stressful events, including low self-efficacy and feelings of poor control, enhance the risk for anxiety disorders, but may be the consequence of poor social support and discouraging parenting behaviour (Brüne, 2010).

Major Depressive Disorder

Major Depressive Disorder (MDD) (Table 2) ranks among the top causes of worldwide disease burden and disability, according to the World Health Organization, in the year 2020 major depression will be the second among the leading causes for

disability (Brüne, 2010). Lifetime risk for MDD is between 7–12% in men and between 20–25% in women (Kessler et al., 2005). The symptoms of unipolar depression include depressed mood, a loss of interest or pleasure in activities, marked change in weight or appetite, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness or guilt, difficulty concentrating or indecisiveness, and thoughts of death or suicide (Barrett et al., 2007). Age at onset peaks around the fourth decade, with a second peak in the sixth decade (Brüne, 2010).

Table 2. DSM-IV-TR diagnostic criteria for Major Depression (Brüne, 2010).

Major Depressive Episode

A. Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.
   Note: Do not include symptoms that are clearly due to a general medical condition, or mood-incongruent delusions or hallucinations.
   (1) depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g. feels sad or empty) or observation made by others (e.g. appears tearful)
   Note: In children and adolescents, can be irritable mood
   (2) markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others)
   (3) significant weight loss when not dieting or increase in appetite nearly every day
   Note: In children consider failure to make expected weight gains
   (4) insomnia or hypersomnia nearly every day
   (5) psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings or restlessness or being slowed down)
   (6) fatigue or loss of energy nearly every day
   (7) feelings of worthlessness o r excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)
   (8) diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)
   (9) recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide
B. The symptoms do not meet criteria for a Mixed Episode
C. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.
D. The symptoms are not due to the direct physiological effects of substance (e.g. a drug of abuse, a medication) or a general medical condition (e.g. hypothyroidism).
E. The symptoms are not better accounted by bereavement, i.e. after the loss of a loved one; the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation.

Studies into genetics of unipolar depression suggest a *continuum* between mild (reactive) depression and severe (melancholic or endogenous) depression. First-degree relatives of patients with unipolar depression have a relative risk for the disorder that is between 1.5 to 3-fold higher that the population risk. The concordance rate in monozygotic (MZ) twins is near 40% and roughly 20% in dizygotic (DZ) twins (Brüne, 2010). MDD is a highly heterogeneous disorder and is genetically more closely related to anxiety disorders than to bipolar disorder.

Stressful life events such as losses of important relationships by death or separation or events characterized by humiliation have been found to be particularly depressogenic (Farmer and McGuffin, 2003, Kendler et al., 2003). Gene-environment interaction studies have shown that some individuals might be genetically more vulnerable to the impact of early life stress such as childhood adversity regarding to the development of depressive symptoms (Aguilera et al., 2009, Nugent et al., 2011).

Comorbidity between anxiety and depression

Feinstein first introduced the term comorbidity in the medical literature in 1970 (Feinstein, 1970). The term refers to the presence of two or more distinct co-occurring disorders in an individual patient. In this regard, although depression and anxiety have historically been seen as distinct conditions; the two disorders are not mutually exclusive and often coexist to varying degrees in the same patient (Roy-Byrne et al., 2000, Belzer and Schneier, 2004, Gorman, 1996). Mixed anxiety-depressive disorder is defined by subsyndromal symptoms of both depression and anxiety in the DSM-IV-TR appendix of disorders for further study. Such category constitutes an attempt to response the critical issue of whether anxiety and depression are indeed distinct disorders that frequently co-occur, or whether they are diverse manifestations of a

broader underlying condition such as general negative affectivity (Belzer and Schneier, 2004).

In this context, there are a number of evidences which have lead some studies to consider anxiety and depression together (Ressler and Mayberg, 2007). First, it is well established that symptoms of anxiety and depression commonly co-occur, with estimations of the comorbidity ranging from 10% to more than 50% (Gorman, 1996, Ressler and Mayberg, 2007, Roy-Byrne et al., 2000). More than half of all individuals with MDD also develop an anxiety disorder during their lifetime (Kessler et al., 1996). Similarly, 10-65% of the individuals diagnosed with panic disorder (PD) experience comorbid MDD (Mosing et al., 2009, Wittchen et al., 2008). Second, there is an overlap of symptoms associated with both anxiety and depression which makes diagnosis classification particularly difficult (Gorman, 1996, Ressler and Mayberg, 2007). Third, the most powerful treatments for both disorders are the same, including antidepressants and cognitive behavioural therapy (Ressler and Mayberg, 2007). Fourth, it has been shown that major depression and several anxiety disorders strongly co-aggregate within families and common genetic factors partially explained a proportion of variance among these disorders (Mosing et al., 2009). Thus, it might be possible that some genetic variants might account for genetic risk for both anxiety and depression. Fifth, several lines of evidence suggest that affective and anxious symptoms arise from dysregulation of the limbic-cortical system that mediate stress-responsiveness (Cameron, 2006, Cameron et al., 2004, Ressler and Mayberg, 2007). In this regard, from a neuroimaging perspective the circuits involved in both sets of disorders can be difficult to distinguish (Ressler and Mayberg, 2007). All these evidences indicate that depression and anxiety may share a common etiological pathway (Belzer and Schneier, 2004, Gorman, 1996, Mosing et al., 2009, Ressler and Mayberg, 2007).

1.2.5. Psychotic experiences (PEs) and the psychosis continuum

Psychotic symptoms refer to the loss of contact with reality and constitute the common feature of psychotic disorders such as schizophrenia or paranoid disorder. Psychotic symptoms can be classified in three dimensions: positive, negative and disorganized. Positive psychotic symptoms involve delusions (fixed, false beliefs) and hallucinations (aberrant, false perceptions). Negative symptoms are deficit states in which basic emotional and behavioural processes are diminished or absent. Negative symptoms are more pervasive than psychotic symptoms and are strongly related to social dysfunction (Aleman and Kahn, 2005). Disorganized symptoms involve bizarre behaviour.

Psychotic symptoms have traditionally been viewed as dichotomous: patients are assessed as either having a particular symptom or not having it (Kwapil et al., 1999). Likewise, psychosis has been traditionally viewed as a categorical entity involving a qualitative change from normality to illness, an assumption hold by the main diagnostic systems such as the DSM-IV-TR (American Psychiatric Association, 2000) or the ICD-10 (World Health Organization, 1993). However, the traditional categorical definition of psychotic symptoms was challenged in 1969 by Strauss (Strauss, 1969). He argued that delusions and hallucinations can be viewed as points on a *continuum* of deviancy, rather than as dichotomous events. He reported that many of his patients had delusions or hallucinations that did not fully qualify as symptoms of clinical psychosis. Strauss also reported that remitted patients often continue to experience milder versions of their psychotic symptoms (Strauss, 1969). Since then, epidemiological, experimental and theoretical reasons have been put forward supporting the dimensional approach to psychosis phenotype.

In this regard the concept of *continuum* constitutes a good representation of variation in mental health in general population. Consequently, the psychosis phenotype has been suggested to occur along a *continuum*: the *psychosis continuum* (Johns and van Os, 2001, Van Os et al., 1999, Verdoux and van Os, 2002). According to this perspective, psychotic symptoms would be distributed along a continuum that extends from normality to schizophrenia (Diagnostic Criteria for schizophrenia is detailed in Table 3) with increasing level of severity (Van Os et al., 1999).

Table 3. Diagnostic Criteria for schizophrenia according to the DSM-IV-TR.

A. **Characteristic symptoms:** Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated): delusions, hallucinations, disorganized speech (e.g. frequent derailment or incoherence), grossly disorganized or catatonic behaviour, negative symptoms, i.e. affective flattening, alogia or avolition.

B. **Social/occupational dysfunction:** For a significant portion of the time since the onset of the disturbance, one or more major areas of functioning such as work, interpersonal relations, or self-care are markedly below the level achieved prior to the onset (or when the onset is in childhood or adolescence, failure to achieve expected level of interpersonal, academic or occupational achievement).

C. **Duration:** Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully treated) that meet Criterion A (i.e. active-phase symptoms) and many include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or two or more symptoms listed in Criterion A present an attenuated form (e.g. odd beliefs, unusual perceptual experiences).

D. **Schizoaffective and Mood Disorder exclusion:** Schizoaffective Disorder and Mood Disorder with Psychotic Features have been ruled out because either (1) no Major Depression Episode, Manic Episode or Mixed Episode have occurred concurrently with the active-phase symptoms; or (2) if mood episodes have occurred during active-phase symptoms, their total duration has been relative to the duration of the active and residual periods.

E. **Substance/general medical condition exclusion:** The disturbance is not due to the direct physiological effects of a substance (e.g. a drug of abuse, a medication) or a general medical condition.

F. **Relationship to a Pervasive Developmental Disorder:** If there is a history of Autistic Disorder or another Pervasive Developmental Disorder, the additional diagnosis of Schizophrenia is made only if prominent delusions or hallucinations are also present for at least a month (or less if successfully treated).

Subtypes of schizophrenia: Paranoid type, Catatonic type, Disorganized type, Undifferentiated type and Residual type.

Classification of longitudinal course: Episodic with/without residual symptoms, continuous single episode, partial/full remission, other or unspecified pattern.

Broadly, there are two potential approaches to the measurement of psychotic (subclinical) symptoms in non-clinical samples: one would be to measure schizotypal traits as an attenuated form of psychotic symptoms, while the other would involve measuring in the general population the occurrence of those symptoms that are seen in psychotic patients. The latter approach assumes that experiencing "symptoms" of psychosis is not inevitably linked with the clinical disorder. Thus, even though the prevalence of the clinical disorder is low, the prevalence of these 'milder forms' of psychosis, namely psychotic-like experiences or simply psychotic experiences (PEs), may be much higher (Johns and van Os, 2001, Stefanis et al., 2002). In this context, a growing body of research indicates that attenuated psychotic experiences are present in a substantial proportion of healthy individuals (Barragan et al., 2011, Kelleher and Cannon, 2011, Van Os et al., 2009). These evidences support the conceptualization of psychosis as a continuous phenotype the distribution of which extends into the general population. Thus, PEs might be present in some individuals from the general population who do not present a psychiatric disorder or need of treatment.

Specifically, the prevalence of psychotic symptoms in studies in community populations ranges from 4% to as much as 28.4% in the National Comorbidity Survey (Eaton et al., 1991). The NEMESIS study in the Netherlands showed that 17.5% of subjects in the general population reported at least one psychotic symptom (van Os et al., 2000). A recent study analyzed the cross-national prevalence of psychotic symptoms using the World Health Organization World Health Survey (WHS) data from a total of 52 countries from all regions of the world and different levels of economic development. In this large study, authors concluded that the prevalence of the presence of at least one psychotic symptom has a wide range worldwide varying as much as from 0.8% to 31.4% (Nuevo et al., 2012).

Furthermore, research aimed to study the risk factors underlying the expression of PEs can greatly contribute to the understanding of the psychotic disorders. First, it has been shown that psychotic experiences may precede the onset of psychosis. The seminal study by Poulton and colleagues demonstrated that 25% of participants with low-grade PEs at age 11 years developed a clinical psychotic disorders by age 26 years (Poulton et al., 2000). More recently, Domínguez and collaborators conducted a prospective cohort study in a general population sample of 845 adolescents (aged 14-17 years) and found that 40% of new onset, clinically relevant psychosis can be traced to the early subclinical psychosis phenotype in the general population (Dominguez et al., 2011b). Therefore, PEs can help to identify subjects at risk (Dominguez et al., 2011a, Kelleher and Cannon, 2011). Second, clinical and subclinical psychotic symptoms are likely to involve common risk factors in their aetiology (Johns and van Os, 2001, Kelleher and Cannon, 2011, Van Os et al., 2009). In this regard, recognized risk factors for psychotic disorders and schizophrenia such as childhood trauma (Janssen et al., 2004, Read et al., 2005, Van Winkel et al., 2008, Varese et al., 2012) or cannabis use (Anglin et al., 2012, Henquet et al., 2005, Houston et al., 2011, Manrique-Garcia et al., 2012, Van Os et al., 2010, Estrada et al., 2011) have been linked to psychotic symptoms or PEs in both clinical and non-clinical samples. Therefore, by studying the subclinical expression of psychosis in population samples it is possible to further understand the aetiology of psychosis without the bias of the treatment and the illness itself (Johns and van Os, 2001, van Os, 2003, Van Os et al., 2009).

1.2. Childhood environment and adult mental health

Every child has the right to health and a life free from violence. Each year, though, millions of children around the world are the victims and witnesses of physical, sexual and emotional violence. Child maltreatment is a huge global problem with a serious impact on the victims' physical and mental health, well-being and development throughout their lives – and, by extension, on society in general.

(World Health Organization, 2006)

1.3.1. Family environment and parental negativity

Because the home environment is a crucial developmental context for children, parental practices and their contribution to children's behaviour have been intensively investigated (Hiramura et al., 2010).

Childhood is a developmental period of rapidly growing neurological, physical and emotional systems. Infants also develop attachment bonds in the first months of life; the attachment figure, in turn, provides regulation of the infant's stressful arousal (Bowlby, 1980, Essex et al., 2001). Cummings and Cicchetti have also suggested that early attachment relationships contribute to a child's internal representation of self, critical in the development of healthy self-esteem (Cummings and Cicchetti, 1990). According to this theory, if early parenting care contributes to an insecure attachment relationship, the detrimental effects of insecure attachment relationship may continue after parenting risk has remitted. Further, it has been proposed that attachment figures have a greater role in the socialization of emotion regulation and expression during the first 2 years of life compared with later developmental periods (of note, this does not imply that social and emotional abilities of the children could not improve later in life).

For example, children may be less vulnerable to an onset of maternal depression occurring later in the child's development, as they have already developed effective internalized emotion regulation and coping strategies and a larger network of support figures (e.g. neighbours, friends and teachers).

Attachment theory posits that children are born with an attachment system that is activated when the child is in or perceives distress (Bowlby, 1969, Bowlby, 1980). When activated, this system leads proximity-seeking behaviours (e.g. crying) toward the caregiver who is most likely to provide comfort and protection. It has pointed out that adults have caregiving systems that are activated when children signal distress; activation of these systems, in turn, triggers sensitive behaviours toward the child. However, the caregiving system could be affected by many stressors such as socioeconomical difficulties, health problems and past experiences of abuse, which could lead to unresponsive parenting (George and Solomon, 1999).

In this regard, the quality of parenting exerts critical influence on children's social-affective development especially during the first years of life. Developmental psychopathology research has focused on two broad domains of parenting behaviours: a) positive parenting qualities such as warmth, responsiveness and synchrony and b) harsh or negative parenting marked by criticism and punitive discipline (Wade et al., 2011).

Positive parenting, such as parental warmth, has been associated with higher levels of peer acceptance and lower aggressive behaviour in children (Clark and Ladd, 2000, Davidov and Grusec, 2006, Mrug et al., 2008, Russell, 2003). Maternal sensitivity, usually defined as the mother's ability to recognize her infant's needs and to respond accordingly, is significantly associated with infant's security of attachment (Ainsworth et al., 1978). Other studies have shown that a sensitive rearing environment can help to

regulate infant's expression of negative affect in the first year of life (Belsky et al., 1991).

In contrast, parental negativity, harshness and criticism have been linked to poorer self-regulation and greater behaviour problems over time (Belsky et al., 1998, Kaiser et al., 2010, Nelson et al., 2006, Rubin et al., 2003). Children of difficult temperament who experience authoritarian maternal behaviour are more likely to demonstrate externalizing difficulties than children with difficult temperament who have less negatively controlling mothers (Bates et al., 1998, Rubin et al., 1998). Also, disorganized attachment patterns (conceptualized as a breakdown in attachment strategy, disorganized children show no coherent attachment pattern toward the parent, instead exhibiting contradictory behaviours or fearful reactions in response to reunion with the parent in the strange situation (Ainsworth et al., 1978)) in infancy have been associated with childhood onset of aggressive behaviour problems (van Ijzendoorn et al., 1999).

At the neurobiological level, it has been reported that the parent–child interactions and the psychological state of the mother can influence the child's HPA axis activity. Beginning early in the first year, when the HPA system of the infant is quite labile, sensitive parenting is associated with either smaller increase in or less prolonged activations of the HPA axis to everyday perturbations (Albers et al., 2008). For example, children of less supportive parents experienced larger increases in glucocorticoids levels by late afternoon. This was particularly outstanding in children who are emotionally negative and behaviourally disorganized (Gunnar and Donzella, 2002).

1.3.2. Childhood maltreatment

Understanding the history of early trauma and abuse requires an appreciation of the social and political processes that govern society (Cunningham, 1988). In 1860, Ambroise Tardieu (1818 – 1879) published the first paper directly related to the abuse of children (Dohary et al., 2010, Tardieu, 1860). Tardieu's initial concern with physical abuse gave way to a greater focus on child sexual abuse late in 19th century. Around the time of Tardieu's work on physical child abuse, Paul Briquet (1796 – 1881) established a link between hysteria and childhood traumatic experiences (Briquet, 1859, Dohary et al., 2010). Jean Martin Charcot (1825 – 1893) recognized that childhood trauma were evident in many of his hysteria patients but he gave it no etiological significance (Charcot and Magnan, 1882).

Pierre Janet (1859 – 1947) explored traumas experienced by his patients including traumatic loss, witnessing violent death, incest, rape, physical abuse and traffic accidents. Although his publications do not show specific interest in child abuse and neglect, he regarded inadequate childrearing practices, which may combine with traumatic experiences, as factors that may contribute to the development of mental disorders (Janet, 1925).

On the other hand, Sigmund Freud (1856 – 1939) initially considered that the experience of child sexual abuse was required to develop later psychological difficulties, namely hysteria (Dohary et al., 2010). Indeed, he described different types and parameters of child sexual abuse (Freud, 2001a). However, Freud's belief in the impact of childhood sexual abuse diminished greatly mainly because he considered that he "was not able to distinguish between my patients' fantasies about their childhood years and their real collections" (Freud, 2001b). Moreover, Freud's growing theoretical formulations concerning oedipal fantasy, shifted focus away from the

impact of child abuse. Nevertheless, while psychoanalytic thinking was largely focused on sexual fantasy, several clinicians and researchers continued to see the etiological relevance of child trauma (Dohary et al., 2010).

During the 1960s there was an increase in social awareness of child maltreatment. Social changes including the liberation of women from domestic realm influenced the increase the number of professionals working with children and families. Also, interest in the physical impact of abusive parenting was re-awoken by paediatric investigators. Some paediatric professionals found unexplainable physical injuries of children such as subdural haematomas. Caffey's review of histories of physical injuries in children presenting multiple fractures in large bones in arms and legs lead him to suggest that the origin of such physical trauma "remained obscure" (Caffey, 1946, Caffey, 1965). Similarly, Knight (Knight, 1986) pointed out a case dated in 1888 which reports injuries in several children from a same family consistent with severe physical abuse but for which the medical professionals developed other theories, including rickets and syphilis. Knight suggested that child physical abuse would have been brought into the diagnostic frame if radiological equipment would have been available at that time. Indeed, Parton believed that the discovery of child abuse was dependent on the development of diagnostic radiology in paediatric medicine (Parton, 1979). But it was Kempe and colleagues who put child physical abuse on the medical radar by proposing the term "battered child syndrome" (Kempe et al., 1962), as they noted, "to the informed physician, the bones tell a story the child is too young or too frightened to tell". This term was defined as a "clinical condition in young children who have received serious physical abuse, generally from a parent or foster parent". To define a more inclusive range of maltreatment and neglect types, the term battered child or baby, as it became known in the UK (Parton, 1979), was eventually changes to "child abuse and neglect". This term stressed the pervasive and long-term effects on

emotional well-being, psychological and physical development of the maltreated child (Dohary et al., 2010). However, child sexual abuse was largely absent from discussion during mid 1900s. This changed during 1980s to early 1990s where there was an increased interest in child sexual abuse prevalence and consequences. Interestingly, this renewed interest in child sexual abuse did not reduce the awareness and research interest in other forms of childhood maltreatment.

Nowadays, childhood adversity including not only childhood maltreatment but also other negative experiences that children may experience such as parental loss or natural catastrophes constitutes a burning topic in research. Research has also expanded beyond mental health consequences, implicating child maltreatment and early trauma as etiologically significant in a range of physical health problems, including liver and heart disease (Felitti et al., 1998). Moreover, just as developments in technology from the 1940s assisted the identification of physical abuse, various technological advances in observing and analyzing the brain have allowed the structural and functional effects of child maltreatment to be examined (Edmiston et al., 2011, Grant et al., 2011, Teicher et al., 2010, De Bellis, 2010).

Despite progress in social awareness and scientific understanding, acknowledgement of the existence and impact of child maltreatment is continually threatened by a propensity to deny and disavow (Dohary et al., 2010). It is important to notice that there is evidence indicating that child maltreatment can be stopped and prevented (Asmussen, 2010). Society needs to be aware of the extremely importance of protecting children and political and health institutions must keep improving ways to ensure that children are safe from any adverse event that could had been avoided.

1.3.2.1. Definition and types of childhood maltreatment

There is no single definition of child maltreatment, as the understanding of what constitutes abuse varies with the child's age, culture and context. However, the experience of significant harm and suffering appears to be at the core of most definitions (Asmussen, 2010).

The World Health Organisation (WHO) (World Health Organization, 2006) defines child maltreatment as:

> "All forms of physical and/or emotional ill-treatment, sexual abuse, neglect or negligent treatment or commercial or other exploitation, resulting in actual or potential harm to the child's health, survival, development or dignity in the context of a relationship of responsibility, trust or power"

The perpretors of child maltreatment may be parents and other family members, caregivers, friends, acquaintances, strangers, other is in authority –teachers, police officers and clergy-, employers, health care workers and other children (World Health Organization, 2006).

Within the concept of childhood maltreatment four categories are traditionally recognised. Firstly, *physical abuse* is generally defined as the use of physical force against a child, which includes a range of violent behaviour such as hitting, beating, kicking, shaking, biting, strangling, scalding, burning, poisoning and suffocating. Much physical violence against children in the home is inflicted with the object of punishing (World Health Organization, 2006).

Secondly, *sexual abuse* is defined as the involvement of a child (also forcing or enticing) in sexual activity, including prostitution, that he or she does not fully comprehend, is unable to give informed consent to, or for which the child is not developmentally prepared. Sexual abuse includes both physical (penetrative acts) and

non-physical acts such as exposing one's sexual parts to a child, exposing children to sexual imagery or encouraging a child to behave in other sexually inappropriate ways. Perpretors can be adults and other children who are –by virtue of their age or stage of development- in a position of responsibility, trust or power over the victim (Asmussen, 2010, World Health Organization, 2006).

Thirdly, *emotional or psychological abuse* refers to the persistent emotional maltreatment of a child that may severely impair child's psychological development. Emotional abuse includes movement restriction, blaming, threatening, frightening, discriminating against or ridiculing, and other non-physical forms of rejection or hostile treatment.

Finally, *neglect* includes both isolated incidents, as well as a pattern of failure over time of the part of a parent or other family member to provide for the development and well-being of the child –where the parent is in a position to do so- in one or more of the following areas: health, education, nutrition, emotional development, shelter and safe living conditions. Neglect can also be divided into physical and emotional neglect as it is the case when using the Childhood Trauma Questionnaire (CTQ; (Bernstein, 1998)) to assess childhood maltreatment.

1.3.2.2. Measurement and prevalence

In the last decades, literature on childhood adversity has profited from methodological advances devoted to the operationalization of child maltreatment. A method used estimate lifetime and annual rates of abuse are population-based surveys, although these studies suffer from a wide range of methodological problems. For example, it is not possible to ask children, particularly very young ones, about their experiences of abuse because they will not understand the questions. Parents can also

be unreliable sources of abuse-related information because they may not know abuse or they may not want to disclose self-incriminating information. Nevertheless, the most common method for investigating rates of abuse and the consequences of childhood adversity involves interviewing adults retrospectively usually by means of self-reported instruments. The retrospective and self-report nature of these methods although adults may forget or block out adverse childhood experiences which may underestimate rather than over repot real incidence rates (Angold et al., 1999, Becker-Blease and Freyd, 2006, Brown et al., 1999, Hardt and Rutter, 2004).

Studies about childhood adversity frequently report the use of self-reported retrospective instruments as a limitation due to the inherent risk of bias. However, as abovementioned, there is evidence that the retrospective assessment of childhood trauma tends to underestimate rather than over report real incidence rates (Hardt and Rutter, 2004). Furthermore, studies have demonstrated the validity and reliability of retrospective reports of trauma in psychotic samples, showing that they are stable across time, unaffected by current symptoms, and are generally concordant with other sources of information (Fisher et al., 2011).

The Childhood Trauma Questionnaire (CTQ; (Bernstein, 1998)) constitutes one of the most commonly used self-reported questionnaires to assess victimization. This instrument consists of 28 items and it provides brief, reliable and valid screening for histories of abuse and neglect. It was designed for population of 12 years and older. It inquiries about five types of maltreatment: emotional, physical and sexual abuse and emotional and physical neglect. Originally developed by Bernstein and colleagues in 1994, the psychometric properties of the CTQ have been further examined (Bernstein et al., 2003, Fink et al., 1995). It is worth it to notice that the use of this instrument has been recommended by a review about childhood trauma and psychotic disorders (Bendall et al., 2008).

The Adverse Childhood Experiences (ACE) Study, in which some 17300 middle-aged, middle-class and mostly employed residents of the state of California (USA) participated, has examined the association between exposure to early adversity and negative outcomes such as chronic diseases or suicides (Felitti and Anda, 2010, Felitti et al., 1998). Also, the ACE Study developed an instrument to briefly assess the exposure to childhood maltreatment in their participants. This instrument consists of 10 items and all of them are introduced with the question "While you were growing up during your first 18 years of life…" (Felitti et al., 1998). The so-called ACE-score ranges from 0 to 10 and indicates the number of childhood adverse events categories (psychological abuse, physical abuse, sexual abuse, emotional neglect, physical neglect, parents divorced or separated, mother treated violently, substance abuse by a household member, mentally-ill household member, incarcerated household member) that the person experienced.

Child maltreatment is unfortunately all too common in most cultures and countries. It is important to note that rates of abuse and neglect vary considerably across cultures and countries because of differences in the ways in which these concepts are defined.

Infants and pre-school children are at the greatest risk of fatal maltreatment as a result of their dependency, vulnerability and relative social invisibility. The risk of fatal abuse is two to three times higher in low-income and middle-income countries than it is in high-income countries. It is also greater in societies with large economic inequalities than in those where wealth is more evenly distributed (World Health Organization, 2006).

Some international studies have shown that, depending of the country, between a quarter and a half of all children report severe and frequent physical abuse including being beaten, kicked or tied up by parents. 63% of the people who participated in the Adverse Childhood Experiences (ACE) Study had experienced at least one category of

childhood trauma, which included a broad range of negative events, specifically: 11% experienced emotional abuse, 28% experienced physical abuse, 21% experienced sexual abuse, 15% experienced emotional neglect, 10% experienced physical neglect, 13% witnessed their mothers being treated violently, 27% grew up with a household member using alcohol and/or other drugs, 19% grew up with a mentally-ill person in the household, 23% lost a parent due to separation or divorce, 5% grew up with a household member in prison (Anda et al., 1998). These estimates might seem high but considering more severe events, within the UK, Office for Standards in Education (OFSTED) estimates that three children per week die as a result of child abuse and neglect (Asmussen, 2010, OFSTED, 2009) and research suggests that at least 16% of the population will experience some form of serious maltreatment during their childhood (May-Chahal and Cawson, 2005). Other population-based studies in the USA, Australia and UK suggested that annual rates range from 4% to 16% for physical abuse and from 1 to 15 per cent for neglect (Gilbert et al., 2009a, Gilbert et al., 2009b). Annual rates for sexual abuse are somewhat lower, but data collected on lifetime rates suggest that approximately 10% of all girls and 5 per cent of all boys experience some form of sexual abuse before they reach the age of 18. Then, girls are at least twice as likely as boys to experience sexual abuse; however, boys are at greater risk or harsh physical punishment and certain forms of neglect (Asmussen, 2010, Gilbert et al., 2009a, Gilbert et al., 2009b, World Health Organization, 2006).

Importantly, these statistics reflect only those cases that came to the attention of authorities and, therefore, the actual number of children who are victims of child abuse and neglect are far greater. Research also suggest that professionals are reluctant to report suspected cases of maltreatment because they are not confident that the child's circumstances will improve because of the report (Gilbert et al., 2009b).

Childhood traumatic experiences tend to cosegregate so that being exposed to one type of adversity increases the risk of exposure to another (Green et al., 2010).

1.3.2.3. Consequences and mechanisms of risk

It is easier to build strong children than to repair broken men

Frederick Douglas (1817-1895)

Exposure to childhood maltreatment may have profound and lasting impact when it occurs at critical ages or developmental transitions, particularly if it also involves disruption in fundamental attachment relationships, betrayal by caregivers or violation of the self (e.g. sexual or emotional abuse) (Ford, 2010). Early stress may compromise core psychobiological self-regulatory capacities (Manly et al., 2001) and places infants at risk for anxiety, affective, psychotic, regulatory and attachment disorders (Manly et al., 2001, Van Winkel et al., 2008). Furthermore, exposure to childhood adversity leads to the early initiation of drug, alcohol and nicotine use and risky sexual behaviours (Fig 5) (Anda et al., 2006) and accounts for 50-75% of the population attributable risk for alcoholism, drug abuse, depression and suicide (Anda et al., 2002, Dube et al., 2003). It also substantially increases risk for ischemic heart disease, chronic obstructive pulmonary disease, liver disease and obesity (Felitti et al., 1998).

Converging epidemiological and neurobiological evidences suggest that early life stress such as abuse and neglect cause enduring brain dysfunction that, in turn, affects health and quality of life throughout the lifespan (Anda et al., 2006). An expanding body of evidence from rodent, primate and human research suggests that early stressors cause long term changes in multiple brain circuits and systems (Bremner et al., 2003, Sanchez et al., 2001). In this regard, as abovementioned, the hypothalamic-pituitary-adrenal (HPA) axis plays a critical role in the stress response. Early stress

cause long-term increases in glucocorticoid responses to stress, as well as decreased genetic expression of cortisol receptors in the hippocampus and increased genetic expression of corticotrophin-releasing factor in the hypothalamus, both of which may contribute to dysregulation of the HPA axis (Ladd et al., 1996). Also, deprivation of developmentally appropriate experience (e.g. emotional neglect) may reduce neuronal activity, resulting in a generalized decrease in neurotrophin production, synaptic connectivity, and neuronal survival resulting in profound abnormalities in brain organization and structure (Perry, 2002, Read et al., 2001, Tomalski and Johnson, 2010).

Of note, exposure to child maltreatment does not have enduring effects in all exposed subjects or the same effects. A part from differences regarding the stressful event such as severity, frequency or timing, interinidividual genotypic variation may partially explain differences in the organism's sensitivity and response to stress (Bellani et al., 2012). This issue will be discussed in another section. Furthermore, some children and adolescents maintain positive adaptation despite experiences of distressing life conditions such as violence, poverty, stress, trauma, deprivation and oppression. In the light of this fact, some authors have hypothesized that some victims of maltreatment may be *relisient* (Collishaw et al., 2007). A growing body of research focuses of factors related to resilience; nevertheless the definition of this concept is still a matter of debate. In summary, resilience can be defined in terms of an individual's capacity, the process he or she goes through and the result. Resilience as a capacity refers to an individual's capacity for adapting to changes and stressful events in a healthy way. Resilience as a process is regarded as a reintegration process and a return to normal functioning with the support of protective factors after encountering a severe stressor. Resilience as a result is defined as the positive and beneficial outcomes resulting from successfully navigating stressful events. Thus, resilience can be defined as the process

of effectively mobilizing internal and external resources in adapting to or managing significant sources of stress or trauma (Lee et al., 2012).

In this context, the ecobiodevelopmental (EBD) framework has been proposed as an integrated framework for promoting health and preventing disease across life span considering the negative consequences that early abusive and neglectful experiences may have on adult undesirable outcomes (Shonkoff, 2010). This framework can help to further understand how from early childhood adversity, an adult individual can be more likely to present difficulties in social relationships, attachment difficulties, engage in risk or non-healthy behaviours (e.g. drug abuse) and finally develop mental and/or physical diseases (Fig 5). From the EBD framework, inextricable interactions among personal experiences (e.g., family and social relationships), environmental influences (e.g., exposures to toxic chemicals and inappropriate electronic media), and genetic

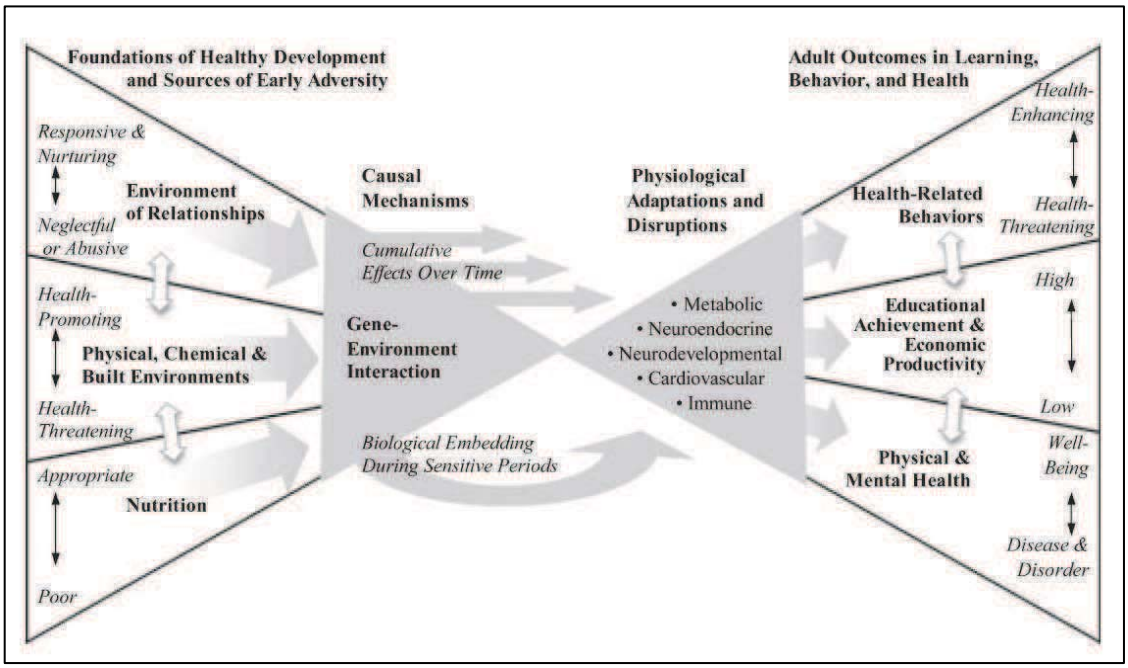

Figure 5. The ecobiodevelopmental framework for understanding the origins of behaviour and health (Shonkoff, 2010).

predispositions shape learning, behaviour, and health across the life span. Childhood adversity appears in this EBD framework as a toxic stress factor (Fig 5). As explained earlier, toxic stress, in contrast to positive and tolerable stress, is defined as the excessive or prolonged activation of the physiologic stress response systems in the absence of the buffering protection afforded by stable, responsive relationships. The exposure to toxic stress early in life plays a critical role by disrupting brain circuitry and other important regulatory systems in ways that continue to influence physiology, behaviour, and health decades later. The EBD framework suggests that early experiences with significant stress are critical, because they can undermine the development of those adaptive capacities and coping skills needed to deal with later challenges; the roots of unhealthy lifestyles, maladaptive coping patterns, and fragmented social networks are often found in behavioural and physiologic responses to significant adversity that emerge in early childhood; and the prevention of long-term, adverse consequences is best achieved by the buffering protection afforded by stable, responsive relationships that help children develop a sense of safety, thereby facilitating the restoration of their stress response systems to baseline.

Recently, evidences of brain changes in individuals exposed to childhood maltreatment have been reported. For example, Edmiston and colleagues examined associations between regional gray matter (GM) morphology and exposure to childhood maltreatment (measured using a childhood trauma self-report questionnaire for physical, emotional, and sexual abuse and for physical and emotional neglect) in a sample of 42 adolescents without psychiatric diagnoses (Edmiston et al., 2011). They found that exposure to childhood maltreatment was associated with corticostriatal-limbic GM reductions. Although the adolescents were mentally healthy, authors concluded that the GM morphologic alterations found may place them at risk for future behavioural difficulties (Edmiston et al., 2011). Also, the application of

molecular biology advances has allowed several researchers to find out that some epigenetic changes, especially changes in DNA methylation, might constitute long-lasting consequences of early trauma in humans (Klengel et al., 2013, McGowan et al., 2009) and animal models (Klengel et al., 2013, Weaver et al., 2004). For example, it has been shown that the rs136078 functional polymorphism of the *FKBP5* gene, an important regulator of stress hormone system, increased the risk of developing stress-related psychiatric disorders in adulthood by allele-specific, childhood trauma-dependent DNA methylation in functional glucocorticoid response elements of FKBP5 (Klengel et al., 2013).

Of course, it is not adversity alone that predicts poor outcomes. From the EBD framework, it is the absence or insufficiency of protective relationships that reinforce healthy adaptations to stress, which, in the presence of significant adversity, leads to disruptive physiologic responses (i.e., toxic stress) that produce "biological memories" that increase the risk of health-threatening behaviours and frank disease later in life (Garner and Shonkoff, 2012, Shonkoff, 2010).

1.4. Genes, environment and their interplay in complex human phenotypes

Over the past half-century there has been a series of changes in the generally prevailing views about the role of genetic and environmental factors in the causation of behaviour traits and mental disorders. Some periods have been characterized by deterministic genetic effects on phenotypes and others by extreme environmentalism. During 1980s to early 1990s, the development and application of molecular genetic strategies to psychiatry research was received with the expectation that the aetiology of many psychiatric disorders would be found (Kidd, 1991). However, many of the initial claims of finding a gene "for" some mental disorders were not replicated and subsequently had to be withdrawn. This was a period of disillusionment and pessimism about the possibility of understanding the role of specific genes in the causation of psychopathology. Nevertheless, from the early 1990s to the present time, the notion of single basic causes has been replaced by the acceptance of the multifactorial origin of most disorders and behavioural traits. This shift in concept was largely adopted in general medicine. In this context, almost all risk factors, whether genetic or environmental, involve probabilistic, rather than deterministic effects. Additionally, there has come the recognition that risk effects extend throughout the normal distribution and not just at the extreme end. Thus, this has been shown with respect to cholesterol levels and the risk of heart attacks (Rutter et al., 2006).

In 2000 Turkheimer claimed "the nature-nurture debate is over" (Turkheimer, 2000). Nowadays it is widely accepted by most experts that common human traits usually result from the combined effects of multiple genes together with environmental factors (Maccabe et al., 2006). These traits are known as *complex traits*. This term refers to any phenotype that does not exhibit a classic Mendelian recessive or

dominant inheritance attributable to a single gene locus. In general, complexities arise when the simple correspondence between genotype and phenotype breaks down, either because the same genotype can result in different phenotypes (due to the effects of chance, environment or interaction with other genes) or different genotypes can result in the same phenotype (Lander and Schork, 1994).

Currently, the prevailing view assumes that for multifactorial complex disorders and behaviours, risk factors are neither necessary nor sufficient to cause disease. The most likely scenario would be that multiple risk factors will have acted to allow the development of disease or a particular behaviour in that person. Some of these risk factors are likely to be genetic and some environmental and both types almost certainly play a contributory role in causing most, if not all, cases of complex diseases and behaviours (Zammit et al., 2012).

Similarly, there is little doubt that an individual's genetic makeup may be associated with vulnerability to psychopathology. However, how genetic variability, gene interactions, gene silencing and imprinting among other genetic mechanisms contribute to psychopathology has only begun to be understood. Also the task of disentangling genetic from environment impacts has proved extremely difficult (Brüne, 2010). Family, twin and adoption studies have firmly established the roles of both genes and environment in mental disorders. It remains difficult, however, to find genes for these disorders, and to characterize the particular environmental circumstances under which psychopathology emerges (Tsuang et al., 2004).

In this context, the field of *quantitative genetics* aims to investigate the influence of genetic and environmental factors while strategies to identify specific genes are investigated by the field of molecular genetics. The quantitative genetics theory assumes that multiple gene influences together with environmental variation, results in quantitative (continuous) distributions of phenotypes. Quantitative genetic methods,

such as twin and adoption methods for human analysis, estimate genetic and environmental contributions to phenotypic variance and covariance in a population. *Behavioural genetics* is a specialty that applies these genetic research strategies to the study of behaviour such as *psychiatric genetics* which investigates genetics of mental illness (Plomin et al., 2008, Plomin et al., 2009).

1.4.1 Basic genetics

Genetically informative studies allow the estimation of the relative contribution of genetic and environmental factors including estimation of the heritability without knowing the biological basis of heredity or molecular genetics. Nevertheless, it is important to understand the biological mechanisms underlying heredity (Plomin et al., 2008).

The information required to grow an organism is stored in the individual *genome*. All genetic information is encoded in a macromolecule called deoxyribonucleic acid (DNA). It is made from monomeric deoxynucleotides that carry one of four kinds of base: adenine (A), guanine (G), cytosine (C) and thymine (T). A is always paired with T and G with C. This "alphabet" or genetic code is identical in all living organisms and therefore highly conservative. Human DNA comprises an estimated 3.5 billion base pairs. DNA consists of a mix of coding DNA and non-coding DNA. The human genome contains perhaps some 20,000 - 25,000 functional genes (Rosa et al., 2010), which is a surprisingly low number. About 55% of coding DNA is expressed in the human brain (Brüne, 2010). A unit of DNA (sequence of nucleotices) that contains the information needed to synthesize a macromolecule with a specific function, usually proteins, is called a *gene*. A gene usually consists of a start (promoter) and a stop region. These regions are important for the initiation or termination of the transcription

process from DNA to RNA. A gene has both introns and exons, such that the introns have to be removed from the primary transcript by RNA splicing to produce fully mature mRNA. RNA is similarly structured as DNA except that T is replaced with uracil (U). Three adjacent bases form a triplet or codon, which code for amino acids to build proteins. Normally, DNA takes the shape of a double helix that in humans represents the basis of 23 pairs of chromosomes, two of which are the sex chromosomes X and Y. Chromosomes are eccentrically squeezed in the centromere region leading to a long (q) and a short (p) arm. The site of a gene on a chromosome is known as the *locus* of that gene. *Alleles* are alternative forms of the gene that occupy the same locus on the chromosome. They are often represented by the letters A and a, or B and b. The simplest system for a locus consists of only two alleles (e.g. A and a) but there also may be a large number of alleles in a system. The *genotype* is the chromosomal set of alleles for an individual. At a single locus, with two alleles, the genotype may be represented by AA, Aa or aa. *Homozygosity* refers to a state of identical alleles at corresponding loci on homologous chromosomes. In contrast, *heterozygosity* refers to a state of unlike alleles at corresponding loci on the chromosomes.

Genetic individual differences contribute to phenotypic variation among all of us. When variants in the DNA sequence are present in the population at a frequency higher than 1% the mutation is called polymorphic and the loci where this mutation take place is called polymorphism. The most common type of DNA variation is single base substitutions, which are termed single nucleotide polymorphisms (SNPs) (Brüne, 2010).

The DNA sequence – meaning the order of base pairs (made up of four chemicals) – that specifies what is inherited. That cannot be altered by the environment. On the other hand, the functional effects of that DNA sequence are

entirely dependent on gene expression can be influenced (sometimes in a major way) by environmental features. In that sense, environments can and do have effects on genes through a process termed epigenesis (Jaenisch and Bird, 2003, Rutter, 2007). Thus, while the DNA content of all cells is much the same, the actions of DNA are crucially dependent on their functional activation, termed expression. This involves the processes of transcription and translatioon plus epigenetic mechanisms. Ultimately, the proteins will bring about the relevant effects on phenotypes, meaning behavioural manifestations in the case of mental traits and disorders (Rutter, 2007). In this way, the association between a gene and a phenotype not just involves the single gene that codes for some product, but rather the effect of multiple inherited DNA elements that influence transcription, translation, plus environmental influences such as drugs and rearing experiences (Rutter, 2007).

1.4.2. Disentangling genes and environment: Twin Studies

1.4.2.1. Biology and prevalence of twinning

There are two types of twins: monozygotic (MZ) or identical and dizygotic (DZ) or fraternal twins. MZ twins results from a single fertilized egg (called a zygote) that splits for unknown reasons, producing two (or sometimes more) genetically identical individuals (Fig 6B). For this reason, MZ twins are said to be natural clones. They are supposed to be 100% genetically identical at the DNA sequence level (Boomsma et al., 2002, Plomin et al., 2008). However, in the course of development of every large multicellular organism, cells will arise with somatic mutations and differential epigenetic control, not to mention the stochastic processes that are part of the generation of immunological and neurological responses (Bell and Spector, 2011, Hall, 2003, Petronis et al., 2003, Machin, 2009). No naturally occurring animal models of MZ

twining exist apart from armadillos, in which identical quadruplets or octuplets arise depending on the strain. Mirror-image twinning happens in about 10-15% of MZ twins. Mirror-image MZ twins have inverse laterality which suggests that the twinning event took place after the cells of the embryonic plate were beginning to lateralise but before formation of the primitive streak. In mirror-image twins minor features are on opposite sides such as whether the first tooth erupts on the right or left side and on which side are hair whorls present. The causes of MZ twinning remain largely unknown (Hall, 2003).

In the womb, babies are covered by three membranes: the placenta, the chorion and the amnios. The number of membranes that will separate the twins during the gestation depends on the time when the zygote splits. For about a third of identical twins, the zygote splits during the first five days after fertilization as it makes its way down to the womb. In this case, the identical twins have different sacs (called chorions) within the placenta (Fig 6B). Two-thirds of the time, the zygote splits after its implant in the placenta and the twins share the same chorion (Fig 6B). Identical twins who share the same chorion may be more similar for some psychological traits than identical twins who do not share the same chorion, although the evidence on this hypothesis is mixed (Fig 6B) (Jacobs et al., 2001, Plomin et al., 2008). When the zygote splits after about two weeks, the twin's bodies may be partially fused – so-called Siamese twins.

DZ or fraternal twins occur when two eggs are separately fertilized; they have different chorions and amnios (Fig 6A). Like other siblings, they are on average 50% similar genetically. DZ twinning arises because more than one dominant ovarian follicle has matured during the same menstrual cycle. The cause of spontaneous DZ twinning seems to be associated with an increased concentration of follicle-stimulating

hormone (FSH) in the mother. This type of twins runs in families and is associated with raised concentrations of FSH (Hall, 2003).

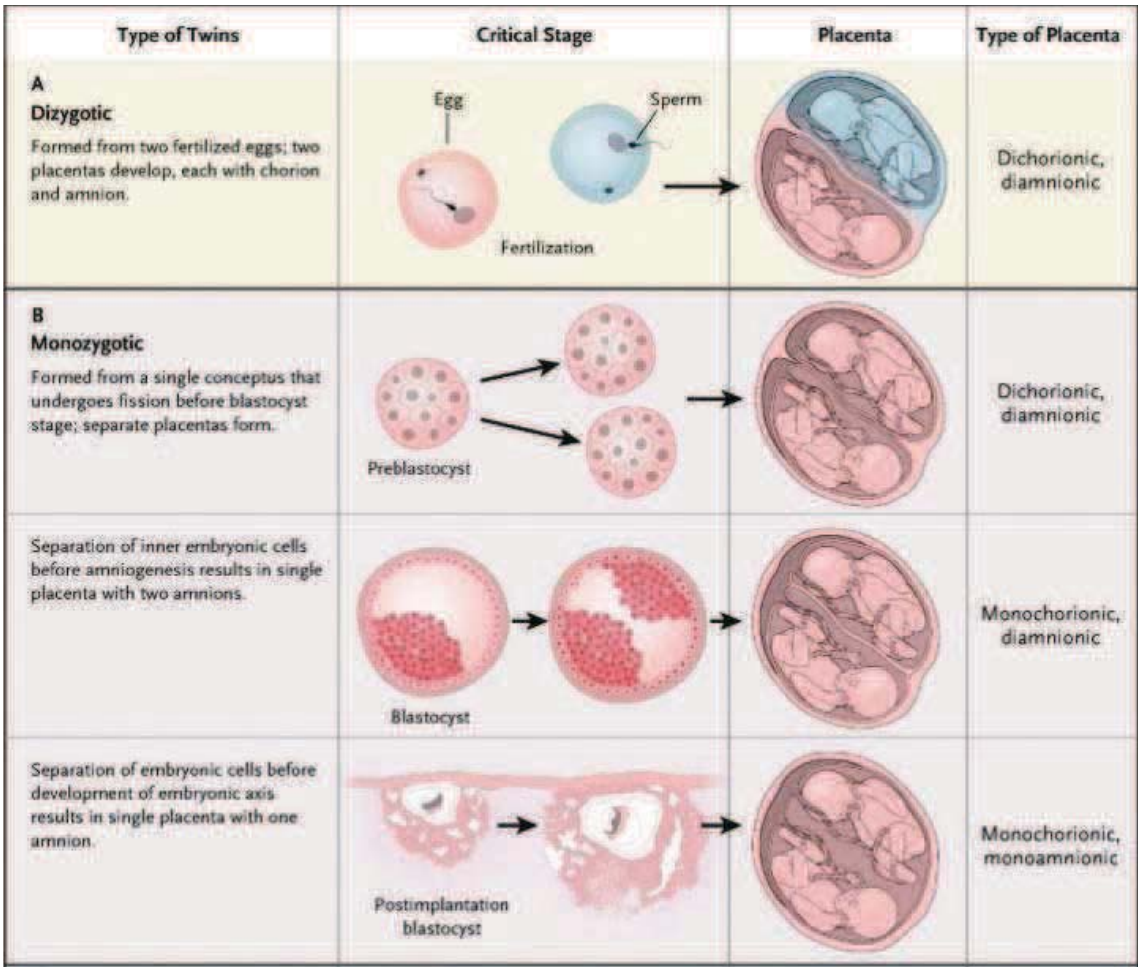

Figure 6. Formation of DZ (A) and MZ (B) twins and types of placenta. A) DZ twins are considered always to be dichorionic. B) MZ twins may be dichorionic, monochorionic, diamniotic or monoamniotic (Adapted from Machin, 2009).

Although most mammals have large litters, primates, including our species, tend to have single offspring. However, primates occasionally have multiple births. Surprisingly, in human pregnancies as many 20 percent of foetuses are twins, but because of the hazards associated with twin pregnancies, often one member of the pair dies very early in pregnancy (Plomin et al., 2008). The rate of MZ twinning has been fairly constant around the world, with variability being attributable to the rate of DZ twinning. The prevalence of spontaneous twinning in live births ranges from about 6 in 1000 in Asia, about 10-20 in 1000 in Europe and the USA, to about 40 in 1000 in Africa.

In Japan only 1 in 250 newborn babies is a twin, whereas in Nigeria 1 in 11 is a twin (Hall, 2003).

In Spain, the twinning rate in 1980 was 7.4 per 1000 deliveries. Thereafter, a continuous increase in the number of multiple deliveries occurred, increasing to 12.4 in 1996 (Fuster et al., 2008). The increase in the twinning rate has been attributed to the change in age structure of mothers (Pison and D'Addato, 2006), as well as to the extensive practice of assisted reproduction techniques (ART), including the use of ovulation promoters, which began in 1978 (Fuster et al., 2008) .

Although twinning is mainly related to the mother's age through variations in the FSH hormone and prenatal mortality, other factors such as birth order, season, socioeconomic factors, ethnic group, and rural–urban differences perhaps related to industrialization, have also been the object of study (Fuster et al., 2008).

1.4.2.2. Twin studies

A part from adoption studies based on singletons (genetically related individuals such as siblings who do not share a common family environment), twin studies constitutes one of the most powerful methods used to disentangle genetic from environmental sources of resemblance between relatives (Boomsma et al., 2002, Plomin et al., 2008, van Dongen et al., 2012). Advances in statistical modelling allow simultaneous analysis of many variables in MZ and DZ twins. Thus, it is possible to carry out multivariate analyses of causes of comorbidity between disorders, the analyses of the relative genetic and environmental contribution to childhood psychopathology over time among others. Nowadays, large twin registers are established in different countries around the world which collect a wide range of traits,

environmental and biological data in twins as well as their family members (Boomsma et al., 2002, van Dongen et al., 2012).

A crucial point in twin research is the determination of the zygosity of the twins. Zygosity refers to whether twins arose from one fertilised egg (i.e. MZ) or from two eggs fertilised by different sperms (i.e. DZ). Different methods have been used to establish zygosity since its knowledge early in 20th century, including physical resemblance, placental examination, dermatoglyphics examination and blood groups (Hall, 2003, Lykken, 1978, Rao and Greene, 1977, Rietveld et al., 2000). Currently the most common methods include zygosity questionnaires (Rietveld et al., 2000) and DNA typing, using variable number tandem repeats (VNTRs), which is considered the most reliable way of defining zygosity (Hall, 2003, Price et al., 2000).

Another relevant question about the validity and reliability of twin studies concerns the generalisation of results coming from twin studies to singletons population. Twins differ from singletons in several ways. They are usually delivered after a shorter gestation time and with a lower birth weight (Blickstein, 2004, Buckler and Green, 2004). This could make twins more at risk for developing diseases (Barker et al., 2002). However, a recent study concluded that despite their adverse intrauterine experience, twins did not seem to fare worse than singletons with respect to adult morbidity and mortality (Oberg et al., 2012). Nevertheless, it is always convenient to examine the representativeness of the twin sample for example comparing means and frequencies of the analysed traits with data from the general population. If twins do not differ in terms of means or frequencies of the analysed traits, there is no reason to question the representativeness of the sample.

Classical Twin Studies

The classical twin study compares phenotypic resemblances of MZ and DZ twins (Boomsma et al., 2002, Rijsdijk and Sham, 2002). Comparing the resemblance of MZ twins for a trait or disease with the resemblance of DZ twins offer the first estimate of the extent to which genetic variation determines phenotypic variation. If genetic factors are important for a trait, genetically identical MZ twin pairs should be more similar than first-degree relatives, who are only 50% similar genetically on average (Plomin et al., 2008). Rather than comparing identical twins with non-twin siblings or other relatives, nature has provided a better comparison group: fraternal or DZ twins. Unlike MZ twins, DZ twins share on average 50% of their genes but shared prenatal environment and have the same age and same sex in some cases. If genetic factors are important for a trait, identical twins must be more similar than fraternal twins. Of note, half of fraternal twin pairs are same-sex pairs and half are opposite-sex pairs. Twin studies usually focus on same-sex fraternal twin pairs because they are a better comparison group for identical twin pairs who are always same-sex pairs (Plomin et al., 2008).

In the classical twin design, one can infer the relative contribution of genetic and environmental factors by comparing the observed correlations (or concordance) between twin members. Of note, in these models genetic and environmental factors are latent variables; i.e. as opposed to observed variables, latent variables have not been directly observed or measured, they are inferred through mathematical models. The sources usually estimated of genetic and environmental variation in behaviour genetics are: *additive genetic influences (A), shared or common environmental influences (C) and non-shared or unique environmental influences (E)*. *A* represents the sum of the effect of the individual alleles at all loci that influence a trait. A is also known as *heritability* (h^2), this

concept is further discussed below. C includes environmental influences that contribute to similarity within twin pairs, while E represent environmental influences that are unique to each individual, plus measurement error (Fig 7) (Plomin et al., 2008, Rijsdijk and Sham, 2002).

Of note, in order to understand the two types of environmental sources estimated in classical twin studies, it is important to distinguish between objective and effective environments (Turkheimer and Waldron, 2000). *Objective environments* refer to environmental events as they can be observed by a researcher. Objectively nonshared events are those, like peer relationships and birth order, that constitute the environment of only one sibling, again regardless of whether they work to make siblings alike or different. Therefore, objectively, whether a particular environmental factors is shared or nonshared refers only to whether or not it has been experienced by one or more members of the twin pair. Thus, socioeconomic status and marital discord, are objectively shared in this sense. In contrast to objective environments, *effective environments* are defined by the outcomes they produce. The estimate of shared environmental variation that results from classical twin studies refers to the effect of environments in creating sibling resemblance, regardless of whether the objective environments were shared or nonshared. Thus, if an objectively shared environmental variable results in nonshared effects, the effective contribution of the objectively shared event is included with the nonshared rather than the shared component of variance (Turkheimer and Waldron, 2000).

According to the methodology of classical twin studies, total phenotypic variance (P) of a given trait is the sum of A, C and E variance components (P=A+C+E). Twin data enable the different variance components to be estimated, because MZ and DZ twins have different degrees of correlation for the genetic component (A) but the same degrees of correlation for the environmental components C and E. MZ twin pairs

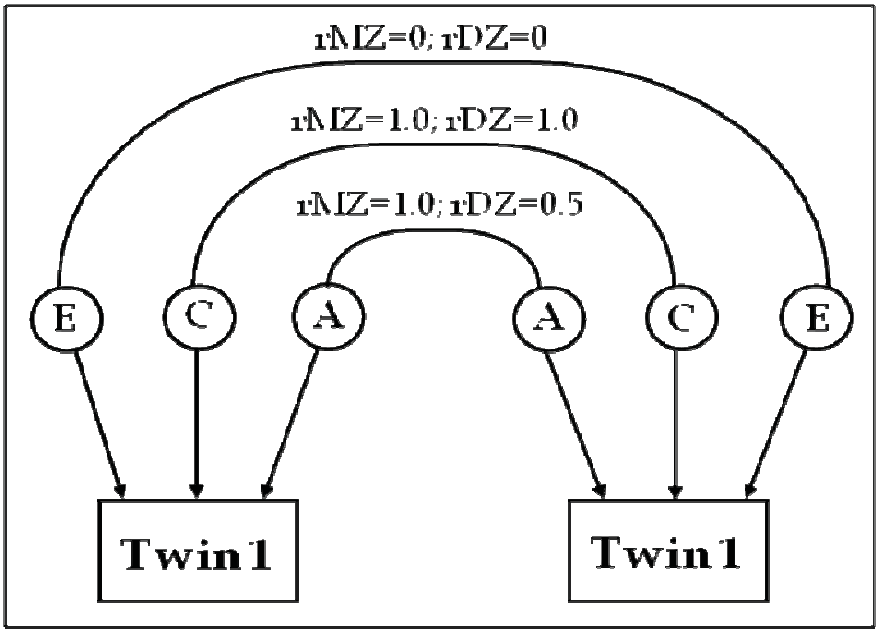

Figure 7. Path diagram for the basic univariate twin model. Cercles represent latent variables (A, C and E), and rectangles represent the observed and measured traits (phenotypes). In the diagram, the phenotype of each twin is decomposed into A, C and E variance compornents corresponding to additive genetic, shared environment and non-shared environmental influences respectively. Additive genetic factors correlate 1 for MZ and 0.5 for DZ pairs. Shared environmental factors correlate 1 for both Mz and DZ pairs and non-shared environmental factors are uncorrelated (r=0).

correlate 1 for A (they are assumed to be genetically identical), whereas DZ twin pairs correlate 0.5. Both MZ and DZ pairs correlate 1 for C and 0 for E (because C contributes to make them more alike and E to make them more different) (Fig 7). In the classical twin method, Falconer's formula was used to estimate heritability (i.e. A) based on twin correlations: $h^2 = 2(r_{MZ} - r_{DZ})$ (Rijsdijk and Sham, 2002). However, this approach is not adequate for testing, for example, sex differences and multivarite data and was replaced by a more advanced method based in Structural Equation Modeling (SEM) (Rijsdijk and Sham, 2002). There are several statistical packages available for SEM but most twin research has been performed using Mx which was specially designed for its application to samples including biologically-related individuals such as twins (Neale, 2003).

Heritability can be defined as the proportion of phenotypic variance in a population that is due to genetic variation (Plomin et al., 2009). Geneticists have emphasized that heritability is a statistic that applies to population variance and not to individuals or to traits as a fixed feature. Thus, heritability refers to genetic variation in populations; it is not a valid concept at individual level (Brüne, 2010). Heritability estimate is in fact a population and time specific estimate. Heritability depends on population because the allele frequencies, the effect sizes of the genetic variants and the mode of gene actions can vary across populations (Visscher et al., 2008). Thus, it is important to find out if that estimate is stable over time (e.g. is the role of genes in a particular trait in childhood as important as in adulthood?), stable over populations (e.g. is the heritability estimate derived from twin studies the same as for non-twin population?). A high heritability means that genetic factors account for much of the variation in the liability to show particular trait in a particular population at a particular point in time (Rutter et al., 2006).

When examining the heritability of diseases (categorical entities), classical twin studies enable us to establish the heritability of the liability to the disorder (Rijsdijk and Sham, 2002). For instance, the predominant role of genetic factors in the aetiology of schizophrenia has been formerly defined by classical twin studies, which have shown substantial heritability for liability to schizophrenia (Cardno et al., 1999).

A related issue to heritability concerns *missing heritability problem* (Maher, 2008). This concept refers to the fact that despite high heritabilities have been reported for several complex disorders, molecular genetic studies, including genome-wide association studies (GWAs)[1], have not been successful in identifying DNA variants responsible for these heritability estimates (Manolio et al., 2009). The gap between

[1] A hypothesis-free genetic method that uses hundreds of thousands of DNA markers distributed throughout the chromosomes to identify alleles that are correlated with a trait (Plomin et al. 2009).

heritability estimates reported and the genetic component detected via GWAs have raised questions about the methods used to estimate heritability as well as genetic architecture of complex phenotypes (Manolio et al., 2009, Zaitlen and Kraft, 2012, Zuk et al., 2012). Also, at least as important to the detection of genetic variants for complex traits is the way complex traits are measured, and the phenotypic information that is modeled (van der Sluis et al., 2010). The highly topical issue of the sources of missing heritability is forcing rethinking about the genetic basis of the pathogenesis of complex disorders.

The validity of the classical twin method depends on several assumptions. One of them is the equal environment assumption (EEA), that is, the assumption that MZ and DZ are equally correlated in their exposure to environmental factors of etiological importance for the trait that is being studied (Kendler et al., 1994). A possible violation of the EEA can arise if MZ twins are treated more similarly than DZ twins and because of that, the resemblance within MZ increases. This violation would result in an over-estimation of the heritability of the observed trait. However, this assumption is continously tested and different studies have found support for its validation in a variety of behavioural traits and disorders (Evans and Martin, 2000, Derks et al., 2006).

Monozygotic Twins Differences Studies

As abovementioned, behavioural genetic studies are able to distinguish between two sources of environmental variation: shared and nonshared environment. It has been shown that nonshared environmental influences are particularly important in a wide range of complex characters such as adult intelligence, neuroticism, anxiety and depression (Boomsma et al., 2002). However, these nonshared environmental influences have been not measured. In this context, the MZ twin differences approach

enables the identification of specific nonshared environmental influences and more importantly, constitute a method to explore environmental processes independent of genetic processes (Pike et al., 1996). This has been referred to as a strong test of the unique environmental experiences that make family members different from each other (nonshared environment) independently of genetics (Caspi et al., 2004, Pike et al., 1996, Viding et al., 2009). Since MZ twins are, nearly always, identical at the DNA sequence level (Boomsma et al., 2002); phenotypic differences observed between MZ twins must be explained by differential exposure to environmental factors. In other words, if differences in the expression of a given trait in MZ twins are associated with differences in exposure to a given environmental factor, this would provide strong evidence that the observed association between the environmental factor and the outcome is not due to genetic confounding. By applying this method, it has been possible to provide evidence for environmental factors which may exert their influence independently of genetic factors while others are genetically mediated. For example, Viding and colleagues (Viding et al., 2009) concluded that negative parental discipline operates as a nonshared environmental risk factor for the development of conduct problems but not for the development of callous-unemotional traits during adolescence.

Concordant and Discordant Affected Monozygotic Twins Studies

MZ twin pairs show a high degree of discordance for complex genetic traits and disorders (Hall, 2003). The concordant and discordant affected MZ twin design is aimed to investigate whether genetic and environmental risk might be differentially associated to particular factors or biological markers for a given disease. This design uses three groups of twins: concordant affected MZ twin pairs (i.e., genetically

identical pairs in which both members have the disorder), discordant affected MZ twin pairs (i.e. genetically identical pairs in which only one member has the disorder) and healthy control twins.

On one hand, the model assumes that the comparison between concordant affected monozygotic (MZ) twin pairs and healthy MZ twins, is likely to reflect a greater genetic liability for these phenotypes in concordant twin pairs than in discordant pairs (Borgwardt et al., 2010, de Geus et al., 2007, Ettinger et al., 2007, Ettinger et al., 2010, Wolfensberger et al., 2008a, Wolfensberger et al., 2008b). However this assumption needs to be tested. In schizophrenia research, MZ twins concordant for schizophrenia are hypothesized to carry a particularly high genetic load for the disorder and specifically greater than discordant pairs, supported by an earlier age of onset, a more severe clinical course, and a less marked association with putative environmental risk factors (Borgwardt et al., 2010, Baare et al., 2001, McNeil et al., 2000, van Haren et al., 2004). Although literature on this regard is still scarce in anxiety and depression disorders, De Geus and colleagues (de Geus et al., 2007) provided support for the notion that concordant MZ twins for anxiety and depression may be subject to a greater genetic risk. They observed higher levels of anxiety, depression and neuroticism among parents of the concordant twins compared to parents of the healthy twins (de Geus et al., 2007).

On the other hand, comparison within discordant MZ twin pairs can be used to identify nonshared environmental influences as it is done in the MZ twin differences design. The concordant and discordant monozygotic (MZ) twin pair design also assumes that any within-pair differences in GMV between MZ twin pairs who are discordant for anxiety and depression could be attributable to *unique* environmental influences (Plomin et al., 2008). For example, using this design De Geus and colleagues observed volume reductions in the temporal love, most notably in the left posterior

hippocampal region in the twins at high risk for anxiety and depression compared to their healthy co-twins (de Geus et al., 2007). Since MZ twins are genetically identical, these findings indicate that the differences in gray matter volume found in this region should arise from differential environmental influences.

Twin Adoption Studies

The most direct way to disentangle genetic and environmental sources of individual differences for a given trait involves adoption. "Genetic parents" are birth parents who relinquish their child for adoption shortly after birth. Resemblance between birth parents and their adopted-away offspring directly assesses the genetic contribution to parent-offspring resemblance. "Environmental parents" adopt children genetically unrelated to them. Resemblance between adoptive parents and their adopted children directly assesses the postnatal environmental contribution to parent-offspring resemblance. This type of studies has examined genetic and environmental contribution to psychological traits such as personality or intelligence (Bouchard et al., 1990, Petrill et al., 2003). Another strategy of the adoption study compares the incidence of a given complex disease, such as schizophrenia (Heston, 1966) between biological parents of affected adoptees and non-affected adoptees. A genetic influence is suggested if the incidence of the disorder is greater for the biological relatives of the affected adoptees than for the biological relatives of the unaffected adoptees.

Adoption studies have become more difficult to conduct as the number of adoptions has declined. Adoption became much less frequent as contraception and abortion increased, and more unmarried mothers kept their infants. Another issue of adoption studies involves representativeness. If biological parents, adopted parents or adopted children are not representative of the rest of the population, the generalizability of the results could be affected.

1.4.3. Gene-environment interaction studies

Gene-environment interaction (GxE) constitutes a mechanism of gene-environment interplay. There are many ways of thinking about GxE (Rutter et al., 2006), but in quantitative genetics the term generally means that there are genetically influenced individual differences in the sensitivity to specific environmental features (Caspi and Moffitt, 2006, Eaves, 1984, Plomin et al., 2008, Rutter and Silberg, 2002). In other words, GxE refers to the genetic control of sensitivity to the environment (Fig 8) (Van Os and Sham, 2003).

That implicates that there are genetically influenced individual differences in the sensitivity to specific environmental features GxE involves a greater effect of genetic risk in a high-risk environment (Plomin et al., 2008). In psychiatric genetics, this type of interaction is called the *diathesis-stress model* (Plomin et al., 2008, Gottesman, 1991)

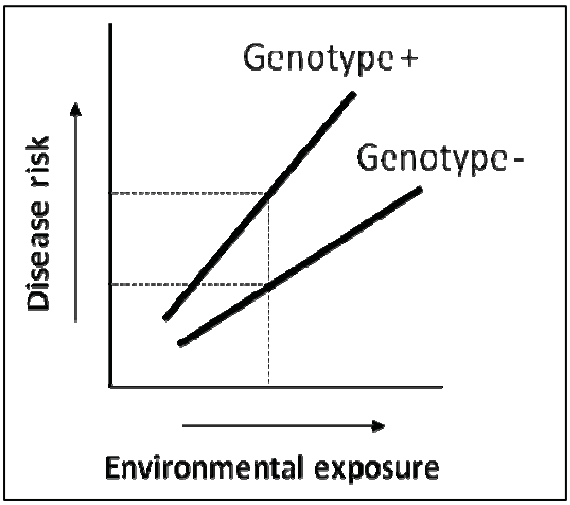

Figure 8. Gene-environment interaction. Genetic control of sensitivity to the environment The exposure to the environmental risk factor increases the disease risk. However, the disease risk is much higher for those carrying the genotype + compared to those carrying genotype - (Van Os and Marcelis, 1998).

In biological terms, GxE can be defined as the joint effect of one or more genes with one or more environmental factors that cannot be readily explained by their separate effects (Thomas, 2010). Interaction between genes and environment means more than simply stating that both are involved in disease aetiology (Van Os and Sham, 2003). This is also related to the terms synergism and parallelism. Biological synergism refers to the

proportion of the population exposed to both genes and environment that developed the illness specifically because of the combination of these exposures; parallelism refers to the proportion of the population exposed to both genes and environment that developed the disease because of either genes or environment (Van Winkel et al., 2008).

The first evidence that genotype moderates the capacity of an environmental risk to bring about mental disorders was reported in 2002 (Caspi et al., 2002, Caspi and Moffitt, 2006). Those findings provided initial evidence that a functional polymorphism in the *MAOA* gene moderates the impact of early childhood maltreatment on the development of antisocial behaviour in males (Caspi et al., 2002).

This publication contributed to change the generally accepted view in behavioural and psychiatric genetics during the era of the 1980s to early 1990s that gene-environment interactions (GxE) were rare and of limited importance (Rutter et al., 2006). A common assumption during that period was that genes would have relatively direct effects on disorder and the hope was that complex mental disorders would turn out to be caused by multiple different single gene conditions (Kidd, 1991). However, as Kendler pointed out, all the susceptibility genes for multifactorial disorders that have been discovered so far have been found to have very slight effects (Kendler, 2005). That initial approach also ignored the evidence from the rest of medicine that many risk factors operated on the basis of dimensional characteristics (Rutter, 2003). The likelihood is that genes affect particular physiological pathways that make a psychiatric condition more or less likely, but it still remains to be demonstrated that genes may cause a mental disorder at all directly (Rutter, 2003).

GxE research has been a hot topic in fields related to human genetics in recent years, perhaps particularly so in psychiatry (Duncan and Keller, 2011). The first decade (2000-2009) of GxE research on candidate genes (defined as a gene whose protein product suggests that it may be involved in a phenotype of interest or a construct

relevant to the phenotype or a gene that has been linked to a phenotype through an association genetic study or a GWA (Hyde et al., 2011)) in psychiatry saw the publication of over 100 findings, many of them in top journals. The publication of GxE studies in high impact journals raised the excitement of these findings which made them appeared as promising mechanism to understand the joint effect between environmental and genetic factors in the aetiology of complex traits such as psychiatric symptoms (Rutter et al., 2006).

Furthermore, expecting GxE effects it is biologically plausible. Genotypes do not exist in a vacuum; their expression must depend to some degree o environmental context (Duncan and Keller, 2011). From an evolutionary perspective, we can expect that gene-environment interaction findings would not only exist but may be quite common in behaviour and psychiatry genetics. Human development is an environmentally-dependent process in which individuals need to adapt to environmental hazards. However, it is implausible that genetic variants do not contribute to individual variation in response to the environment, since this response is associated with pre-existing individual differences in temperament, personality and psychophysiology, all of which are known to be under a certain degree of genetic influence (Rutter et al., 2006). Indeed, it would be astonishing if GxE did not exist, that would imply that reactions to the environment are among the only nonheritable phenotypes (Duncan and Keller, 2011). In this regard, twin studies have shown that at least some responses to the environment are heritable (Duncan and Keller, 2011, Kendler and Baker, 2007).

Statistically, interaction effects take place between three or more variables and it represents a departure from a pure main effects model. An effect of interaction occurs when a relation between two or more variables is moderated by (at least one) other variable. In other words, the strength or the sign (direction) of a relation between (at

least) two variables is different depending on the value (level) of some other variable(s). Note that the term "moderated" in this context does not imply causality but represents a simple fact that depending on what subset of observations (regarding the "moderator" variable(s)) you are looking at, the relation between the other variables will be different.

Within epidemiology, the term interaction is used to describe the situation where the association between one exposure (risk factor) and disease varies according to the presence or absence of another exposure (Zammit et al., 2010). This same definition can be applied to the GxE usually tested in behavioural and psychiatric genetics although the outcome can be also a quantitative trait apart from disease.

Also, some authors have pointed out that interaction is model dependent meaning that the model used to test the interaction affects the interpretation of the results. In this regard, interaction effects can be modelled on either *additive* or *multiplicative* scales (Zammit et al., 2012). Additive models use risk differences (RD) while multiplicative models use risk ratios (RR). For example, statistical interaction between A and B under an additive model means that the risk of disease if both A and B are present is not equal to the additive effect (sum) of the risk if exposed to A only and that if exposed to B only, that is, risk from the joint exposure could be greater than the sum (addition). This model used risk differences or differences between means. When interaction is found under this model, the findings indicate that the combined effect of A and B interact greater than additively (Table 4). Similarly, statistical interaction between A and B under a multiplicative model (e.g. studying risk ratios using logistic regression) means that the risk of disease if both A and B are present is not equal to the multiplicative effect (product) of the risk if exposed to A only and that if exposed to B only. Under this model, interaction effects would be greater than multiplicative and usually involve the use of odds ratios.

Table 4. Statistical model used to study interactions between two risk factors, A and B (Zammit et al., 2012).

Statistical Model	Relationship	Definition	Interaction
Additive	Additive	Risk (A and B) = Risk (A only) + Risk (B only) - Risk (Neither A nor B)	No (null hypothesis)
	Greater than additive	Risk (A and B) > Risk (A only) + Risk (B only) - Risk (Neither A nor B)	Yes
Multiplicative	Multiplicative	Risk ratio (A and B) = Risk ratio (A only) x Risk ratio (B only)	No (null hypothesis)
	Greater than multiplicative	Risk ratio (A and B) > Risk ratio (A only) x Risk ratio (B only)	Yes

Also, there is a particular type of interactions, called *qualitative* or *crossover* interactions, which are believed to be of major importance (Gail and Simon, 1985, Zammit et al., 2012, Zammit et al., 2010). In a qualitative interaction the effects go in opposite directions, for example, exposure to a particular environment is deleterious in carriers of a particular allele and protective in non-carriers and vice-versa). As opposite, in a quantitative or non-crossover interaction, there is a change in magnitude but not in direction of effects (Gail and Simon, 1985).

It is important to note that finding evidence for a gene-environment interaction effect does not provide *per se* information about the underlying biological or pathophysiological mechanism involved in the studied phenotype.

In the last years, the enthusiasm for GxE research has recently been tempered by increasing scepticism (Duncan and Keller, 2011, Munafo and Flint, 2009, Eaves, 2006, Zammit et al., 2012). Dismissal about GxE studies has recently arisen mainly due to the failure to replicate, as happened before with genetic association studies (Munafo and Flint, 2009). It has been argued that the lack of replication can be related to the greater number of potential statistical tests that are possible when interaction effects are included in any analysis, which greatly increases the risk of false positives which can be nominally significant but do not represent true insight (Munafo and Flint, 2009).

Critics also worry about publication bias meaning the tendency to publish significant results more readily than nonsignificant ones (Duncan and Keller, 2011). Thus, the debate is not whether GxEs may be expected in psychiatry but which GxE findings are replicable and illuminating and which are spurious and lead to wasted resources, false hope and increased scepticism. Duncan and Keller claimed that true progress in understanding GxEs in psychiatry requires researchers to keep on standards that will increase certainty in reported results. By doing so, the second decade of GxE research in psychiatry can live up to the promises made by the first (Duncan and Keller, 2011).

GxE has to be distinguished from another mechanism of gene-environment interplay, gene-environment correlation (Fig 9). Gene-environment correlation (rGE) crefers to the influence of genes in environmental exposure concerns genetic influences on individual's exposure to environmental factors. In other words, detection of rGE effects indicates that the occurrence of the environmental exposure depends, at least partially, on genetic factors. A gene may increase the likelihood that a person would be exposed to an environmental risk factor, which in turn, increases the likelihood to develop a particular trait or disease (Van Os and Sham, 2003). For example, liability to the use of cannabis is influenced by genetic factors, especially heavy use (Kendler and Prescott, 1998). Therefore, since heavy cannabis use is a risk factor for schizophrenia (Henquet et al., 2005), part of the apparently environmental

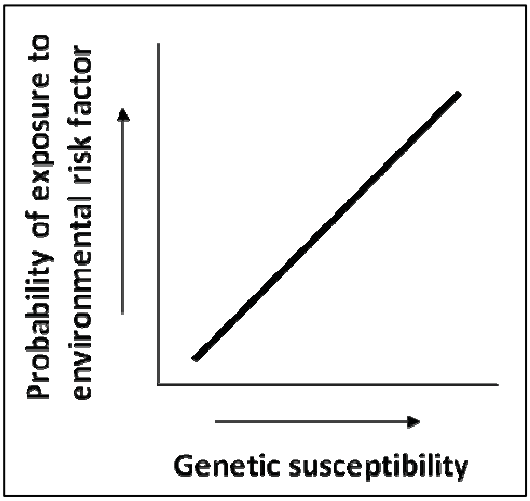

Figure 9. Gene-environment correlation. Genetic control of exposure to the environment. The greater the genetic susceptibility, the higher the probablity to be exposed to the environmental risk factors (Van Os and Marcelis, 1998).

risk may be of genetic origin (Van Os and Sham, 2003). Of note, this does not denies the effect of the environmental factor *per se*, in this example, cannabis. Three types of rGE can be differentiated: passive, active and evocative rGE (Table 5) (Plomin et al., 1977). Passive rGE refers to the association between the genotype a child inherits from her/his parents and the environment in which is raised. Passive rGE requires interactions between genetically related individuals. Evocative (or reactive) rGE refers to the association between and individual's genetically influenced behaviour and the reaction of those in the individual's environment to that behaviour. Active (or selective) rGE refers to the association between individual's genetically influenced traits or behaviours and the environmental niches selected by the individual.

Table 5. Three types of gene-environment correlation (Plomin et al., 2008)

Type	Description	Source of environmental influence
PASSIVE	Children receive genotypes correlated with their family environment	Parents and siblings
EVOCATIVE	Individuals are reacted to on the basis of their genetic propensities	Anybody
ACTIVE	Individuals seek or create environments correlated with their genetic proclivities	Anybody

For example, consider musical ability. If musical ability is heritable, musically gifted children are likely to have musically gifted parents who provide them with both genes and an environment conducive to the development of musical ability (passive rGE). Musically talented children might also be picked out at school and given special opportunities (evocative rGE). Even if one does anything about their musical talent, gifted children might seek out their own musical environments by selecting musical friends or otherwise creating musical experiences (active rGE) (Table 5) (Plomin et al., 2008).

There is evidence describing these types of rGE but these effects are still difficult to detect (Rutter et al., 2006). Nevertheless, it has been argued that the identification of

rGE may suggest targets for environmental intervention even in highly heritable disease (Jaffee and Price, 2007).

1.4.3.1. The BDNF and COMT as candidate genes for gene-environment interaction studies

The BDNF gene

Brain-Derived Neurotrophic Factor (BDNF) (Fig 10) is a neurotrophin that promotes the growth and differentiation of developing neurons in central and peripheral nervous systems (Buckley et al., 2007). BDNF is also implicated in the survival of neuronal cells in response to stress (Chen et al., 2006, Chen et al., 2004b, Van Winkel et al., 2008). It has been shown that early stress can influence BDNF expression and produce long-lasting effects on neurotrophic processes, thereby impacting on neuronal maturation and plasticity in later life (Van Winkel et al., 2008).

In this context, the *BDNF* gene, located at chromosome 11p14, contains a functional polymorphism that has been particularly widely studied in genetic association and GxE studies in psychiatry research (Gratacos et al., 2007). This single-nucleotide polymorphism (SNP) consists of a methionine (Met) substitution for valine (Val) at codon 66 (Val66Met) (Chen et al., 2006). The Val variant is associated with higher neuronal BDNF secretory activity than is the Met variant. Additionally, the co-

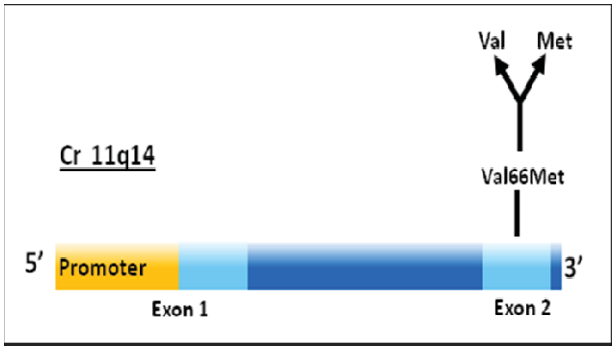

Figure 10. Schematic view of the Val66Met polymorphism of the *BDNF* gene.

expression of Val and Met alleles in heterozygotes results in less efficient intracellular trafficking and processing, leading to decreased BDNF secretion (Chen et al., 2004b).

Evidence from animal studies suggests that individuals carrying the Met/Met genotype are more likely to develop anxiety-related behaviours in response to stressful events (Chen et al., 2006). In humans, it has been shown that Met homozygotes and heterozygotes who have experienced childhood adversity could also be more genetically vulnerable to the development of affective symptoms, in comparison to Val homozygotes (Aguilera et al., 2009). The BDNF-Val66Met polymorphism has been related not only to psychosis but has also been shown to moderate the impact of childhood adversity on the later expression of affective symptoms.

The COMT gene

The enzyme catechol-O-methyl transferase (COMT) is involved in catabolism of monoamines that are influenced by psychotropic medications, including neuroleptics and antidepressants (Craddock et al., 2006). Furthermore, this enzyme plays an important role in the degradation of dopamine in the brain, particularly in the prefrontal cortex (Akil et al., 2003, Craddock et al., 2006, Matsumoto et al., 2003, Van Winkel et al., 2008). The *COMT* gene, located in chromosome 22q11, encodes this enzyme and contains a functional polymorphism (COMT-Val158Met) that results in two common variants of the enzyme: Val and Met (Fig 11) (Chen et al., 2004a). The Val variant is associated with increased COMT activity, which results in a combination of reduced dopamine neurotransmission in the prefrontal cortex, associated with impairments in working memory, attention and executive functioning (Meyer-Lindenberg et al., 2005), and increased levels of dopamine in mesolimbic areas. Individuals carrying the Met/Met genotype have the lowest COMT activity and

heterozygotes are considered to be of intermediate activity, as the two alleles are codominant (Mannisto and Kaakkola, 1999).

However, despite considerable research effort, it has not proved straightforward to demonstrate and characterise a clear relationship between genetic variation at COMT and psychiatric phenotypes (Craddock et al., 2006).

Increase in COMT activity has been reported to be associated with anxiety disorders and anxiety-spectrum phenotypes (Hettema et al., 2008, Lonsdorf et al., 2010) as well as with depression (Massat et al., 2005); however, there are also contradictory reports (Arias et al., 2006, Serretti et al., 2003, Wray et al., 2008). The *COMT* val158met polymorphism has been shown to influence emotional processing as well as amygdala responsivity to anxiety- and depression-related emotional stimuli with, however, again contradictory results (Domschke and Dannlowski, 2010). In schizophrenia, most work on COMT has been predicated on the hypothesis that the Val158Met polymorphism is a direct risk factor for schizophrenia. The classic hypothesis that schizophrenia results from enhanced dopaminergic neurotransmission predicts that the Met allele will be directly associated. In contrast, the hypothesis that excess dopamine function in the mesolimbic system is secondary to low dopamine function in the prefrontal cortex predicts association to the Val allele. However, the overall evidence from published studies is not compatible with either simple hypothesis (Craddock et al., 2006). Furthermore, COMT-Val158Met has been shown to interact with other genes and

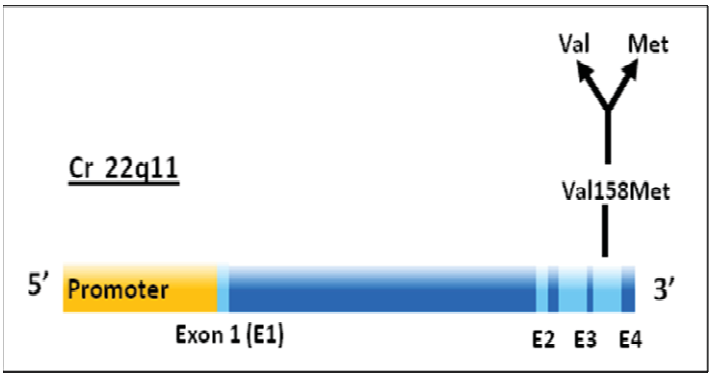

Figure 11. Schematic view of the Val158Met polymorphism of the *COMT* gene.

environmental risk factors in the development of psychotic symptoms (Estrada et al., 2011, Peerbooms et al., 2012) and cognition (Goldberg et al., 2013). Thus, the role of this polymorphism in the risk for psychopathology still remains to be fully understood.

1.5. Justification of the PhD aims

Presentation of the manuscripts included in this thesis

Taking into account all abovementioned, some of the main relevant issues that remain unclear are: the specific genetic contribution, the role of early psychosocial environment risk factors and the putative interplay between these two factors in the development of subclinical complex phenotypes such as child behaviour problems, psychotic experiences and anxiety and depression. Also, whether these psychopathological characteristics assessed in general population samples may present neuroimaging brain correlates similar to that observed in clinical samples has been poorly explored. For these reasons, our work was aimed to address these issues by the following studies:

We explored to what extent the experience of parental negativity in early childhood (age 4) accounted for behaviour problems during adolescence (age 12) and what was the relative contribution of genetic and environmental factors to this longitudinal association. Up to our knowledge, this is the first longitudinal genetically sensitive study investigating this association across two different developmental stages (manuscript presented in section 7.1).

As commented in the introduction, childhood maltreatment constitutes an environmental risk factor for several psychiatric symptoms and disorders. However, the mechanisms underlying its impact and why not everyone exposed to early adversity develops psychopathological symptoms later in life remains largely unknown. Using samples from the general population, we aimed to further explore the frequently reported link between childhood maltreatment and subclinical psychotic symptoms, from a dimensional approach. We tested the putative moderator role of BDNF gene (manuscript presented in section 7.2) and also the moderator role of COMT

gene and cannabis (manuscript presented in section 7.3). We also explored whether this association is due to genetic confounding using a MZ twin differences approach (manuscript presented in section 7.4).

Finally, putative structural and functional correlates of depression and anxiety and psychotic experiences and its influence in emotional processing were explored in twin samples drawn from the general population using neuroimaging techniques (manuscripts presented in section 7.5 and 7.6).

2. Hypothesis and Objectives

HYPOTHESIS

Early exposure to psychosocial stress will account for variability in psychopathological dimensions such as child behaviour problems, anxiety and depression and psychotic experiences in adulthood in individuals from the general population. Variation on these psychological problems will involve complex mechanisms of interaction between environmental and genetic factors and will be associated with changes in brain structure or functioning.

2.1 Main objective

To study, in representative samples from the general population, how early environmental factors, including parental negativity and childhood maltreatment, directly or in interaction with genetic factors explain psychopathological variation including both childhood behavioural problems and adult psychotic, depressive and anxious symptoms. In addition, we aimed to analyse, using neuroimaging techniques, possible structural and functional brain changes associated to these symptoms.

2.2 Specific objectives

1) To examine in a sample of 4075 pair of twins drawn from a large population-based twin study from the United Kingdom to what extent parental negativity at age 4 can account for child behaviour problems at age 12 applying a cross-lagged model; we will also explore the genetic and environmental architecture of the putative longitudinal association between parental negativity and child behaviour problems from age 4 to age 12 and whether child-driven and parent-driven effects can be identified in such relationship.
2) To explore whether childhood adversity (childhood abuse and childhood neglect) have a differential impact on the presence of psychotic experiences

(PEs) in a Spanish sample consisting of 533 individuals drawn from the general population. Furthermore, a possible moderating effect of the BDNF-Val66Met polymorphism on the relationship between childhood adversity and PEs was also investigated by means of linear multiple regression models.

3) To investigate whether the impact of the childhood adversity and cannabis effects on the development of PEs varies according to COMT-Val158Met polymorphism genotypes in a sample drawn from the general population (n=533) by means of multiple linear regression models.
4) To examine in a population-based twin sample consisting of 115 pairs (85 MZ twin pairs) i) whether childhood adversity was associated with positive and negative psychotic experiences in a twin sample from the general population, ii) to what extent MZ twins were similar for their exposure to childhood adversity and presence of psychotic experiences and iii) whether differences in exposure to childhood adversity were associated with differences in the expression of psychotic experiences in a subsample of MZ twins using the MZ twin differences method.
5) To investigate in a MZ twin sample (n=53 twins) drawn from a population-twin sample whether genetic and environmental liabilities make different contributions to abnormalities in gray matter volume (GMV) in anxiety and depression using a concordant and discordant MZ twin pairs design.
6) To explore in a MZ twin sample (n=53) drawn from a population-twin sample brain activation to facial emotion in subjects reporting positive and negative PEs using an fMRI community-based twin study. Furthermore, whether scores in psychotic experiences interact with anxiety and depression and genetic risk for these disorders was also explored by means of full factorial designs.

3. Supervisor's Report on Impact Factor

Dr Lourdes Fañanás
Anthropology Unit, Department of Animal Biology
Faculty of Biology

Supervisor's Report on Impact Factor

The doctoral thesis "**Genes, environment and their interplay in the development of psychopathological characteristics and their neuroimaging correlates in general population"** is based on the original results obtained by Silvia Alemany. These results are based on the analyses of genetic and environmental factors in i) a sample of Spanish twins from the general population; ii) a twin cohort from the general population of UK; and iii) a sample of Spanish individuals from the general population.

These results have been published or have been submitted to international peer reviewed journals. The impact factors of these journals demonstrate the quality of the research conducted, and are as following:

1. **Genetic origin of the relationship between parental negativity and behaviour problems from early childhood to adolescence: a longitudinal genetically informative design,** accepted for publication (in press) in *Development and Psychopathology*. This multidisciplinary journal is devoted to the publication of original, empirical, theoretical and review papers which address the interrelationship of typical and atypical development in children and adults. It is indexed in Journal Citation Reports (Social Sciences Edition) with a current impact factor of 4.397 and classified in the first decile of the area of Developmental Psychology (ranking: 5/68).

2. **Childhood abuse and the BDNF-Val66Met polymorphism: Evidence for gene-environment interaction in the development of adult psychosis-like experiences**, published in *The British Journal of Psychiatry*. This journal is one of the world's leading psychiatric journals. It covers all branches of the subject, with particular emphasis on the clinical aspects of each topic. *The British Journal of Psychiatry* is indexed in Journal Citation Reports (Sciences Edition) with a current impact factor of 6.619 and classified in the first decile of the area of Psychiatry (ranking: 4/117).

3. **Psychosis-inducing effects of cannabis are related to both childhood abuse and COMT genotypes,** accepted for publication (in press) in *Acta Psychiatrica Scandinavica*, a journal that acts as an international forum for the dissemination of high-quality scientific articles representing clinical and experimental work in psychiatry. This journal is indexed in Journal Citation Reports (Science Edition) with a current impact factor of 4.220 and classified in the first quartile of the area of Psychiatry (ranking: 15/117).

4. **Childhood adversity and psychosis: examining whether the association is due to genetic confounding using a monozygotic twin difference approach,** accepted for publication (in press) in *European Psychiatry*. This journal presents the results of original research relative to those domains which are presently of interest to psychiatry such as psychopathology, nosography, chemotherapy, psychotherapy, clinical methodology, biological disorders and mental pathology, psychophysiology, neuropsychology, as well as animal behaviour. This wide scope emphasizing, nevertheless, the publication of original articles,

is aimed at 1) encouraging the exchange of ideas and research within Europe, and 2) establishing within the international psychiatric community an improved level of scientific communication. *European Psychiatry* is indexed in Journal Citation Reports (Science Edition) with a current impact factor of 2.766 and classified in the second quartile of the area of Psychiatry (ranking: 30/117).

5. **Regional gray matter reductions are associated with genetic liability for anxiety and depression: a MRI Twin Study,** accepted for publication (in press) in *Journal of Affective Disorders*. This journal publishes papers concerned with affective disorders in the widest sense: depression, mania, anxiety and panic. It is interdisciplinary and aims to bring together different approaches for a diverse readership. It is indexed in Journal Citation Reports (Science Edition) with a current impact factor of 3.517 and classified in the first quartile of the area of Psychiatry (ranking: 19/117).

6. **Examining the relationship between anxiety, depression and psychotic experiences and brain activation to facial emotion: An fMRI community-based twin study,** currently in preparation.

Accordingly, I confirm the quality of the published and submitted articles.

Signed by Dr Lourdes Fañanás

Barcelona, 11 February 2013

4. Global Discussion and conclusions

Global discussion

The present dissertation, which can be framed in the fields of behavioural and psychiatric genetics (Plomin et al., 2008), was aimed to study how early environmental factors such as parental negativity and childhood adversity, directly or in interaction with genetic factors account for psychopathological variation (subclinical and clinical psychiatric symptoms) in general population. The phenotypes of interest included: childhood behaviour problems, adult psychotic, depressive and anxious symptoms and their neuroimaging correlates. Furthermore, from the different studies included in this dissertation, additional research questions were explored. Conclusions derived from the work included in this thesis are discussed below.

The use of population-based samples for studying psychopathological characteristics

In the last decades, the conventional categorical perspective that mental disorders constitute discrete entities has been challenged by epidemiological and genetic evidences suggesting that some of these phenotypes might be best conceptualised as dimensional constructs (Coghill and Sonuga-Barke, 2012, Linscott and van Os, 2010, Plomin et al., 2009). It is accepted that both approaches have advantages and disadvantages, however it has been pointed out that there is not enough data to support a shift of the categorical-based diagnostic paradigm (Moller, 2008).

In the particular case of psychosis, some authors remain reluctant to accept the conceptualization of psychosis as a continuum (Lawrie et al., 2010) while others strongly support this notion (Kaymaz and van Os, 2010, Cuesta et al., 2009, Linscott and van Os, 2010, van Os, 2003). However, a growing body of research, reports the occurrence of subclinical psychotic symptoms (also known as psychotic experiences) in community-based samples (Kelleher et al., 2012, Nelson et al., 2012). Furthermore, self-

reported questionnaires have been designed and validated with the specific aim of assessing psychotic experiences in general population samples (Konings et al., 2006, Stefanis et al., 2002).

In this regard, the present dissertation was aimed to characterize general population samples at psychopathological level. We found evidence indicating that child behaviour problems, anxious and depressive symptoms and psychotic experiences (PEs) were present in our samples drawn from the general population which is in agreement with a dimensional approach to psychopathology. Although this was not specifically assessed, in general, the experience of these symptoms did not dramatically affect daily life functioning among the participants since all of them were working or studying and socially well adapted. Thus, our results support a continuous distribution of behaviour problems and psychosis in general population where individuals differ in the frequency or intensity of the experience of these symptoms. Similarly, some individuals from the general population presented in the moment of assessment a diagnosis or history of diagnosis of a major depressive disorder (MDD) or an anxiety disorder. In some cases, this disorder had not being previously diagnosed or treated. These facts evidence that, at least some psychiatric disorders or symptoms, present a high variability in their expression in the general population.

Furthermore, evidence derived from the studies in this dissertation, indicates that some environmental risk factors previously linked to the more severe clinical expression of these disorders are also associated to subclinical symptoms. For instance, we observed that childhood adversity was associated with the development of psychotic experiences in three studies conducted in two different samples. Thus, as it has been shown in clinical samples (Read et al., 2001, Van Winkel et al., 2008, Varese et al., 2012), childhood trauma also plays a role in the development of PEs in general population. This supports the notion of an *etiological continuum*, that is, both clinical

and subclinical symptoms would share some of the same risk factors in their aetiology. Thus, the study of the factors and mechanisms underlying psychiatric symptoms in non-clinical samples contributes to the understanding of the severe expression of these phenotypes and present the advantage of not obtaining results which may be biased by treatment or the illness itself.

Taking in consideration all above-mentioned, we can conclude that it is valid to use population-based samples to enhance our understanding of the aetiology of mental disorders. Furthermore, the phenotype observed in general population constitutes an interesting object of study by itself since these subclinical or moderate expressions of psychopathology may cause suffering, may precede a more severe phenotype and are much more common than the clinical disorders. In this regard, a study developed in US pointed out that over one third of the cases were mild (Kessler et al., 2005). Also, in the case of young samples, assessment of subclinical symptoms would contribute to the identification of individuals at risk for psychiatric disorders.

The long-terms effects of early adversity

One of the main aims of this dissertation was to study the impact of early adverse experiences. Based on a longitudinal twin study, we observed that parental negativity at age 4 was associated with child behaviour problems 8 years later. Thus, experiences occurring during the first years of life can partially account for behavioural variance in adolescence. We referred to the self-regulation framework (Calkins and Keane, 2009) to explain this association. Behaviour problems are likely to reflect difficulties in behavioural adjustment that may be underlined by deficits in self-regulatory processes.

In this context, failures in the acquisition of basic processes such as emotion regulation and cognitive control early in life would ultimately lead to the expression of

behaviour problems. Our results indicating a bidirectional relationship (i.e. there were both effects coming from the parents and effects elicited by child behaviour) between parental negativity and behaviour problems from early childhood (age 4) to adolescence (age 12), support the role of parenting in the early origins and maintenance of behaviour problems. Furthermore, our results indicated that this cascade of events was mainly underlined by genetic factors. Biological foundations related to the physiological and neurobiological mechanisms of the self-regulation process may well include genetic influences, therefore adding plausibility to our results (Calkins and Keane, 2009, Posner and Rothbart, 2009).

Similarly, adverse childhood experiences occurring during the first 18 years of life showed to have an impact in adult development of PEs. Since brain and behaviour development constitute extremely environment-dependent processes (Blakemore, 2012, Jernigan et al., 2011, Lenroot and Giedd, 2011), exposure to adverse, stressful or just inappropriate events (including lack of stimulation or care) has disruptive effects the consequences of which can extend into adulthood (Ford, 2010, Tomalski and Johnson, 2010). Early adversity exerts its enduring effects by disrupting the optimal development of brain structure and circuitry and, consequently, the development of cognitive, emotional and social abilities. By impairing or limiting the full and optimal development of these neurobiological processes and emotional, cognitive and social abilities, early adversity can ultimately be linked to adult negative outcomes including psychopathology (Shonkoff, 2010).

Neurobiologically, an affective pathway has been proposed to underlie the specific association between childhood adversity and psychosis, preferentially positive psychotic clinical or subclinical symptoms (Van Winkel et al., 2008). According to this hypothesis, childhood adversity will exert its psychosis-inducing effects through dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis. As mentioned in the

introduction, the HPA axis is one of the most important brain circuits involved in regulating adaptive responses to stress (Lupien et al., 2009). In this context, the intrusive nature of abusive experiences may make them especially likely to deregulate the HPA axis. We found evidence for a significant association between child abuse and PEs independent of neglect, which shown no significant association with psychotic experiences. This is in agreement with a previous study where no impact of neglect on the expression of psychosis was found when controlling for the impact of abuse (Fisher et al., 2010). In this way, events characterized by abuse have shown the most robust association with psychotic symptoms (Janssen et al., 2004, Morgan and Fisher, 2007, Whitfield et al., 2005). Moreover, it has been postulated that abusive experiences could have an aetiological significance in psychosis (Harris, 1987), and research has described higher rates of abusive maltreatment than neglect among individuals with psychosis (Hlastala and McClellan, 2005).

These facts highlight the importance of protecting childhood and adolescence since individuals at these ages do not have still acquired cognitive and psychological skills and abilities to copy with adverse situations, especially very disruptive ones such as abuse. Probably, the best way that researchers have to increase awareness, prevention and management of childhood maltreatment is keep showing the consequences of childhood maltreatment and further investigating parameters such as timing, frequency, intensity, definition and specifity of such environmental exposures in order to identify vulnerable subjects and prevent undesirable outcomes. On the other hand, neuroimaging studies and molecular biology appear as promising fields to investigate the mechanisms underlying the association between childhood adversity and psychopathology (Edmiston et al., 2011, Klengel et al., 2013).

The complex dialogue between genetic and environmental factors

Interestingly, some of environmental influences such as early adversity might be *genetically mediated*. For example, in the study of parental negativity and behaviour problems over time we found that this association was mainly of genetic origin, that is, genetic factors play a major role in this association even though parental negativity constitutes an environmental risk factor. Of note, it is important to point out that the fact that an association between an environmental risk factor and a given phenotype would be mainly accounted by genetic factors does not deny the environmental effects of the environmental factor. These findings lead us to reflect about the gene-environment dialogue. Initially, the field of behaviour genetics was concerned with partitioning population variance into that due to genetics and that due to environmental influences. The implication was that these two types of factors were separated. Also, it was assumed that gene-environment interactions were usually of so little importance that they could safely be ignored (Rutter, 2007). However, the increasing number of studies, especially during the last decade, evidencing gene-environment correlation and interaction effects, raised the question about the supposed separateness of genes and environment (Rutter, 2007). In this way, from the studies included in this dissertation where the association between childhood adversity and PEs is explored, we observed that gene-environment (*BDNF*-Val66Met polymorphism and childhood abuse) and gene-environment-environment (*COMT*-Val156Met polymorphism, childhood abuse and cannabis use) interactions might be involved in the individual variation in response to childhood adversity. However, we also found evidence supporting an association between childhood adversity and PEs after controlling by genetic confounding, meaning that childhood adversity might have an environmental impact in the individual risk to develop PEs regardless of the genetic background of the individual. These evidences might seem contradictory but reflect

something already claimed by experts in the field of behaviour genetics. As stated by Plomin and colleagues, "when considering the variation of phenotype, genes can affect the phenotype independent of environmental effects, and environments can affect the phenotype independent of genetic effects. In addition, genes and environments can interact to affect the phenotype beyond the independent prediction of genes and environment" (Plomin et al., 2008). Therefore, the direct effect of genes, the direct effect of environment and the possible interaction effects between genes and environment on phenotypic variation are not mutually exclusive. Moreover, it might be possible that all these possibilities are involved in the causation of complex characters such as psychopathological characteristics. The findings derived from the studies included in the present dissertation are in line with this perspective because provide evidence of different ways through genes, environment and their interplay can modulate the final expression of the phenotype. Thus, while there is no doubt regarding that genes and environmental factors can have a direct influence in the phenotype, there is a *gene-environment interdependence* that must be considered when studying complex characters (Rutter, 2007). Since, these two factors are expected to act together in nature, even when studying genetic or environmental influences separately it is important to consider their interdependence because genes may be under environmental control and vice versa. Exploring the effect of one type of factors always require considering the role of the other. Genes require the effects of environment to have an influence in behaviour and, at the same time, the impact of environmental factors would be influenced by the genetic background of the individual.

In this context, this thesis includes two studies reporting novel gene-environment interaction effects related to the development of positive PEs in general population. One hand, Met carriers of the *BDNF*-Val66met polymorphism were found to be more likely to report positive PEs when exposed to child abuse compared to

carriers of the Val allele. On the other hand, we found evidence for a qualitative gene-environment-environment interaction effect indicating that the psychosis-inducing effects of cannabis were related to the exposure to child abuse and *COMT* genotypes. Specifically, cannabis and *COMT* genotypes had a negligible effect on positive PEs among those individuals not exposed to child abuse. However, in individuals exposed to child abuse and not using cannabis, positive PEs scores increased as a function of the number of copies of the Met allele of *COMT* gene. An opposite pattern was found in individuals exposed to child abuse and using cannabis, positive PEs scores increased as a function of the number of copies of the Val allele of the *COMT* gene. All these findings, if replicated, can greatly contribute to the understanding of how genetic variation is related to individual variation in vulnerability to the exposure to environmental risk factors.

Of note, recent critics about gene-environment interaction studies force us to reflect on several aspects (Duncan and Keller, 2011, Munafo and Flint, 2009, Zammit et al., 2012, Zammit et al., 2010). The main concern refers to the lack of replication which can only be solved by refining the attempts to replicate. However, there are different views about the definition and way of testing additive and multiplicative interaction effects. Also there is confusion about the effect size of GxE findings. In this regard, it would important to unify concepts and definitions, at least, within a same field such as psychiatric genetics in order to facilitate the interpretation and replication of results.

Using neuroimaging techniques to find brain correlates of psychopathological characteristicsin general population

In the last decades, neuroimaging techniques appeared as a promising methodology to explore putative brain correlates of risk and presence of psychiatric symptoms and disorders (Linden, 2012). Consequently, the number of studies using

neuroimaging techniques has increased substantially. In this regard, since brain structure and function are affected in psychiatric disorders, it is important to disentangle the sources of variation in these phenotypes from clinical to subclinical symptoms (Blokland et al., 2012). This led us to question whether genetic and environmental factors operate through the same causal neurobiological pathways. In this way, initially, the magnetic resonance imaging (MRI) twin studies presented in this dissertation were expected to highlight brain areas and functions that might be particularly vulnerable to environmental influences. For this purpose, only MZ twins were included. Furthermore, we expected to find intrapair differences in the discordant MZ twin group for anxiety and depression. As above-mentioned, the MZ twin differences design is extremely powerful to detect environmental factors since phenotypic differences within MZ twin pairs must be explained by differential exposure to environmental factors. Thus, the application of this design and its extension, the concordant and discordant MZ twin design, appear as a promising method to identify human brain morphological and functional aspects plastic to environmental influences. Unfortunately, we did not find statistically significant differences within MZ twin pairs, probably due to the sample size. Nevertheless, we made use of the fact of including a concordant MZ twin group for anxiety and depression, presumable representative of individuals carrying a high genetic load for these disorders. Therefore, this design allows us to infer whether a particular brain difference between affected and healthy individuals, might be linked to genetic risk to the disorder (de Geus et al., 2007). The implication of these kind of findings is that admixture of individuals which disorder or risk for the disorder is mainly accounted by genetic factors with those which disorder or risk for the disorder is mainly accounted by environmental factors, might lead to negative or confusing results. Therefore, genetically sensitive studies such twin studies, can greatly contribute to

elucidate whether brain abnormalities can be mainly accounted for by genetic or environmental risk for the disorder, symptoms or subclinical phenotypes. Of course, this approach does not deny the possibility that gene-environment interaction effects would be underlying the presence of brain abnormalities.

The availability of functional neuroimaging data regarding brain activation to facial emotion and our interest regarding the development of PEs in general population samples led us to explore a whether the presence of PEs would influence emotional processing. In this regard, according to previous studies, the presence of PEs was associated with the activity of the anterior cingulate cortex during exposure to facial emotion (Modinos et al., 2010). Interestingly, we also found that the presence of positive PEs influenced (moderated) brain activation to facial emotion in our group of MZ twins concordant for lifetime anxiety and depression. This finding may be suggesting a shared pattern of emotional processing across different psychopathological dimensions assessed in general population.

Limitations

Finally, several limitations need to be considered when interpreting the present work. Firstly, it is worth it to mention that data from general population samples might be biased because of the type of individuals who are willing to participate in scientific research (e.g. low levels of suspicion). Also, the most likely scenario is that a small proportion of the sample may present (current or past) history of psychiatric symptoms or disorders but in low to moderate levels, that is, not reaching the most severe expression of such disorders. It is important to consider these characteristics when interpreting and, especially, generalizing results. Secondly, the sample size of the Spanish twin sample is modest, especially in the case of the neuroimaging studies. Indeed, as abovementioned, the small number of brain images of discordant MZ twin

pairs (n=10 pairs) may explain why no significant intrapair differences were detected in this group. Therefore, these studies need replication and should be interpreted with caution. Thirdly, in the studies of gene-environment interaction included in this thesis, only one polymorphism –although with functional implications- of each gene analysed was available and only one gene was included in each study. Similarly, the number of environmental factors of interest was limited to one or two at maximum. These facts limit the exploration of gene-environment interaction effects. However, the choice of the polymorphism, gene and environmental factors to analyze in each study was made considering a priori hypothesis based on previous literature or in a plausible neurobiological explanation. Furthermore, a number of confounders were considered in each study (sex, age, schizotypy and trait anxiety). Finally, no main genetic effects were found in the gene-environment interaction studies conducted, that is, we did not detect a direct and independent effect of the studied genetic variant on the phenotype. This constitutes another critic made to gene-environment interaction studies, the generalized lack of significant main genetic effects (Duncan and Keller, 2011, Zammit et al., 2012, Zammit et al., 2010). This critic arise from the premise that, statistically, it is more difficult to find interaction effects than main effects (McClelland and Judd, 1993), thus, a robust GxE effect finding obtained in a well-powered study should always find significant main effects, including significant main genetic effects. However, such findings would indicate a direct and independent effect of the genetic variant studied on the phenotype. Although statistically possible or even expectable, would we expect main genetic effects from a biological point of view? As Rutter and Silberg pointed out years ago, the genes that influence sensitivity to the environment may be quite different from those that bring about main effects (Rutter and Silberg, 2002). This biologically plausible reasoning might help to explain the lack of main genetic effects in gene-environment interaction studies.

Conclusions

1) Exposure to parental negativity at age 4 was significantly associated with behaviour problems 8 years later. This association showed to be mainly accounted for by genetic factors. This adds evidence to the fact that early exposures occurring in the first four years of life can have effects several years later, even across different developmental stages (from early childhood to adolescence). Furthermore, by tracking the impact of environmental influences using a genetically sensitive design (longitudinal twin study) we observed that genetic factors were largely responsible for the maintenance of this association over time. This highlights the importance of gene-environment interaction and correlation mechanisms.

   Interestingly, the association was not only parent-driven since child-driven effects were also identified indicating that the longitudinal association between parental negativity seems to be bidirectional.

2) Individuals exposed to adverse events during childhood are more likely to develop psychotic experiences (PEs) in adulthood. Specifically, child abuse but not child neglect is associated with positive but not negative (although a trend toward significance was observed) PEs. This indicates certain specificity on the association between child adversity and psychosis being the events characterized by abuse of particular importance in the development of positive PEs rather than negative PEs.

   Furthermore, the BDNF-Val66Met polymorphism showed to moderate the association between child abuse and positive PEs indicating that Met carriers were more likely to report positive PEs when exposed to childhood abuse

compared to individuals carrying the Val/Val genotype. This gene-environment interaction effect may be involved in individual variation in response to childhood abuse, i.e. Met carriers of the BDNF gene might be neurobiologically more vulnerable to the impact of child abuse regarding the expression of positive PEs.

3) A significant three-way interaction was found between child abuse, cannabis use and the COMT-Val158Met polymorphism in the development of positive PEs. The use of cannabis after exposure to childhood abuse had opposite effects on positive PEs depending on the COMT genotypes.

   When individuals were exposed to low rates of childhood abuse, cannabis use and the Val158Met polymorphism of the *COMT* gene had a negligible effect on the presence of positive PEs scores. In individuals exposed to childhood abuse who used cannabis, positive PEs score increased as a function of the Val allele dose of the *COMT* gene. However, among individuals exposed to childhood abuse who did not use cannabis, the positive PEs score increased as a function of the Met allele copies of the *COMT* gene. This pattern of results coincides with the epidemiological definition of *qualitative interaction*.

   In the light of these findings, child abuse seems to have a role in the association between cannabis use and psychosis. Furthermore, the effects of child abuse and cannabis were moderated by the COMT genotypes indicating a gene-environment-environment interaction.

4) Using a MZ-twin differences approach which allows controlling for genetic confounding, we found a significant environmental effect of childhood adversity on the development of positive and negative PEs. This suggests that

although some individuals may be genetically vulnerable to the impact of childhood adversity, as abovementioned, early adverse events can independently contribute to the development of PEs.

5) Concordant affected MZ twin pairs for anxiety and depression are hypothesized to carry a particularly high genetic loading for these disorders. When comparing MRIs of concordant affected MZ twins for lifetime anxiety and depression against MRIs of healthy MZ twins, we found that concordant twins had significantly lower GMV mainly in bilateral fusiform gyrus and bilateral amygdala compared to healthy control twins. This finding suggests that genetic risk for anxiety and depression may underlie GMV reductions in fusiform gyrus in amygdala.

   No intrapair significant differences in whole brain GMV were detected in discordant twins, thus, our study does not provide evidence for *unique* environmental factors accounting for GMV changes in anxiety and depression.

6) The occurrence of PEs is related to changes in brain response to facial emotion. Specifically, activation and deactivation of the anterior cingulate cortex (ACC) to negatively valenced stimuli was associated with negative and positive dimensions of PEs. These findings support the hypothesis of the presence of emotion dysregulation in the psychosis *continuum.* Furthermore, positive dimension of PEs moderates emotional processing in concordant affected MZ twin pairs for lifetime anxiety and depression. This suggests that these psychopathological dimensions may share altered emotional functioning.

5. Summary

INTRODUCCIÓN GENERAL

1) El desarrollo cerebral y su interacción con el ambiente

La evolución del cerebro está ligada a la historia de la optimización de la capacidad humana de adaptarse, explotar y manipular el entorno o ambiente, incluyendo el ambiente social, que juega un papel crucial en animales sociales como es el caso de los seres humanos (Brüne, 2010). El cerebro pasa por diferentes etapas caracterizadas por cambios neurobiológicos como el desarrollo de sinapsis durante la primera infancia y un proceso de eliminación de sinapsis durante la adolescencia (Kolb and Wishaw, 2003). De forma paralela a estas fases, el comportamiento social y las habilidades cognitivas del individuo también evolucionan de modo que es necesario que se produzcan determinados cambios neurobiológicos que permitan el desarrollo de habilidades y comportamientos más complejos. A medida que el cerebro se desarrolla y madura los sistemas neuronales se vuelven receptivos e interactúan con el ambiente. Actualmente está ampliamente aceptado que la estructura del cerebro en cualquier momento es un producto de la interacción entre factores genéticos, epigenéticos y ambientales (Brune, 2010). En este sentido, aunque gran parte del desarrollo cerebral está determinado genéticamente, la expresión final y la consolidación de los cambios neurobiológicos acabarán siendo esculpidos por diferentes influencias ambientales. Esto es posible gracias a la plasticidad cerebral, es decir, a la capacidad cerebral de cambiar estructural y funcionalmente en función de las influencias ambientales. El hecho de que el cerebro sea plástico a las influencias ambientales, capacita al individuo para aprender y adaptarse al entorno. Al mismo tiempo, sin embargo, algunas influencias ambientales pueden tener consecuencias

negativas, tales como las condiciones de estrés, especialmente cuando el estrés se produce a temprana edad.

Durante el desarrollo se producen desajustes entre las demandas del entorno y las capacidades cerebrales, por ejemplo, habilidades cognitivas, emocionales o sociales. Estos desajustes producen cambios fisiológicos y neurobiológicos. Aunque algunos forman parte aprendizaje y desarrollo normal del individuo, se ha de tener en cuenta que si el desajuste es excesivo, podría tener consecuencias negativas (Lenroot and Giedd, 2006). En este sentido, a pesar de que la mayoría de los mecanismos psicológicos humanos son "programas abiertos" y dependen en gran medida de la estimulación ambiental adecuada para desarrollarse correctamente, la exposición a adversidad ambiental o la falta de estimulación adecuada pueden causar disfunciones que finalmente pueden incluso llevar a la patología (Brune, 2010).

En cuanto a la adversidad ambiental, las amenazas al bienestar del individuo, ya sea físico o psicológico, son inevitables en la experiencia de la vida. Los estudios en animales y humanos han demostrado que durante la primera infancia el cerebro es particularmente sensible al estrés, probablemente debido a que durante este período el cerebro experimenta importantes cambios neurobiológicos (Lupien et al., 2009). El estrés puede ser definido como un estado psicológico en el que el individuo percibe experiencias o retos a la integridad física o el bienestar emocional como abrumadores, es decir, que sobrepasan sus recursos y capacidad de adaptación (Gunnar y Quevedo, 2007). Desde un punto de vista biológico, el estrés desencadena la activación de un sistema clave en la respuesta al estrés, el eje hipotálamo-pituitario-adrenal (HPA), que culmina en la producción de glucocorticoides (Lupien et al., 2009). Cuando el cerebro detecta una amenaza, se activa una respuesta fisiológica que involucra respuestas autonómicas, neuroendocrinas, metabólicas e inmunológicas. Desde el hipotálamos se libera la hormona liberadora de corticotropina (CRH) y la arginina vasopresina (AVP)

desencadenando la posterior secreción de la hormona adrenocorticotropa (ACTH) por la glándula pituitaria. Esto conduce a la producción de glucocorticoides por la corteza suprarrenal. Los receptores para estos esteroides se expresan en el cerebro y, además, pueden actuar como factores de transcripción y regular la expresión génica. Por lo tanto, los glucocorticoides pueden tener efectos a largo plazo sobre el funcionamiento de las regiones del cerebro que regulan su liberación. Además, los glucocorticoides son importantes para la maduración normal del cerebro. Tras la activación del eje HPA, y una vez que el factor estresante percibido ha disminuido, mediante diversos mecanismos de retroalimentación, el organismo retorna a su homeostasis inicial (Lupien et al., 2009).

La respuesta anormal al estrés, incluyendo el desarrollo de síntomas depresivos y de ansiedad, se atribuyen a disfunciones en el sistema nervioso central (SNC), especialmente en personas con cierta predisposición genética (Gottesman y Hanson, 2005). La exposición a estrés crónico puede dañar una amplia variedad de moduladores de la plasticidad cerebral. A nivel bioquímico, puede causar una reducción en la expresión de genes asociados con la plasticidad sináptica, lo que resulta en la disminución de la actividad cortical frontal (Kuipers et al., 2003).

2) Características psicopatológicas en población general

La conducta anormal y los trastornos mentales, constituyen el objeto de estudio de la psiquiatría. A nivel *fenomenológico*, se delimitan y describen estos fenotipos y esta información se utiliza para clasificación de los trastornos mentales. La *psicopatología*, en un sentido general, se define como el estudio empírico y teórico de la conducta anómala, su expresión y sus causas. El objetivo de la psicopatología es ofrecer una descripción y tipología general de los trastornos mentales (Parnas et al., 2013). Por

definición la conducta anormal y sus signos y síntomas psicopatológicos asociados a los que nos referimos como "trastornos mentales", son desadaptativos en tanto que causan sufrimiento e interfieren con la vida diaria del individuo y/o su entorno (Brüne, 2010).

La aproximación categorial vs. la aproximación dimensional a la psicopatología

La clasificación categórica de los trastornos mentales es ampliamente aceptada y los instrumentos diagnósticos basados en esta aproximación son los más utilizados en la práctica clínica. Sin embargo, esta aproximación sigue siendo cuestionada sobretodo por aquellos que abogan por la aproximación dimensional a la psicopatología. El interés en el debate categorías versus dimensiones ha crecido recientmente probablemente debido a que se está preparando la quinta versión del *Diagnostic and Statistical Manual of Mental Disorder* (DSM-V) (Coghill y Sonuga Barke, 2012).

Por un lado, el enfoque categorial considera que los trastornos mentales son entidades discretas (categorías) que se diferencias tanto cuantitativamente como cualitativamente de la conducta normal. Por otro lado, el enfoque dimensional considera el trastorno mental como una expresión extrema de la variación conductual normal en la población. De este modo, trastorno y conducta normal sólo se diferenciarían en grado de severidad pero no constituirían entidades distintas (Coghill y Sonuga Barke, 2012).

La publicación de la *International Classification of Disease* por la Organización Mundical de la Salud (ICD-9 (World Health Organization, 1993)) y del *Diagnostic and Statistical Manual of Mental Disorder Third Edition* por la Asociación Americana de Psiquiatría (DSM-III (American Psychiatric Association, 1980)) representaron el comienzo de la era moderna de la psiquiatría. La forma de identificar los trastornos mentales cambió con la introducción de estos manuales con criterios diagnósticos

claros y explícitos. La facilidad de uso, de comunicación entre clínicos, de planificación de tratamiento y sobretodo de toma de decisión clínia son algunas de las ventajas que han permitido que la clasificación categorial de los trastornos mentales y su representación en el ICD y el DSM sea ampliamente aceptada tanto en la práctica clínica como en investigación. Sin embargo, la heterogeneidad y la comorbilidad psicopatológica, entre otras cuestiones, han llevado algunos autores a cuestionar el enfoque categórico (Sonuga-Barke, 1998). La aplicación estricta de los criterios diagnósticos categoriales también puede dar lugar a que algunos individuos con síntomas significativos pero que no cumplen criterios para ser diagnosticados de un determinado trastorno, no reciban un tratamiento adecuado.

La aproximación dimensional a la psicopatología constituye una alternativa al enfoque categorial. A nivel dimensional, los trastornos mentales se conceptualizan como un *continuum* que iría desde la salud o la normalidad hasta la expresión más severa del trastorno mental. Por lo tando, se asume una continuidad entre la normalidad y la psicopatología y se utilizan datos empíricos para cuantificar y separar distintas dimensiones psicopatológicas (Kendell, 1975). Los hallazgos epidemiológicos han demostrado consistentemente que la mayoría de trastornos mentales muestran distribuciones continuas en la población sin punto claro dónde discernir normalidad y la psicopatología (Rutter, 2003). Estas evidencias han contribudio a que se acepte la conceptualización dimensional de fenotipos como la depresión o la conducta antisocial pero, hasta hace poco, trastornos más graves como el autismo o la esquizofrenia se han considerado entidades totalmente independientes de la variación conductual normal (Rutter, 2003).

Aunque las dos aproximaciones presentan ventajas y desventajas y tienen defensores y detractores, es posible utilizar un enfoque u otro dependiendo del objetivo clínico o científico. Por ejemplo, el cociente intelectual (CI) funciona mejor

como una dimensión si el interés es en la predicción del rendimiento académico o el funcionamiento social, incluso en la vida adulta. Por otra parte, funciona mejor como una categoría si el objetivo es la investigación de las causas neurobiológicas del retraso mental grave porque es probable que sean diferentes de los mecanismos neurobiológicos que explican las diferencias individuales en el CI normal (Rutter, 2003).

Problemas de conducta durante la infancia y la adolescencia

En psicopatología infantil a menudo se distingue entre problemas de conducta externalizantes e internalizantes (Achenbach, 1991). La co-ocurrencia de estos dos tipos de problemas de conducta es muy frecuente y se pueden desarrollar a edades muy tempranas ya en la primera infancia (Hill, 2002); por esta razón, este tipo de conductas colocan al niño en una situación de riesgo y pueden tener consecuencias graves ya que afectan a las relaciones con los padres y compañeros e incluso pueden acabar desarrollando conductas agresivas y delictivas (Coie y Dodge, 1998, Kovacs y Devlin, 1998). En este sentido, se ha puesto de manifiesto que los problemas emocionales y de conducta a edades tempranas pueden preceder a trastornos antisociales y de depresión en la etapa adulta (Moffitt, 1993). Además, ambos tipos de problemas conductuales comparten factores de riesgo comunes tales como el ambiente familiar negativo, la depresión materna o deficiencias cognitivas (Angold y Costello, 1993, Rutter, 1997).

Los problemas de conducta externalizantes incluyen el comportamiento hostil y físicamente agresivo hacía los otros, la impulsividad, la hiperactividad y la no aceptación de límites (McMahon, 1994). Los niños utilizan a menudo estos comportamientos para solucionar conflictos con los compañeros pero con el desarrollo de habilidades cognitivas y capacidad de regular emociones, estas conductas externalizantes tienden a disminuir durante la etapa pre-escolar (Coie and Dodge,

1998). La expresión clínica de los problemas de conducta externalizantes está recogida en el DSM-IV-TR (American Psychiatric Association, 2000) en el trastorno de conducta y el trastorno negativista desafiante. Los problemas de conducta internalizantes incluyen quejas somáticas, ansiedad, depresión y retraimiento social. Al contrario que las conducta externalizantes, las internalizantes tienden a incrementar gradualmente desde la infancia (Bongers et al,. 2003).

Ansiedad y depresión

Los trastornos de ansiedad incluyen el trastorno de pánico, el trastorno obsesivo-compulsivo (TOC), el trastorno de estrés postraumático (TEPT), la fobia social o trastorno de ansiedad social, la fobia específica y el trastorno de ansiedad generalizada (TAG). Los trastornos de ansiedad se caracterizan por la presencia de altos niveles de preocupación y anticipación de peligros inminentes que no podrán ser afrontados, estos síntomas se viven con mucho sufrimiento. A nivel fisiológico, los síntomas suelen acompañarse de taquicardia, hiperventilación, mareos y náuseas y sudoración. A diferencia de la ansiedad leve y breve causada por un evento estresante (como hablar en público o una primera cita), los trastornos de ansiedad duran por lo menos 6 meses y pueden empeorar si no se tratan. Los trastornos de ansiedad ocurren comúnmente junto con otras enfermedades mentales o físicas, incluyendo el abuso de alcohol o sustancias, que pueden enmascarar los síntomas de ansiedad o empeorarlos (Brune, 2010). Como grupo, los trastornos de ansiedad tienen una prevalencia de hasta un 30%, siendo la prevalencia a 12 meses de aproximadamente un 15% (Brune, 2010). Un estudio reciente realizado en EEUU indica que la prevalencia de vida para fobia específica es de 13,8%, para la fobia social es del 13%, para el trastorno de pánico es del 5,2%, para el trastorno obsesivo-compulsivo es del 2,7% y para la agorafobia con o sin trastorno de pánico es de 2,6% (Kessler et al., 2012).

El trastorno depresivo mayor (TDM) se encuentra entre las principales causas de baja laboral y discapacidad en todo el mundo. Según la Organización Mundial de la Salud (OMS) en el año 2020 el TDM será la segunda de las principales causas de discapacidad (Brune, 2010). El riesgo de padecer un TDM a lo largo de la vida se encuentra entre 7-12% en los hombres y entre un 20-25% en las mujeres (Kessler et al., 2005). Los síntomas de la depresión unipolar incluyen: estado de ánimo deprimido, pérdida de interés o de la capacidad de disfrutar de actividades que habitualmente eran placenteras, cambio marcado de peso o apetito, insomnio o hipersomnia, agitación o enlentecimiento psicomotor, fatiga o pérdida de energía, sentimientos de inutilidad o de culpa, dificultad para concentrarse o indecisión y pensamientos de muerte o suicidio (Barrett et al., 2007).

Aunque la depresión y la ansiedad históricamente han sido consideradas condiciones distintas, estos dos grupos diagnósticos a menudo coexisten en diversos grados en el mismo paciente (Roy-Byrne et al, 2000, Belzer y Schneier, 2004, Gorman, 1996). De hecho, diversos estudios investigan estos dos trastornos de forma conjunta ya que existen evidencias que sugieren diversos puntos en común en ansiedad y depresión incluso a nivel etiológico (Ressler y Mayberg, 2007). En primer lugar, la co-ocurrencia de síntomas ansiosos y depresivos es más la norma que la excepción con estimaciones de la comorbilidad que van desde 10% a más del 50% (Gorman, 1996, Ressler y Mayberg, 2007, Roy-Byrne et al., 2000). Más de la mitad de todos los individuos con TDM también desarrollan un trastorno de ansiedad a lo largo de la vida (Kessler et al., 1996). En segundo lugar, existe un solapamiento de síntomas asociados tanto con ansiedad como con depresión que puede hacer el diagnóstico particularmente difícil (Gorman, 1996, Ressler y Mayberg, 2007). En tercer lugar, los tratamientos más eficaces para ambos trastornos son los mismos, incluyendo los antidepresivos y la terapia cognitivo conductual (Ressler y Mayberg, 2007). En cuarto

lugar, ansiedad y depresión tienden a co-segregar en familias y parece ser que gran parte de la covarianza entre estos trastornos se explicaría por factores genéticos comunes (Mosing et al., 2009). En quinto lugar, diversas líneas de investigación sugieren que tantos los síntomas afectivos como los ansiosos surgen de la desregulación del circuito límbico-cortical, mediador de la respuesta al estrés (Cameron, 2006, Cameron et al., 2004, Ressler y Mayberg, 2007). En este sentido, desde los estudios de neuroimagen, los circuitos implicados en ambos grupos de trastornos pueden ser difíciles de distinguir (Ressler y Mayberg, 2007). Todas estas evidencias indican que la depresión y la ansiedad pueden compartir una vía etiológica común (Belzer y Schneier, 2004, Gorman, 1996, Mosing et al., 2009, Ressler y Mayberg, 2007).

Experiencias psicóticas y el *continuum* de psicosis

La pérdida de contacto con la realidad constituye la característica común de los trastornos psicóticos, como la esquizofrenia o el trastorno paranoide. Los síntomas psicóticos pueden clasificarse en tres dimensiones: positiva (alucinaciones y delirios), negativa (estados deficitarios dónde los procesos emocionales y las conductuales están disminuidas o ausentes) y desorganizada (conducta bizarra).

Los síntomas psicóticos se han considerado tradicionalmente de forma dicotómica: los pacientes los presentan o no (Kwapil et al, 1999). Del mismo modo, los trastornos psicóticos representan una entidad categórica, el grupo de las psicosis, y así están recogidos en los principales sistemas diagnósticos como el DSM-IV-TR (American Psychiatric Association, 2000) o la CIE-10 (World Health Organization, 1993). Sin embargo, estudios epidemiológicos han demostrado que estos síntomas, en menor intensidad y/o frecuencia, son experimentados por una proporción importante de la población general a lo largo de la vida sin que haya necesidad de tratamiento ni interferencia con la vida diaria (Barragan et al., 2011, Johns and van Os, 2001, Kelleher

and Cannon, 2011). Estos hallazgos apoyan la definición del fenotipo psicótico desde una perspectiva dimensional con la que es compatible que formas atenuadas de los mismos síntomas que se observan en el trastorno clínico estén también presenten en algunos individuos de la población general que no cumplen criterios diagnósticos para ningún trastorno psiquiátrico. En este sentido, el concepto de *continuum* representa adecuadamente la variabilidad del fenotipo de psicosis, que iría desde la normalidad (ausencia total de síntomas) hasta la esquizofrenia (la expresión más grave de la psicosis) (Johns y van Os, 2001, Van Os et al, 1999, Verdoux y van Os, 2002). Así, los síntomas psicóticos se distribuirán a lo largo de un *continuum* que iría desde la normalidad a la esquizofrenia con un aumento gradual (cuantitativo más que cualitativo) del nivel de gravedad de los síntomas experimentados (Van Os et al., 1999).

La prevalencia de síntomas psicóticos investigada en población general oscila entre un 4% hasta un 28,4% (Eaton et al., 1991). Un estudio reciente analizó la prevalencia de síntomas psicóticos en 52 países del mundo con distintos niveles de desarrollo económico utilizando datos de la OMS (Nuevo et al., 2012). En este amplio estudio, los autores concluyeron que la prevalencia de síntomas psicóticos en todo el mundo está en un rango del 0,8% al 31,4% (Nuevo et al., 2012). Además, la investigación dirigida a estudiar los factores de riesgo que subyacen a la expresión de experiencias psicóticas (EPs; una forma de denominar la presencia de síntomas psicóticos subclínicos en población general) puede contribuir enormemente a la comprensión de los trastornos psicóticos. En primer lugar, se ha demostrado que las experiencias psicóticas pueden preceder a la aparición de la psicosis. Un estudio demostró que el 25% de los participantes que presentaban EPs a los 11 años desarrollaron un trastorno psicótico clínico a la edad de 26 años (Poulton et al., 2000). Por lo tanto, la presencia de EPs puede ser útil para la identificación de individuos en situación de riesgo (Domínguez et al., 2011b, Kelleher y Cannon, 2011). En segundo

lugar, es probable que la expresión clínica y subclínica de la psicosis compartan factores de riesgo comunes en su etiología (Johns y van Os, 2001, Kelleher y Cannon, 2011, Van Os et al., 2009). Por ejemplo, factores de riesgo ampliamente reconocidos para los trastornos psicóticos como el maltrato en la infancia (Janssen et al., 2004, Lee et al., 2005, Van Winkel et al., 2008, Varese et al., 2012) o el uso de cannabis (Anglin et al., 2012, Henquet et al., 2005, Houston et al., 2011, Manrique-García et al., 2012, Van Os et al., 2010) se han relacionado con síntomas psicóticos y EPs en muestras clínicas y de población general. Por lo tanto, mediante el estudio de la expresión de la psicosis subclínica en muestras de población general es posible contribuir a la comprensión de la etiología de la psicosis sin el sesgo del tratamiento y de la enfermedad en sí misma (Johns y van Os, 2001, van Os, 2003, Van Os et al. , 2009).

3) Ambiente temprano y salud mental en el adulto

Ambiente familiar y negatividad parental

El ambiente familiar es un contexto fundamental para el desarrollo de los niños, por esta razón las prácticas parentales y su contribución a la variabilidad conductual han sido intensamente estudiadas (Hiramura et al., 2010). La infancia es un período de rápido crecimiento neurológico, físico y emocional durante el cual los niños desarrollan vínculos de apego ya en los primeros meses de vida (Bowlby, 1980, Essex et al, 2001). Cummings y Cicchetti sugieren que las relaciones tempranas de apego contribuyen a la representación interna del niño, un proceso fundamental en el desarrollo de una autoestima saludable (Cummings y Cicchetti, 1990). En este sentido, las figuras de apego juegan un papel crucial en la socialización y la regulación y expresión de las emociones durante los primeros 2 años de vida en comparación con los períodos posteriores de desarrollo (esto no quiere decir que las habilidades sociales y

emocionales de los niños no puedan mejorar en el futuro). Por ejemplo, la aparición de la depresión materna en niños mayores de 2 años tiene menores consecuencias negativas que en niños de menor edad ya que durante los 2 primeros años de vida el niño ya ha aprendido a regular mejor sus emociones, empieza a desarrollar estrategias de afrontamiento y tiene una red más grande de figuras de apoyo (por ejemplo: amigos y profesores) (Cummings y Cicchetti, 1990). La teoría del apego sostiene que los niños nacen con un *sistema de apego* que se activa cuando el niño percibe alguna amenaza (Bowlby, 1969, Bowlby, 1980), entonces el niño busca la proximidad con sus figuras de apego buscando protección. En este mismo sentido, se ha señalado que los adultos poseen sistemas de "cuidador" que se activan cuando perciben las señales de angustia y demanda de ayuda en los niños, la activación de estos sistemas desencadenan conductas de cuidado y protección hacia el niño. Sin embargo, el sistema de "cuidador" de los adultos puede verse afectado por diversos estresores como dificultades económicas o problemas de salud o experiencias pasadas de maltrato infantil que pueden conducir a conductas parentales indiferentes o disfuncionales (George y Solomon, 1999).

En este sentido, la calidad del estilo parental ejerce una influencia crítica en el desarrollo socio-afectivo de los niños, especialmente en los primeros años de la vida (Wade et al, 2011). A nivel neurobiológico, se puesto de manifiesto que las interacciones entre padres e hijos y el estado psicológico de la madre pueden influir en la actividad del eje HPA del niño (Albers et al., 2008). Por ejemplo, los hijos de padres que muestran menos apoyo presentan incrementos mayores en los niveles de glucocorticoides (Gunnar y Donzella, 2002). La negatividad parental, incluyendo niveles altos de severidad, normas muy estrictas y frecuentes críticas, se han vinculando a niveles de autorregulación y auto-control pobres y a mayor riesgo de

desarrollar problemas de conducta a lo largo de la vida (Belsky et al., 1998, Kaiser et al., 2010, Nelson et al., 2006, rubin et al., 2003).

Maltrato infantil, consecuencias y mecanismos de riesgo

No existe una definición única de maltrato infantil. Lo que constituye abuso o negligencia varía con la edad del niño, la cultura y el contexto. Sin embargo, la experiencia de un daño significativo y el sufrimiento parece ser el núcleo de la mayoría de las definiciones (Asmussen, 2010). La OMS (World Health Organization, 2006) define el maltrato infantil como:

"Todas las formas de malos tratos, el abuso sexual, descuido o negligencia físico y/o emocional o explotación comercial o de otro tipo, que resulta en un daño real o potencial para la salud del niño, su supervivencia, desarrollo o dignidad en el contexto de una relación de responsabilidad, confianza o poder"

El maltrato infantil es frecuentemente dividido en las siguientes categorías: abuso físico, abuso emocional o psicológico, abuso sexual, negligencia física y negligencia emocional. Los causantes del maltrato infantil pueden ser padres y otros familiares, cuidadores, amigos, conocidos, desconocidos, oficiales de policía, miembros de la iglesia, personal doméstico, trabajadores sanitarios u otros niños (Organización Mundial de la Salud, 2006). El maltrato infantil es, por desgracia, muy común en la mayoría de culturas y países. Algunos estudios apuntan que entre el 30% y el 50% de los niños han padecido maltrato físico (Organización Mundial de la Salud, 2006). Estas estimaciones pueden parecer elevadas pero considerando únicamente los acontecimientos más graves la *Office for Standards in Education* (OFSTED) del Reino Unido estima que tres niños mueren cada semana como consecuencia del maltrato y la negligencia (OFSTED, 2009), asimismo esta investigación sugiere que por lo menos el 16% de la población sufrirá algún tipo de maltrato grave durante su infancia (May-

Chahal y Cawson de 2005). Cabe destacar que estas estadísticas reflejan sólo los casos que llegaron al conocimiento de las autoridades y, por lo tanto, el número real de niños que son víctimas de abuso y negligencia pueden ser mayores. Desgraciadamente, algunos autores también han señalado que los profesionales pueden ser reticentes a denunciar algunos presuntos casos de malos tratos porque dudan de que las circunstancias del niño mejoren debido a la denuncia o informe (Gilbert et al., 2009b).

La exposición al maltrato en la infancia pueden tener un impacto profundo y duradero cuando se produce en edades críticas o transiciones del desarrollo, sobre todo si también implica interrupción en las relaciones de apego (Ford, 2010). El estrés temprano puede poner en peligro mecanismos psicobiológicos cruciales para las capacidades de autorregulación (Manly et al., 2001). Asimismo, el maltrato infantil se ha asociado al riesgo para desarrollar ansiedad, trastornos psicóticos y afectivos (Manly et al., 2001, Van Winkel et al., 2008). Además, la exposición a la adversidad infancia también se ha asociado a la iniciación temprana de conductas sexuales de riesgo y al consumo de sustancias nocivas para la salud (nicotina, alcohol y drogas) (Anda et al., 2006).

4) Interacción gen-ambiente en el desarrollo de fenotipos humanos complejos

Actualmente, está ampliamente aceptado que los trastornos mentales son fenotipos complejos de origen multifactorial entre los que se incluyen factores de riesgo tanto genéticos como ambientales (Zammit et al., 2012). Del mismo modo, se asume que los factores genéticos están involucrados en la vulnerabilidad individual para desarrollar psicopatología. Sin embargo, aún estamos lejos de comprender los mecanismos específicos mediante los cuales los genes se expresan e interaccionan con

las influencias ambientales para contribuir a la expresión de síntomas psicopatológicos. Igualmente, la tarea de tratar de discernir las influencias genéticas y las ambientales ha resultado ser extremadamente difícil (Brune, 2010). Con este propósito se han llevado a cabo estudios de adopción y estudios basados en familias y gemelos que han permitido poner de manifiesto que una amplía diversidad de fenotipos son el producto tanto de factores ambientales como genéticos, lo que conocemos como fenotipos complejos. En este contexto, el campo de la genética cuantitativa tiene como objetivo investigar y cuantificar la contribución relativa de los factores genéticos y ambientales, mientras que las estrategias para identificar genes específicos son investigadas por la genética molecular. La teoría de la genética cuantitativa asume que múltiples variantes genéticas junto con variación ambiental, resultará en una distribución cuantitativa (continua) fenotípica. La genética de la conducta es una especialidad que aplica los métodos de la genética cuantitativa para el estudio del comportamiento humano, igualmente, la genética psiquiátrica investiga las bases genéticas de los trastornos mentales (Plomin et al., 2008, Plomin et al., 2009).

Diferenciando genes y ambiente: Estudios de gemelos

Los estudios de gemelos constituyen uno de los métodos más potentes para separar las influencias genéticas y ambientales en un fenotipo determinado (Boomsma et al., 2002, Plomin et al., 2008, van Dongen et al., 2012).

Existen dos tipos de gemelos, los idénticos o monozigóticos (MZ) y los mellizos o dicigóticos (DZ). Los gemelos MZ provienen de un solo óvulo fertilizado (llamado zigoto) que se divide, por razones aún desconocidas, produciendo dos (o a veces más) individuos genéticamente idénticos. Por esta razón, se dice que son clones naturales. Excepto algunos casos raros, estos individuos se consideran genéticamente idénticos a nivel de la secuencia del ADN (Boomsma et al., 2002, Plomin et al., 2008). Por otro lado,

los gemelos DZ provienen de dos óvulos fertilizados por espermatozoides diferentes, por lo tanto hay dos zigotos que a nivel genético comparten aproximadamente un 50% de su carga genética como los hermanos no gemelos.

El estudio de gemelos clásico compara las semejanzas fenotípicas de gemelos MZ y DZ (Boomsma et al., 2002, Rijsdijk y Sham, 2002). La comparación de la correlación fenotípica para un rasgo determinado entre gemelos MZ y DZ ofrece una primera estimación de la medida en que la variación genética determina la variación fenotípica. Si los factores genéticos son importantes en la variación de un rasgo, los gemelos MZ deberían parecerse más que los familiares de primer grado, que sólo comparten de promedio la mitad de su carga genética (Plomin et al., 2008). En lugar de comparar los gemelos MZ con hermanos no gemelos u otros familiares, la naturaleza nos ha proporcionado un grupo de comparación mucho mejor: los gemelos DZ. A diferencia de los gemelos MZ, los DZ gemelos comparten una media del 50% de sus genes, sin embargo, igual que los gemelos MZ, comparten ambiente prenatal y tienen la misma edad, y el mismo sexo en algunos casos. Si los factores genéticos son importantes para un rasgo, los gemelos MZ deben ser más similares que losDZ.

En el diseño clásico de gemelos, es posible cuantificar la contribución relativa de factores genéticos y ambientales mediante la comparación de las correlaciones observadas (o concordancias) entre los gemelos MZ y DZ. Hay que tener en cuenta que en estos modelos los factores genéticos y ambientales son variables *latentes*, es decir, a diferencia de las variables observadas, las variables latentes no han sido directamente observada o evaluadas ya que son inferidas a través de modelos matemáticos. Los componentes habitualmente estimados en los estudios clásicos de gemelos incluyen: las influencias genéticas aditivas (A), las influencias ambientales compartidas o comunes (C) y las influencias ambientales no compartidos o únicas (E). Este modelo se conoce como el modelo ACE en el que A representa la suma de los efectos de alelos

individuales de todos los loci que influyen el fenotipo estudiado. Este componente también se conoce como *heredabilidad* (h^2). El componente C incluye las influencias ambientales que contribuyen a la semejanza entre los miembros de un par de gemelos, mientras que el componente E representa las influencias ambientales que son únicas para cada individuo y que contribuyen a hacer los miembros de un par de gemelos más distintos entre ellos (Plomin et al., 2008, Rijsdijk y Sham, 2002).

De acuerdo con la metodología clásica de los estudios de gemelos, la varianza fenotípica total (P) de un fenotipo determinado es la suma de A, C y E (P = A + C + E). Los datos de gemelos posibilitan la estimación de los diferentes componentes de la varianza del fenotipo a estudiar, ya que los gemelos MZ y DZ tienen diferentes grados de correlación para el componente genético (A) pero los mismos grados de correlación para el medio ambiente C y E. Los gemelos MZ correlacionan 1 para A (asumimos que son genéticamente idénticos), mientras que en los gemelos DZ correlacionan 0,5. Ambos pares de MZ y DZ correlacionan 1 para C (contribuye a su similitud) y 0 para E (contribuye a que sean diferentes). La fórmula de Falconer se utiliza para estimar la heredabilidad (es decir, A) utilizando las correlaciones fenotípicas de MZ y DZ (Rijsdijk y Sham, 2002):

$$h^2 = 2(rMZ - rDZ)$$

Sin embargo este método no es adecuado para explorar diferencias en la arquitectura genética de un fenotipo debidas al sexo o descomponer la varianza de varios fenotipos en A, C y E en el mismo modelo. Estos objetivos más complejos se estudian mediante análisis multivariantes basados en Modelos de Ecuaciones Estructurales (Rijsdijk y Sham, 2002).

La heredabilidad se puede definir como la proporción de la varianza fenotípica que puede ser explicada por variación genética en una población determinada en un momento determinado para un fenotipo determinado (Plomin et al., 2009). Los

genetistas han hecho hincapié en que la heredabilidad es un índice estadístico que se aplica a la variación de la población y no a los individuos. Por lo tanto, la heredabilidad es un concepto poblacional y no individual (Brune, 2010). La heredabilidad depende de la población debido a que las frecuencias de los alelos, los tamaños del efecto de las variantes genéticas y el modo de acción de los genes puede variar entre poblaciones (Visscher et al., 2008). Asimismo, la heredabilidad puede ser diferente en etapas del desarrollo diferentes y también en contextos diferentes (variación ambiental) de modo que es también importante investigar si la heredabilidad estimada para un fenotipo es estable en el tiempo (por ejemplo, para un rasgo particular, ¿es el papel de los genes tan importante en la infancia como en la edad adulta?), estable entre poblaciones (por ejemplo, ¿es la estimación de la heredabilidad derivada de los estudios de gemelos extrapolable a las muestras de no gemelos?) y entre contextos (por ejemplo, ¿puede ser la heredabilidad distinta en un ambiente empobrecido y uno enriquecido?). Una heredabilidad alta significa que los factores genéticos explican una proporción importante de la variación de un fenotipo en una población en particular en un momento en particular (Rutter et al., 2006).

Estudios de interacción gen-ambiente: El concepto de interacción gen-ambiente (GxA) puede variar dependiendo de la disciplina (Rutter et al., 2006), pero en genética cuantitativa el término generalmente se refiere a la existencia de diferencias individuales en la sensibilidad a la exposición de factores ambientales genéticamente influidas (Caspi y Moffitt, 2006, Aleros, 1984, Plomin et al., 2008, Rutter y Silberg, 2002). En otras palabras, GxE se refiere al control genético de la sensibilidad al ambiente (Van Os y Sham, 2003).

La primera publicación reportando un efecto de GxA evidenciaba que los portadores de un genotipo determinado del gen de la MAOA eran más vulnerables al impacto del maltrato infantil en cuanto al desarrollo de comportamiento antisocial

(Caspi et al., 2002). Esta publicación supuso el cambio de una opinión generalmente aceptada en el campo de la genética de la conducta en la década de 1980 que consideraba que los efectos de GxA eran poco frecuentes y de escasa importancia (Rutter et al., 2006). La investigación en GxE en psiquiatría genética ha sido un tema especialmente relevante durante las últimas décadas (Duncan y Keller, 2011). En la primera década (2000-2009) de investigación de efectos de GxE en genes candidatos (definidos como un gen que sintetiza una proteína involucrada en la etiología del trastorno de interés (Hyde et al., 2011)) se publicaron más de 100 hallazgos, muchos de ellos en revistas de alto impacto. Estas publicaciones elevaron el entusiasmo por los estudios de GxA que aparecieron como un mecanismo prometedor para comprender como los factores genéticos y ambientales contribuían a la etiología de los fenotipos complejos como los trastornos y síntomas psiquiátricos (Rutter et al., 2006).

Además, los efectos de GxE son plausibles desde un punto de vista biológico. Los genotipos no existen en el vacío, su expresión depende en cierto grado del contexto ambiental (Duncan y Keller, 2011). Desde una perspectiva evolutiva, los efectos GxA no sólo existen sino que esperaríamos que fueran muy comunes. El desarrollo del comportamiento humano es un proceso que depende estrechamente de las influencias ambientales; los individuos deben adaptarse al entorno y afrontar amenazas ambientales de todo tipo. Sería poco probable que la respuesta a los estresores ambientales no estuviera genéticamente influida ya que el modo en que los individuos manejan las amenazas ambientales está asociado al temperamento, la personalidad y la psicofisiología de cada individuo. Todas estas características se encuentran bajo cierto grado de influencia genética (Rutter et al., 2006). De hecho, sería sorprendente que los efectos GxE no existieran, porque esto implicaría que las respuestas al ambiente constituirían fenotipos altamente complejos no influidos por los factores genéticos lo que actualmente resulta inverosímil (Duncan y Keller, 2011). A este respecto, los

estudios de gemelos han demostrado que un gran número de respuestas al ambiente son heredables (Duncan y Keller, 2011, Kendler y Baker, 2007).

Cabe señalar que encontrar evidencia de un efecto GxA no proporciona información específica sobre el mecanismo biológico o fisiopatológico subyacente al desarrollo del fenotipo estudiado.

En los últimos años, el entusiasmo por la investigación GxE ha disminuido debido a que estos estudios han recibido diversas críticas (Duncan y Keller, 2011, Munafo y Flint, 2009, Aleros, 2006, Zammit et al., 2012). Estas críticas se deben principalmente a la falta replicación en los hallazgos reportados, como ocurrió antes con los estudios de asociación genética (Munafo y Flint, 2009). Algunos autores señalan que la falta de replicación puede estar relacionada con la facilidad de realizar un gran número de tests estadísticos pero sólo reportar los positivos. Esto conlleva una probabilidad alta de publicar falsos resultados positivos, es decir, al realizar un gran número de tests, es factible que algunos sean significativos sólo por azar pero que no representen un hallazgo robusto o clínicamente significativo (Munafo y Flint, 2009).

Actualmente, el debate acerca de los estudios GxA no se centra en la plausibilidad de los efectos de GxA sino en la metodología y la rigurosidad con que estos efectos deben ser testados, interpretados, publicados y reproducidos (Duncan y Keller, 2011).

HIPÓTESIS Y OBJETIVOS

Hipótesis

La exposición temprana a estrés psicosocial explicará parte de la variabilidad observada en características psicopatológicas, incluyendo problemas de conducta infantiles, experiencias psicóticas y síntomas de depresión y ansiedad, en individuos de la población general. La variación en estas características psicopatológicas involucra mecanismos complejos de interacción gen-ambiente y se asocia a cambios en la estructura y funcionamiento del cerebro.

Objetivo principal

Estudiar cómo los factores ambientales, incluyendo la negatividad parental y el maltrato infantil, directamente o en interacción con factores genéticos, explican parte de la variación psicopatológica observable en población general incluyendo: problemas de conducta infantil, experiencias psicóticas, síntomas depresivos y de ansiedad, y sus correlatos cerebrales obtenidos mediante técnicas de neuroimagen.

Objetivos específicos

1) Examinar en una muestra de 4075 pares de gemelos procedentes de un estudio poblacional que incluye todos los gemelos nacidos en Reino Unido, la asociación entre negatividad parental a los 4 años y problemas de conducta infantiles a los 12 años aplicando un modelo *cross-lagged* que permite a su vez, explorar la arquitectura genética y ambiental de la esta asociación longitudinal. También se exploró si existían efectos bidireccionales en la asociación.

2) Explorar si el maltrato infantil (abuso y negligencia infantil) tiene un impacto diferencial sobre la presencia de las experiencias psicóticas (EPs) en una muestra española extraída de la población general (n=533). Por otra parte, se investigó la existencia de un posible efecto de moderador por parte del polimorfismo BDNF-Val66Met sobre la relación entre maltrato infantil y el desarrollo de EPs mediante análisis de regresión lineal múltiple.

3) Investigar si el impacto del maltrato infantil y el consumo de cannabis en el desarrollo de EPs varía en función de los genotipos del polimorfismo COMT-Val158Met en una muestra extraída de la población en general (n=533) mediante análisis de regresión lineal múltiple.

4) Examinar en una muestra de gemelos provenientes de la población general (n=230): i) si el maltrato infantil se asocia con el desarrollo de EPs positivas y negativas, ii) en qué medida los gemelos monozigóticos (MZ) son similares en cuanto a la exposición a maltrato infantil y a la presencia de EPS y iii) si las diferencias en la exposición a maltrato infantil se asocian con diferencias en la expresión de EPs en una submuestra de gemelos MZ (n=85 pares de gemelos) utilizando un diseño de diferencias en gemelos MZ.

5) Investigar en una muestra de gemelos MZ (n=53) (incluyendo pares concordantes afectados de ansiedad y depresión, pares discordantes para ansiedad y depresión y gemelos sanos controles) extraída de la muestra de gemelos de población general si la vulnerabilidad genética y ambiental para el desarrollo de ansiedad y depresión se asocia de forma diferencial a anormalidades en el volumen de sustancia gris en estructuras cerebrales. Este objetivo se testó utilizando el diseño de gemelos MZ concordantes y

discordantes aplicado a datos provenientes de imágenes cerebrales obtenidas por resonancia magnética.

6) Explorar en una muestra de gemelos MZ (n=53) extraídos de la muestra de gemelos de población general si la presencia de EPs influía la activación cerebral en respuesta a un paradigma emocional (caras humanas expresando distintas emociones) utilizando técnicas de neuroimagen funcional. Además, se investigó si la presencia de EPs moderaban la respuesta cerebral emocional en individuos afectados de ansiedad y depresión utilizando diseños factoriales.

CONCLUSIONES

1) La exposición a la negatividad parental a los 4 años se asoció significativamente con problemas de conducta 8 años después. Esta asociación se explicaba fundamentalmente por factores genéticos. Este hallazgo apoya evidencias previas que señalan que experiencias vividas durante la primera infancia pueden afectar al desarrollo y expresión de conductas años más tarde, incluso en etapas del desarrollo distintas, en nuestro caso, la primera infancia y la adolescencia. Además, al tratarse de un estudio genéticamente informativo y longitudinal fue posible determinar que los factores genéticos eran en gran medida responsables del mantenimiento de esta asociación a lo largo del desarrollo. Este resultado subraya la importancia de la interacción entre genes y ambiente y, especialmente, de los mecanismos de correlación gen-ambiente.

   De modo interesante, la asociación no sólo incluía el efecto de la negatividad parental hacia los hijos sino también el efecto de los problemas de conducta en los niños en la expresión de negatividad parental, de modo que esta asociación parece ser bidireccional.

2) Las personas expuestas a eventos adversos en la infancia tienen más probabilidades de desarrollar experiencias psicóticas (EPs) posteriormente. Específicamente, el abuso infantil pero no la negligencia se asoció con el desarrollo de EPs positivas, indicando cierta especificidad entre el maltrato infantil y el desarrollo de EPs positivas siendo las experiencias caracterizadas por abuso de especial relevancia en esta asociación.

Por otra parte, se observó un papel moderador por parte del polimorfismo BDNF-Val66Met en la relación entre abuso infantil y el desarrollo de EPs positivas. Este efecto de GxA indicaba que los portadores del alelo Met del gen BDNF eran más vulnerables a los efectos negativos del abuso infantil comparados con los portadores del genotipo Val/Val. Es decir, los portadores del alelo Met del gen BDNF podrían ser neurobiológicamente más vulnerables a los efectos del abuso infantil en relación con la expresión de EPs positivas.

3) Se encontró una triple interacción GxAxA entre el abuso infantil, el consumo de cannabis y el polimorfismo COMT-Val158Met en el desarrollo de las EPs positivas. Concretamente, el consumo de cannabis en individuos expuestos a abuso infantil tiene efectos opuestos dependiendo de los genotipos del gen COMT.

En individuos no expuestos o expuestos a niveles bajos de abuso infantil, el consumo de cannabis y el polimorfismo Val158Met del gen COMT no tenían ningún efecto en cuanto al desarrollo de EPs positivas. Sin embargo, entre los individuos expuestos a niveles altos de abuso infantil y que consumen cannabis, la puntuación en EPs positivas aumentaban en función de las dosis del alelo Val del gen COMT. El efecto opuesto se observó en individuos expuestos a abuso infantil pero que no consumían cannabis, sus puntuaciones en EPs positivas aumentaban en función de las dosis del alelo Met del gen COMT. Este patrón de resultados coincide con la definición epidemiológica de interacción GxA cualitativa.

4) Utilizando un diseño de diferencias en gemelos MZ que permite controlar por factores genéticos confusores, se observó un efecto ambiental significativo por parte del maltrato infantil en el desarrollo de EPs positivas y negativas. Este hallazgo sugiere que, si bien algunas personas pueden ser genéticamente vulnerables al impacto del maltrato infantil como se mencionaba anteriormente, los eventos adversos tempranos, en ciertas circunstancias, pueden contribuir al desarrollo de EPs positivas independientemente de la carga genética del invidividuo.

5) Se ha propuesto que los gemelos MZ concordantes para ansiedad y depresión representarían un grupo con una vulnerabilidad genética para estos trastornos particularmente alta. Cuando comparamos las imágenes de resonancia magnética cerebral (RMs) de los gemelos MZ concordantes con los gemelos MZ sanos, observamos que el primer grupo presentaba un volumen de sustancia gris significativamente menor a nivel bilateral del giro fusiforme y de la amígdala. Este hallazgo sugiere que la reducción de sustancia gris en el giro fusiforme y en la amígdala en ansiedad y depresión estaría asociado al riesgo genético para estos trastornos.

   No se observaron diferencias significativas a nivel intrapar cuando se examinaron las RMs del grupo de gemelos MZ discordante para ansiedad y depresión. Por lo tanto, nuestro estudio no proporciona evidencias de la posible contribución de factores ambientales únicos en las anormalidades cerebrales asociadas a la ansiedad y la depresión.

6) La presencia de las EPs influye a la respuesta cerebral a la emoción facial. Específicamente, la activación del córtex cingulado anterior durante la

exposición de caras de enfado se asoció a las puntuaciones de EPs negativas mientras que la desactivación del córtex cingulado anterior durante la exposición de caras de miedo se asoció a las puntuaciones de EPs positivas.

Estos resultados apoyan la hipótesis de que existiría una desregulación emocional presente en el *continuum* de la psicosis.

Por otra parte, la dimensión positiva de las EPs moderaba la respuesta emocional fundamentalmente a nivel del cerebelo, en el grupo de gemelos MZ concordantes para ansiedad y depresión. Esto sugiere que estas dimensiones psicopatológicas pueden compartir procesamiento emocional alterado.

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7. Publications

7.1. Genetic origin of the relationship between parental negativity and behaviour problems from early childhood to adolescence: a longitudinal genetically informative design. Alemany S, Rijsdijk FV, Haworth CMA, Fañanás L, Plomin R. *Development and Psychopathology*, 2013. 25: 487-500.

Resumen en Castellano

La relación entre negatividad parental y problemas de conducta infantiles desde la primera infancia hasta la adolescencia es de origen genético: Un diseño longitudinal genéticamente informativo

Alemany S, Rijsdijk FV, Haworth CMA, Fañanás L, Plomin R.

Development and Psychopathology, 2013. 25: 487-500.

Cómo los factores genéticos y ambientales contribuyen a la relación entre la negatividad parental y los problemas de conducta en los hijos desde la infancia hasta la adolescencia permanece aún sin esclarecerse. El presente estudio aplica un modelo cross-lagged en una muestra de 4075 pares de gemelos con el objetivo de explorar i) el papel de los factores genéticos y ambientales en la relación entre la negatividad parental y los problemas de conducta desde los 4 años hasta los 12 años, ii) si los efectos provenientes de los padres y los efectos provenientes de los hijos explicaan independientemente la asociación, y iii) si existen diferencias sexuales en esta asociación longitudinal. Ambos fenotipos mostraron una influencia genética sustancial tanto a la edad de 4 años como a la edad de 12 años. También la covarianza entre estos fenotipos en cada edad se explicaba principalmente por factores genéticos comunes. Las vías causales que representaban los efectos provenientes de los padres y los efectos provenientes de los hijos independientemente y significativamente explicaban la asociación entre negatividad parental y problemas de conducta. Se encontraron diferencias significativas pero de moderada relevancia clínica entre niños y niñas en cuanto a los efectos provenientes de los padres. Estos resultados prácticamente no variaban si la capacidad cognitiva general se añadía como covariable. En resumen, la asociación longitudinal entre negatividad parental y problemas de conducta parece ser de naturaleza bidireccional y se explica principalmente por factores genéticos.

Development and Psychopathology **25** (2013), 487–500

doi:10.1017/S0954579412001198

Genetic origin of the relationship between parental negativity and behavior problems from early childhood to adolescence: A longitudinal genetically sensitive study

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Abstract

Little is known about how genetic and environmental factors contribute to the association between parental negativity and behavior problems from early childhood to adolescence. The current study fitted a cross-lagged model in a sample consisting of 4,075 twin pairs to explore (a) the role of genetic and environmental factors in the relationship between parental negativity and behavior problems from age 4 to age 12, (b) whether parent-driven and child-driven processes independently explain the association, and (c) whether there are sex differences in this relationship. Both phenotypes showed substantial genetic influence at both ages. The concurrent overlap between them was mainly accounted for by genetic factors. Causal pathways representing stability of the phenotypes and parent-driven and child-driven effects were significantly and independently accounting for the association. Significant but slight differences were found between males and females for parent-driven effects. These results were highly similar when general cognitive ability was added as a covariate. In summary, the longitudinal association between parental negativity and behavior problems seems to be bidirectional and mainly accounted for by genetic factors. Furthermore, child-driven effects were mainly genetically mediated, and parent-driven effects were a function of both genetic and shared-environmental factors.

Several lines of research have converged in showing a robust association between parenting components such as parental negativity and child and adolescent behavior problems (Hill, 2002). Both cross-sectional (Hiramura et al., 2010; Kaiser, McBurnett, & Pfiffner, 2010) and longitudinal studies (Burt, McGue, Krueger, & Iacono, 2005; Larsson, Viding, Rijsdijk, & Plomin, 2008; Leve et al., 2009; Viding, Fontaine, Oliver, & Plomin, 2009) have indicated that negative parenting constitutes a risk factor for child and adolescent externalizing disorders such as conduct disorder, oppositional defiant disorder, and attention-deficit/hyperactivity disorder, as well as internalizing problems such as emotional and social difficulties. Because the home environment is a crucial developmental context for children, parental practices and their contribution to children's behavior have been intensively investigated (Hiramura et al., 2010). Positive parenting, such as parental warmth, has been associated with higher levels of peer acceptance and lower aggressive behavior in children (Clark & Ladd, 2000; Davidov & Grusec, 2006; Mrug et al., 2008; Russell, Robinson, & Olsen, 2003); negative parenting has been linked to externalizing symptoms and social difficulties in children (Belsky, Hsieh, & Crnic, 1998; Kaiser et al., 2010; Nelson, Hart, Yang, Olsen, & Jin, 2006). Supporting these findings, experimental treatment research has shown that improving parental discipline strategies resulted in reduced externalizing problems in children (Bagner, Sheinkopf, Vohr, & Lester, 2010; Dishion & Kavanagh, 2000; Gardner, Sonuga-Barke, & Sayal, 1999; Kilgore, Snyder, & Lentz, 2000).

We gratefully acknowledge the ongoing contribution of the parents and children in the Twins Early Development Study. The study is supported by a program grant (G0500079) from the UK Medical Research Council; our work on school environments is also supported by a grant from the US National Institutes of Health (HD44454). The third author is supported by an MRC/ESRC fellowship (G0802681). The first author thanks the Institute of Health Carlos III for her PhD grant (FI00272). The first and fourth authors thank the Ministry of Science and Innovation (SAF2008-05674-C03-00), Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), and Comissionat per a Universitats i Recerca del DIUE of the Generalitat de Catalunya (2009SGR827) for their support.

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Bidirectional Effects in the Association Between Parenting and Behavior Problems

However, it has been shown that children's behavior can also elicit certain reactions in others (Pettit & Arsiwalla, 2008). Two directions of effects in the association between parenting and behavior problems have been identified, effects coming from the parents, called *parent-driven effects*, and effects elicited by the children, called *child-driven effects* (Pettit & Arsiwalla, 2008). Evidence for a bidirectional parent–child relationship is consistent with the reciprocal effects models (Bell, 1968) where parents' behaviors influence children's develop-

ment but children's behaviors also influence parents' behaviors in a series of cycles over time.

In the case of behavior problems, difficult children may influence their parents negatively, resulting in parents being less involved and providing less positive or developmentally appropriate environments for their children (Shaw, Gilliom, Ingoldsby, & Nagin, 2003). Such patterns of parent–child relationship can lead to a downward cycle of interpersonal dysfunction, called *coercive relationships* (Collins & Laursen, 1999; Patterson, 1982).

The Cross-Lagged Model in Longitudinal Genetically Sensitive Studies

These findings have encouraged researchers to develop models that simultaneously account for both types of effects. In this sense, cross-lagged models are typically used because they are designed to examine the longitudinal association between two different measures independent of stability and the concurrent associations between the measures. When the cross-lagged model is applied in a genetically informative sample, it is possible to estimate the genetic and environmental influences on the associations between the measures. For example, Neiderhiser, Reiss, Hetherington, and Plomin (1999) analyzed the association between parental conflict–negativity and adolescent antisocial behavior and depressive symptoms using a genetically sensitive cross-lagged model in a sample consisting of biologically related individuals, assessed at two ages, 3 years apart. They concluded that the association between the two phenotypes was explained primarily by genetic factors.

The work of Neiderhiser and colleagues (1999) inspired other researchers to extend and refine their pioneering model. Recently, Neiderhiser's model was refined by Luo, Haworth, and Plomin (2010) by adding a Cholesky decomposition that ultimately allows the decomposition of the cross-lagged paths per se into their genetic and environmental components also controlling for the stability and reverse cross-lagged association. However, the two cross-lagged associations tested in Luo et al. (2010) were presented in two separate models that do not allow the test of bidirectionality.

In this sense, the model developed by Burt et al. (2005) is advantageous because the cross-lagged model is nested in a genetic model. By nesting the phenotypic relationships between the variables analyzed over time, it is possible to test the difference between bidirectional relationships. Burt et al. (2005) analyzed the associations between parent–child conflict and child externalizing problems from ages 11 to 14. They found evidence for a bidirectional relationship. Furthermore, although the Burt et al. (2005) model does not allow the decomposition of the cross-lagged paths per se, it is possible to decompose into genetic and environmental factors the transmitted variance from the analyzed phenotypes over time, which ultimately enables us to explore whether the longitudinal association is genetically or environmentally mediated. In this particular study, the association between parent–child conflict and child externalizing problems from 11 to 14 years of age was mostly driven by environmental factors, although genetic factors were also implicated (Burt et al., 2005).

The cross-lagged model developed by Burt et al. (2005) has been applied in two other studies. Larsson et al. (2008) examined the association between parental negativity and child antisocial behavior at ages 4 and 7. Similarly to Burt et al. (2005), the association was best explained by bidirectional processes, although in their case child effects were genetically mediated while environmental factors mediated parent-driven effects on child antisocial behavior (Larsson et al., 2008). Recently, Moberg, Lichtenstein, Forsman, and Larsson (2011) investigated the direction and etiology of the association among different parental styles, parental emotional overinvolvement and parental criticism, and internalizing behavior from ages 16–17 to 19–20. They found evidence for genetically influenced child-driven effects underlying this association but only in girls.

In summary, both parent-driven and child-driven effects have been found in the association between parenting components and child and adolescent behavior problems with mixed results regarding the genetic or environmental mediation of these processes and the specifity of the direction in the association across genders.

Our Study

To extend the literature on the etiology of reciprocal effects and the genetic and environmental architecture of the association between parental negativity and behavior problems, we analyzed data at ages 4 and 12 from a large population-based twin study, the Twins Early Development Study (TEDS; Trouton, Spinath, & Plomin, 2002) by means of a genetically sensitive cross-lagged model (Burt et al., 2005). For the first time in a longitudinal genetically sensitive study we have explored the directional relationships between parental negativity and behavior problems from early childhood to adolescence. Previous genetically sensitive research examining similar relationships applying a cross-lagged model has focused on spans of 3 years within the same developmental period (Burt et al., 2005; Larsson et al., 2008; Moberg et al., 2011; Neiderhiser et al., 1999). Furthermore, phenotypic studies examining risk factors or developmental trajectory and stability of behavior problems over different developmental stages are relatively scarce and mostly focused on continuity of behavior problems over time (Fanti & Henrich, 2010; Trentacosta & Shaw, 2009; Van Hulle et al., 2009). Therefore, it remains poorly understood whether associations between parental measures and behavior problems extend across developmental stages such as early childhood and adolescence. The present study will investigate genetic and environmental etiologies of the links between parental negativity and behavior problems across 8 years, from childhood to adolescence. The cross-lagged approach will also yield information about the etiology of stability of behavior problems from childhood to ado-

lescence, controlling for the association and stability with parental negativity.

In addition, sex differences in the genetic and environmental architecture of the phenotypes and their association were assessed capitalizing on TEDS' inclusion of opposite-sex twins. Although research has often explored the relationship between different parental components and behavior problems, less attention has been given to whether these familial factors impact girls and boys differently (Blatt-Eisengart, Drabick, Monahan, & Steinberg, 2009). Some studies have suggested that the greater prevalence of behavior problems among boys than among girls (Hill, 2002) is due to higher rates of exposure to risk factors such as parental negativity among boys or boys' greater sensitivity to them (Rutter, Caspi, & Moffitt, 2003). Furthermore, it has been pointed out that direction of effects can depend on child gender (Moberg et al., 2011). Our longitudinal study extends into adolescence, when secondary sexual characteristics emerge (Spear, 2003). Therefore, we address the possibility of sex differences in the etiological relationship between parental negativity and behavior problems from childhood to adolescence.

Finally, apart from parenting characteristics, general cognitive ability is a fundamental developmental resource in successful adaptative behavior (Masten, 2001). For example, children with cognitive difficulties are at greater risk of developing behavior problems (Deutch & Bubser, 2007; Hill, 2002; Tong et al., 2010). Because the current study was focused on the relationship between parental negativity and behavior problems, we considered the potential role of cognitive difficulties.

Research questions

The present study addresses five research questions:

1. How much of the variance of parental negativity and behavior problems is due to genetic and environmental factors at age 4 and age 12?
2. How do genetic and environmental factors influence the concurrent overlap at each age between parental negativity and behavior problems?
3. How do parental negativity and behavior problems at age 4 contribute to parental negativity and behavior problems at age 12 (parent-driven effects, child-driven effects, and stability of the phenotypes)?
4. How do genetic and environmental factors in parental negativity and behavior problems at age 4 contribute to parental negativity and behavior problems variables at age 12?
5. Are there sex differences in the genetic and environmental architecture of the longitudinal associations between parental negativity and behavior problems from early childhood to adolescence?

Hypotheses

Based on the literature, we hypothesized that we would identify both parent-driven and child-driven effects in the association between parental negativity and behavior problems indicating a bidirectional relationship over time. In addition, we predict that genetic factors will mediate the effects of behavior problems at age 4 on parental negativity at age 12, whereas we expect that the effects of parental negativity at age 4 on behavior problems at age 12 will be more environmentally mediated (Larsson et al., 2008).

Method

Participants

Participants were drawn from TEDS, a large longitudinal population-based study of all twins born in England and Wales between 1994 and 1996 (Oliver & Plomin, 2007; Trouton et al., 2002). Parents completed behavioral rating scales for both twins at ages 4 and 12. Zygosity was determined using a standard zygosity questionnaire, which has been shown to have 95% accuracy (Price et al., 2000). Furthermore, zygosity has been confirmed for most same-sex pairs using DNA markers (Freeman et al., 2003). TEDS has been shown to be reasonably representative of the UK population (Kovas, Haworth, Dale, & Plomin, 2007).

The sampling frame for the present study was 7,660 twins, born in 1994, 1995, or 1996, using data available from parents' ratings of parental negativity and behavior problems at age 4 and 12.

A total of 584 twin pairs were excluded from the analyses because of medical or neurological conditions, outlier scores, or unknown (unreliable) zygosity. Thus, the total number of twin pairs included in the analyses was 4,075 twin pairs: 659 monozygotic (MZ) male twin pairs, 835 MZ female twin pairs, 622 dizygotic (DZ) male twin pairs, 715 DZ female twin pairs, and 1,244 DZ opposite-sex twins. Mx uses a full-information maximum likelihood method to handle missing data, which allows the use of missing data with minimum bias.

Measures

Behavior problems were assessed by means of parent reports of the Strengths and Difficulties Questionnaire (SDQ; Goodman, 1997) when children were 4 and 12 years old. The SDQ is a brief behavioral screening of 25 items for individuals aged between 3 and 16 years old. Raters are asked to indicate on a 3-point response scale (ranging from *not true* to *certainly true*) how well each item described the child's behavior over the past 6 months. The questionnaire consists of five subscales (emotional problems, peer problems, conduct problems, hyperactivity, and prosocial behavior). Example items are "Restless, overactive, cannot stay still for long" and "Often lies or cheats." We found that the first four subscales were highly and significantly correlated at both age 4 (average correlation = 0.57) and age 12 (average correlation = 0.66). Due to the high overlap between these behavioral problem measures, both in our sample and in other studies (Angold, Cost-

ello, & Erkanli, 1999; Timmermans, van Lier, & Koot, 2010), we combined the first four subscales to yield a total behavior problems score.

Parental negativity was assessed when children were 4 and 12 years of age, using the Parental Feelings Questionnaire (Deater-Deckard, 1996). This questionnaire consists of 4 items rated on a 5-point scale (ranging from *definitely untrue* to *definitely true*) where parents report their negative feelings about their children. The items representing negative feelings were used to create a total score of parental negativity. At age 4, for the firstborn twins the statements were: "Sometimes I feel very impatient with him/her," "Sometimes I wish he/she would go away for a few minutes," "Sometimes he/she makes me angry," and "Sometimes I am frustrated by him/her." For the second-born twins parents were asked "Do you feel this way more or less with your second-born twin?" and these questions were rated on a 5-point scale ranging from *a lot more* to *a lot less*. This differential scoring method was aimed to accentuate within-family differences. The score of the firstborn twins was obtained by summing up the items and then standardizing across the whole population to zero mean and unit variance. For the second-born twins, the standardized scores of the firstborn twins were added to the standardized sum of the differential scores of the second-born twins, and then this composite was standardized (Knafo & Plomin, 2006). At age 12, assessment of parental negativity included the same 4 items, but parents were asked to report on their feelings about each twin separately without comparing them. The scores of each of the 4 items were summed to obtain a total score of parental negativity, which was also standardized.

As mentioned above, the potential role of general cognitive ability as a covariate was investigated. General cognitive ability (*g*) was assessed at each age through administration of nonverbal and verbal cognitive test batteries. At age 4, *g* was calculated as the standardized sum of the verbal and nonverbal scores. Nonverbal cognitive performance was assessed by means of the Parent Report of Children's Abilities (Saudino, Oliver, Petrill, Richardson, & Rutter, 1998). At age 12, twins were administered (online) two verbal tests, the Wechsler Intelligence Scale for Children (third edition) Multiple Choice Information and Vocabulary Multiple Choice subtests (Wechsler, 1992), and two nonverbal reasoning tests, the Wechsler Intelligence Scale for Children (thirrd edition) Picture Completion (Wechsler, 1992) and Raven's Standard and Advanced Progressive Matrices (Raven & Raven, 1996, 1998). More details on the cognitive assessments are reported elsewhere (Davis, Haworth, & Plomin, 2009; Haworth et al., 2007).

Statistical Analyses

Structural equation modeling of twin data is based on the differential genetic relationship between pairs of twins: MZ twin pairs are 100% similar genetically, and DZ twins are 50% similar genetically for additive genetic effects on average. When these twins are raised in the same family, the twin method assumes that there are no differences in their environmental relatedness, that is, both types share 100% of shared environmental effects and 0% of nonshared environmental effects. The difference in MZ and DZ correlations (resemblance in measured traits) can be used to estimate the relative contribution of additive genetic effects (A), shared environmental effects (C), and nonshared environmental effects (E) to the total phenotypic variance of a given trait. A represents the sum of the effect of the individual alleles at all loci that influence a trait. C includes environmental influences that contribute to similarity within twin pairs, and E represent environmental influences that are unique to each individual, plus measurement error (Plomin, DeFries, McClearn, & McGuffin, 2008; Rijsdijk & Sham, 2002).

The current study examines the association between parental negativity and behavior problems from ages 4 to 12 fitting a cross-lagged model (Burt et al., 2005; see Figure 1). This model constrains all the associations between and within the two phenotypes across ages to take the form of phenotypic

Fig. 1 - B/W online, B/W in print

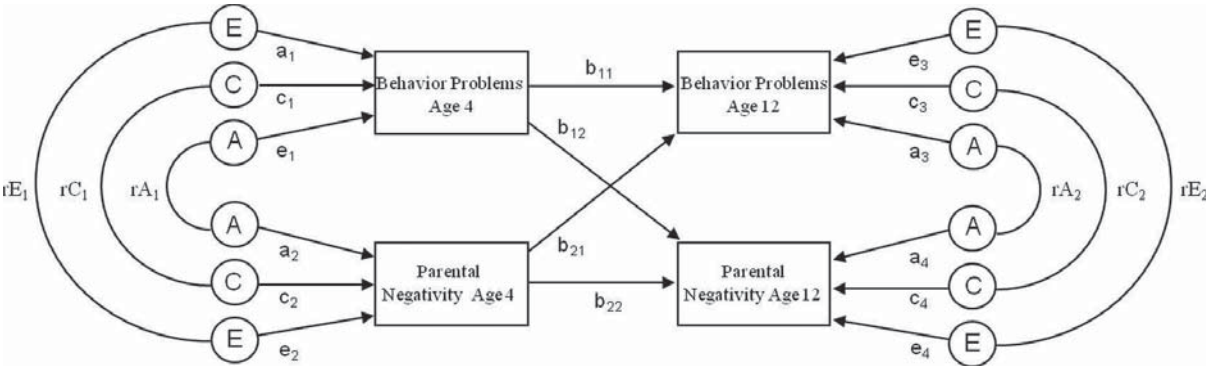

Figure 1. A path diagram of the cross-lagged model. Circles represent latent variables, additive genetic factors (A), shared environmental factors (C), and nonshared environmental factors (E). Rectangles represent the measured variables (i.e., parental negativity and behavior problems at ages 4 and 12). Standardized paths estimates for these variables (i.e., a_1, c_1, e_1, a_2, c_2, e_2, a_3, c_3, e_3, a_4, c_4, e_4), genetic and environmental correlations (i.e., rA_1, rC_1, rE_1, rA_2, rC_2, rE_2), cross-age stability paths (i.e., b_{11}, b_{22}), and cross-lagged paths (i.e., b_{12}, b_{21}) are also presented in the diagram.

partial regression coefficients. The paths connecting the same phenotype from age 4 to age 12 represent the cross-age stability paths (Figure 1, b_{11} and b_{22}). These paths estimate the 8-year stability for parental negativity and behavior problems when controlling for the preexisting association between the two phenotypes at age 4. The paths connecting one phenotype with the other from age 4 to age 12 are the cross-lagged paths of the model (Figure 1, b_{12} and b_{21}). The cross-lagged paths estimate the independent contribution of parental negativity at age 4 on behavior problems at age 12 (parent-driven effects) and, similarly, the independent contribution of behavior problems at age 4 on parental negativity at age 12 (child-driven effects), controlling for the stability of the two phenotypes.

At each age, the variance of each phenotype and their covariation is decomposed into A, C, and E. Moreover, at age 12, the genetic and environmental influences on the phenotypes can be broken down into age-specific and transmitted variance from age 4 phenotypes and their covariation. This also enables an estimate of how much of the variance of age 12 phenotypes is transmitted through the cross-age stability and cross-lagged paths and whether this transmitted variance is mainly loading into genetic or environmental factors of age 12 phenotypes. Therefore, it is possible to examine how genetic and environmental influences on age 4 phenotypes contribute to genetic and environmental influences on age 12 phenotypes. These analyses constitute one of the most salient features of the cross-lagged model because it allows us to elucidate whether the longitudinal association is of genetic or environmental origin.

Since the sample includes male and female MZ and DZ pairs and opposite-sex pairs, it is possible to test whether there are sex differences in the genetic and environmental architecture of the phenotypes or in their longitudinal association by fitting different sex-limitations models. The current study fitted four sex-limitations models to test for quantitative sex differences (differences in the relative contribution of genetic and environmental factors to the phenotypes), phenotypic variance differences between sexes, and causal pathway differences between sexes. Quantitative sex differences were examined by allowing the parameter estimates (i.e., A, C, and E) to differ across genders (Model 1). A constrained model, where all variance components were set to be equal across genders, was also fitted (Model 2). Next, we fitted a scalar model to examine phenotypic variance sex differences. This model allows sex differences in phenotypic variances but constrains A, C, and E parameters to be equal across genders (Model 3). Finally, we fitted a scalar model constraining A, C, and E parameters to be equal across genders but allowing sex differences in the phenotypic variance and causal pathways (Model 3).

All analyses (estimating correlations and genetic model-fitting parameters) were performed by means of the structural equation modeling program Mx (Neale & Maes, 2003). Models were fitted on scores adjusted for age, sex, and *g*. These models were compared to models fitted on scores only adjusted by sex and age to test whether *g* was modifying the associations in the cross-lagged model.

Goodness of fit of the models was assessed by likelihood-ratio chi-square tests, which is the difference between –2 log likelihood (–2 LL) of the saturated model and that of the restricted model, with the degrees of freedom (*df*) of this test being the difference between the number of estimated parameters of the two models (a significant *p* value indicating a bad fit). Competing (nested) models can be compared in a similar way. In addition, the Akaike information criterion (AIC = $\chi^2 - 2 \times df$) was used to compare the fit of (nonnested) competing models (with lower AIC values indicating better fit).

Results

Descriptive statistics

Because the pattern of the results and the estimates were almost exactly the same either adjusting by *g* or not, the results presented are based on scores adjusted by sex and age (results adjusted by sex, age, and *g* are available on request from first author).

Means, standard deviations, and number of respondents for age- and sex-adjusted scores of parental negativity and behavior problems at ages 4 and 12 are presented in Table 1. The means and standard deviations are nearly identical for males and females. The means of parental negativity slightly increase at age 12.

Phenotypic correlations

The age-specific phenotypic correlation between behavior problems and parental negativity increased substantially from age 4, males: $r = .29$, 95% confidence interval (CI) (0.26–0.33); females: $r = .29$, 95% CI (0.26–0.30), to age 12, males: $r = .50$, 95% CI (0.47–0.53); females: $r = .49$, 95% CI (0.46–0.51). There was stability over time for both behavior problems, males: $r = .47$, 95% CI (0.46–0.48); females: $r = .45$, 95% CI (0.43–0.48), and parental negativity, males: $r = .37$, 95% CI (0.33–0.38); females: $r = .34$, 95% CI (0.33–0.36). The across-trait and time correlations were small but significant for both behavior problems at age 4 and parental negativity at age 12, males: $r = .28$, 95% CI (0.21–0.31); females: $r = .27$, 95% CI (0.24–0.30), and parental negativity at age 4 and behavior problems at age 12, males: $r = .21$, 95% CI (0.18–0.24); females: $r = .17$, 95% CI (0.14–0.20). The pattern of phenotypic correlations between the measures was similar for both sexes.

Twin correlations

The twin correlations for behavior problems and parental negativity at age 4 and at age 12 are also presented in Table 1 by zygosity and sex. For behavior problems at age 4, the MZ twin correlation is twice as high as the DZ correlation, suggesting genetic influence on the trait. For parental negativity, both MZ and DZ twin correlations are quite high, indicating genetic and common environmental influences. At age 12,

Table 1. *Means, standard deviations, and sample sizes of measures of parental negativity and behavior problems at age 4 and 12 adjusted by sex, age, and general cognitive ability*

	Males			Females			Correlations				
	N	*M*	*SD*	*N*	*M*	*SD*	MZM	DZM	MZF	DZF	DZO
Age 4											
Behavior problems	3802	6.65	1.22	4340	6.67	1.14	.72 (.70–.75)	.38 (36–.44)	.72 (.68–.74)	.33 (.27–.34)	.40 (.35–.44)
Parental negativity	3802	6.71	1.24	4340	6.71	1.27	.77 (.75–.80)	.55 (.50–.60)	.76 (.74–.79)	.55 (.54–.60)	.51 (.50–.53)
Age 12											
Behavior problems	3806	6.06	4.04	4344	6.28	3.41	.76 (.73–.78)	.49 (.45–.54)	.79 (.77–.81)	.53 (.48–.58)	.44 (.40–.45)
Parental negativity	3806	7.14	1.04	4344	7.13	1.02	.87 (.85–.89)	.71 (.69–.74)	.87 (.86–.88)	.70 (.67–.73)	.66 (.63–.69)

Note: Twin intraclass correlations (95% confidence intervals) for parental negativity and behavior problems at age 4 and 12. MZ, monozygotic; M, male twin pairs, DZ, dizygotic; F, female twin pairs; O, opposite twin pairs.

both MZ and DZ correlations increase for both parental negativity and behavior problems. All correlations were statistically significant. Twin correlations were generally similar for males and females and for same-sex and opposite-sex twins.

Model-fitting analyses

Four sex-limitation models were fitted (see Table 2). The best fitting model constrained genetic and environmental influences to be the same across males and females (as suggested by the twin correlations in Table 1) but allowed for sex differences in variances and causal pathways (Model 4, Table 2). Model 4 showed the lowest AIC value and a nonsignificant decline in fit compared to Model 1 ($p = .21$).

Research Question 1: How much of the variance of parental negativity and behavior problems is due to genetic and environmental factors at each age?

The proportion of variance of behavior problems and parental negativity at ages 4 and 12 explained by additive genetic factors (a^2), common environment (c^2), and unique environment (e^2) is presented in Figure 2.

Behavior problems at age 4 are highly heritable (69%) and almost no variance is explained by common environment ($c^2 = .03$). At age 12, common environmental influences become more important (11%) and the genetic influences decreased slightly (60%). The proportion of variance explained by unique environmental influences was similar at age 4 ($e^2 = 28\%$) and age 12 ($e^2 = 29\%$).

For parental negativity, around half of the variance was explained by genetic factors (49%) at age 4 but by common environment at age 12 (45%). Nevertheless, genetic factors were also important at age 12, accounting for 38% of the variance of parental negativity. The influence of unique environmental influences was similar at both ages (23% and 17%, respectively).

Research Question 2: How do genetic and environmental factors influence the concurrent overlap between parental negativity and behavior problems at each age?

The genetic and environmental overlap between behavior problems and parental negativity at each age can be found in the outer sides of Figure 2.

The predicted correlation between behavior problems and parental negativity at age 4 is obtained by summing the paths that join the two phenotypes: $(\sqrt{.69} \times .47 \times \sqrt{.49} = .23) + (\sqrt{.03} \times -.70 \times \sqrt{.28} = -.06) + (\sqrt{.28} \times .31 \times \sqrt{.23} = .08) = .25$. Thus, the phenotypic correlation of .25 between the two phenotypes at age 4 was mainly due to genetic factors (.23/.25 = 92%), whereas environmental influences (C and E) are largely specific to each trait and do not contribute to the similarity between the traits.

At age 12, following the same calculation, the correlation between the two phenotypes was .42. Similar to age 4, concurrent associations at age 12 between parental negativity

Table 2. *Model fitting results for parental negative feelings and antisocial behavior at age 4 and 12*

Model	–2 LL	*df*	χ2	*df*	*p*	AIC	Compared to Model	Differences in Fit of Competing Models: Δχ2	Δ*df*	*p*
Saturated model	109779.45	32196	—	—	—	—	—	—	—	—
1. Cross-lagged model, sex differences	110499.86	32370	720.41	174	<.001	372.41	—	—	—	—
2. Cross-lagged model, no sex differences	110597.86	32382	818.41	186	<.001	446.41	1	97.99	12	<.001
3. Cross-lagged model, Scalar	110517.18	32372	737.73	176	<.001	385.73	1	17.32	2	<.001
4. Model 3 allowing for sex differences in causal paths	110510.78	32378	**731.33**	**182**	**<.001**	**367.33**	**1**	**10.92**	**8**	**.21**

Note: The chi-square, degrees of freedom, and *p* values (columns 4–6) are the difference in the –2 log likelihood statistics (–2 LL) of each model and the saturated model. The best fitting model is indicated in bold.

and behavior problems were mainly due to genes (52%), but there was an increase in the common environmental factors shared by the two phenotypes, with shared environments explaining 26% of the phenotypic correlation.

Research Question 3: How do parental negativity and behavior problems at age 4 influence parental negativity and behavior problems at age 12 (cross-lagged and cross-age stability pathways)?

Cross-lagged partial regression coefficients located in the center of Figure 2 indicate the association between the two variables connected by each path controlling for the preexisting relationship between behavior problems and parental negativity at age 4. The best fitting model allowed causal pathways to differ across genders; therefore, estimates for cross-lagged and cross-age stability pathways are different for males and females.

Behavior problems at age 4 significantly predict parental negativity at age 12, males: $r = .13$; 95% CI (0.10–0.16); females: $r = .14$; 95% CI (0.11–0.16). The converse association was also significant, males: $r = .09$; 95% CI (0.05–0.12); females: $r = .03$; 95% CI (0.01–0.06). The influence of each pathway on variances at age 12 can be obtained by squaring the partial regression coefficients. Thus, parent-driven effects (parental negativity at age 4 → behavior problems at age 12) explained 0.8% of parental negativity at age 12 in males (calculated by $.09^2$) and 0.1% in females ($.03^2$). Child-driven effects (behavior problems at age 4 → parental negativity at age 12) explained 1.7% and 2% of behavior problems at age 12 for males and females, respectively.

Regarding the stability of the phenotypes, both phenotypes measured at age 12 were significantly influenced by

Fig. 2 - B/W online, B/W in print

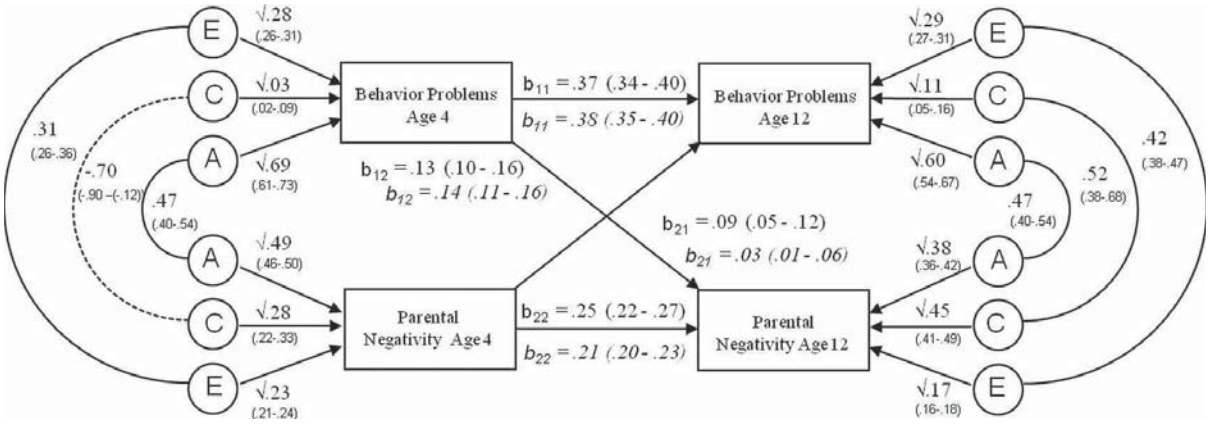

Figure 2. A path diagram representing the association between behavior problems and parental negativity from age 4 to age 12 and the standardized path estimates of the additive genetic (A), shared environmental (C), and nonshared environmental effects (E). The squared A, C, and E path estimates at age 12 represent the total (transmitted + time specific) variance. Solid lines indicate significant pathways. Standardized estimates for cross-age stability paths (i.e., b_{11}, b_{22}) and cross-lagged paths (i.e., b_{12}, b_{21}) are presented in the center of the diagram for males and females (italics).

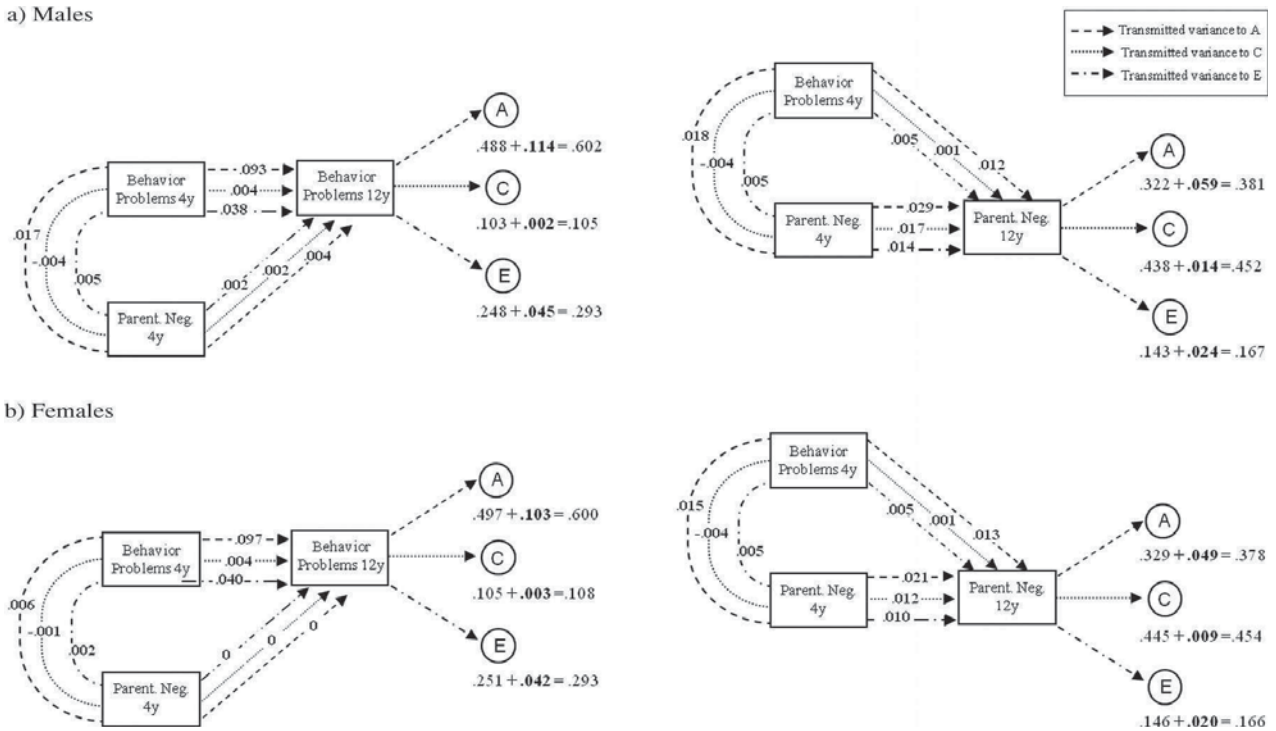

Figure 3. Diagrams presenting the breakdown of the total genetic (A), common (C), and unique environmental (E) influences of behavior problems and parental negativity at age 12 in (a) males and (b) females. These values do not represent path estimates, but instead represent the different proportions of transmitted A (dashed line), C, and E variance. Total A, C, and E variances are decomposed into time-specific and transmitted (in bold) variances. For example, total genetic influences of behavior problems at age 12 in males equals .602. This value is the sum of the time-specific (.488) and transmitted variance (.114). Following the dashed line, genetic transmitted variance to behavior problems at age 12 can be tracked, specifically .114 equals the sum of the genetic transmitted variance from the same phenotype at age 4 (.093), parental negativity at age 4 (.004), and their covariance (.017)(.093 + .004 + .017 = .114). Common and unique environmental transmitted variance can also be tracked following the dotted line and the dotted and dashed line, respectively.

Fig. 3 - B/W online, B/W in print

the same phenotype at age 4 independent of the other phenotype. The cross-age stability path from behavior problems at age 4 independently explained 13.7% and 14.4% of the variance of behavior problems at age 12 in males and females, respectively, males: $r = .37$; 95% CI (0.34–0.40); females: $r = .38$; 95% CI (0.35–0.40). Parental negativity at age 4 independently explained 6.3%, $r = .25$; 95% CI (0.22–0.27), of the variance of parental negativity at age 12 in males and 4.4%, $r = .21$; 95% CI (0.20–0.23), in females.

Research Question 4: How do genetic and environmental influences on parental negativity and behavior problems at age 4 contribute to parental negativity and behavior problems at age 12?

From the cross-lagged model, it is possible to break down the genetic, shared, and nonshared environmental influences on phenotypes at 12 years into age-specific variances and transmitted variance from each of the phenotypes at 4 years and from their covariance at 4 years. The breakdown of age-specific and transmitted genetic, shared environmental, and nonshared environmental influences on behavior problems at age 12 is graphically presented in Figure 3. The purpose of Figure 3 is to focus on parental negativity and behavior problems at age 12, showing the amount of age-specific and transmitted variance in each A, C, and E estimate. The sum of these two components constitutes the total A, C, and E estimates that are shown in Figure 2.

Specifically, in Figure 3a (males), age-specific genetic, shared, and nonshared environmental factors account for 84% of the variance of behavior problems at age 12, ($a^2 = .49$) + ($c^2 = .10$) + ($e^2 = .25$) = .84. Thus, 16% of the variance is transmitted from genetic (.114), shared (.002), and nonshared environmental factors (.045), influencing behavior problems, parental negativity, and their covariation at age 4 (.114 + .002 + .045 = .161). Most of the transmitted variance of behavior problems at age 12 is genetic (.114/.16 = 70.8%), and it is mainly due to cross-age stability effects (.093/.114 = 81.6%). For females (Figure 3b), transmitted variance to behavior problems at age 12 represents 15% of the total variance of the phenotype (.103 + .003 + .042 = .148). Most of the transmitted variance is genetic in origin (.103/.148 = 69.6%), and it mainly comes from the same phenotype at age 4 (.097). The amount of transmitted variance through the cross-lagged path representing parent-driven effects was negligible for females (<.0005).

Regarding parental negativity at age 12, age-specific variance represents 90% of the total variance, ($a^2 = .32$) + ($c^2 = .44$) + ($e^2 = .14$) = .90, for males. Transmitted variance (10%) again mainly loads on genetic factors (.06/.10 = 60%), which primarily comes from the same phenotype at age 4 (.029). For females, transmitted variance represents 8% (.049 + .009 + .020 = .078) of the total variance of parental negativity at age 12. Again, genetic factors account for most of the transmitted variance (.049/.078 = 62.8%), which largely comes from the same phenotype at age 4 (.021).

Research Question 5: Are there sex differences in the genetic and environmental architecture of the longitudinal associations between parental negativity and behavior problems from early childhood to adolescence?

The best fitting model (Model 4 in Table 2) constrained all genetic and environmental contributions to be constant across genders but allowed phenotypic variances and causal pathways (cross-lagged and cross-age stability pathways) to differ for males and females. The estimates of the causal pathways were significant and similar in both males and females. However, the cross-lagged path representing parent-driven effects from parental negativity at age 4 to age 12 behavior problems was significantly greater for males (0.09) than for females (0.03; $\Delta\chi^2 = 7.17$; $\Delta df = 1$; $p = .007$), although the confidence intervals of the estimates overlap. In addition, the cross-age stability path for parental negativity was significantly greater for males (0.25) than for females (0.21; $\Delta\chi^2 = 5.04$; $\Delta df = 1$; $p = .025$), although the confidence intervals for the estimates overlap.

Discussion

This first longitudinal genetically sensitive study investigating the cross-lagged association between parental negativity and behavior problems aimed to assess the causal direction and genetic and environmental etiology of these associations from early childhood to adolescence. The findings indicate bidirectional cross-lagged associations; that is, both parent-driven and child-driven effects independently account for the associations between parental negativity and behavior problems across these ages. Furthermore, child-driven effects were mainly genetically mediated and parent-driven effects were a function of both genetic and shared-environmental factors. There were small sex differences in the genetic and environmental architecture of the longitudinal association between parental negativity and behavior problems, which are discussed below. Overall, the stability of the parental negativity and behavior problems and the association between them from early childhood to adolescence seems to be mainly of genetic origin.

Here we discuss the findings in relation to the five research questions outlined in the introductory section.

Research Question 1: How much of the variance of parental negativity and behavior problems is due to genetic and environmental factors at age 4 and age 12?

As reported by previous studies, the heritability found for behavior problems ranged from 40% and 70% and did not differ across genders (Hill, 2002; Simonoff, 2001). Looking more carefully into the genetic and environmental etiology of behavior problems, there is a change in the role of shared environmental influences, which account for negligible variance of behavior problems at age 4 but account for 14%–15% of the variance at age 12. This increase in common environmental influences in behavior problems at age 12 can be par-

tially explained by the increase in conflicts with parents, which has been pointed out during adolescence, especially around puberty (Steinberg & Morris, 2001).

Although parental negativity is typically considered as an environmental measure (or risk), we found that almost half of its variance was explained by genetic factors. This result is consistent with previous heritabilities reported for similar parental measures (Deater-Deckard, Fulker, & Plomin, 1999; Neiderhiser et al., 2004; Pike & Plomin, 1996; Vinkhuyzen, van der Sluis, de Geus, Boomsma, & Posthuma, 2010). Environmental measures are influenced by genes because they involve, at least in part, reactions to heritable characteristics (Reiss, 1995). In this context, our results may be reflecting gene–environment correlation effects in which a child's behavior problems may evoke or seek parental negativity. Child-driven effects, which support this explanation, are discussed below.

Research Question 2: How do genetic and environmental factors influence the concurrent overlap at each age between parental negativity and behavior problems?

At each age, overlap between parental negativity and behavior problems were mainly accounted by genetic factors, indicating that the same genes that make parents feel negatively about their children also influence behavior problems. These results are similar to one study (Larsson et al., 2008). However, in two other studies, genetic covariation also contributed to covariation between parental measures and behavior problems, but most of the association was mainly accounted by environmental factors (Burt et al., 2005; Moberg et al., 2011). One hypothesis about these different results could be a developmental shift in the covariation between negative parenting and behavior problems because these latter two studies were based on adolescent samples.

Research Question 3: How do parental negativity and behavior problems at age 4 influence parental negativity and behavior problems at age 12 (cross-lagged and cross-age stability pathways)?

Both phenotypes were moderately stable from ages 4 to 12, and the stability estimates were similar to those reported in previous studies examining similar associations 3 years apart, even though in our study the association was studied 8 years apart (Burt et al., 2005; Larsson et al., 2008; Moberg et al., 2011).

The key cross-lag analyses indicate that both child-driven and parent-driven effects independently contribute to the association between parental negativity and behavior problems from ages 4 to 12. Regarding the longitudinal effect size of these effects, behavior problems at age 4 accounted for 1.7% and 2% of parental negativity at age 12 in males and females, respectively (child-driven effects). Parental negativity at age 4 only accounted for 0.8% and 0.1% of behavior problems at age 12 in males and females, respectively (parent-driven effects). Although these effect sizes are small, phenotypes that account for around 2% of the variance during a 3-year interval are not unusual because the paths are independent of the association between parental negativity and behavior problems at age 4 as well as independent of the stability of both measures across age (Burt et al., 2005; Larsson et al., 2008; Moberg et al., 2011). Moreover, in our case, these effects emerged across an 8-year age span. The effect size of parent-driven effects, although significant, is smaller than child-driven effects. The recent study by Moberg et al. (2011) reported evidence for child-driven effects but not for parent-driven effects. Despite these differences in effect size between child-driven effects and parent-driven effects, our study provides support for a bidirectional relationship between parental negativity and behavior problems from early childhood to adolescence. These results are consistent with previous studies (Burt et al., 2005; Larsson et al., 2008). This bidirectional relationship has been described as a downward spiral where parenting both impacts and is impacted by child behavior (Burt et al., 2005). This downward spiral relates to the concept of a coercive parent–child relationship (Collins & Laursen, 1999) where difficulties in children behavior coupled with stressed-out parents who finally relent and fail to provide support and adequate negative consequences for bad behaviors. Ultimately, parents end up reinforcing child behavior problems. This illustrates a pathway through which ineffective parental management and early difficult and demanding child characteristics foster the development or consolidation of behavior problems later in life (Patterson, 1982; Pettit & Arsiwalla, 2008).

Research Question 4: How do genetic and environmental influences on parental negativity and behavior problems at age 4 contribute to parental negativity and behavior problems at age 12?

In line with previous research, stability of behavior problems was mainly attributable to genetic factors, specifically; around 68% of the transmitted variance through this cross-age stability path was due to genetic factors (Figure 3; Eley, Lichtenstein, & Moffitt, 2003; Haberstick, Schmitz, Young, & Hewitt, 2005; Larsson et al., 2008; Neiderhiser et al., 1999).

In regard to the etiological nature of the bidirectional effects, the parent-driven path was a function of both genetic and environmental factors. In contrast, the child-driven path was largely a function of genetic factors. Therefore, as we expected based on previous research (Burt et al., 2005; Larsson et al., 2008), child-driven effects were mainly genetically mediated and parent-driven effects were a function of both genetic and shared-environmental factors. Furthermore, the relevant role played by genetic factors in the association between parental negativity and behavior problems is consistent with some previous studies examining similar phenotypes (Leve et al., 2009; Neiderhiser et al., 1999; Pike & Plomin, 1996).

Research Question 5: Are there sex differences in the genetic and environmental architecture of the longitudinal associa-

tions between parental negativity and behavior problems from early childhood to adolescence?

Similar to previous studies (Burt et al., 2005; Larsson et al., 2008), we found generally similar results for males and females. However, a hint of sex differences in the association between parental negativity and behavior problems over time and the genetic and environmental contribute to this association. Looking into these sex differences more carefully, they arise from the cross-lagged path representing parent-driven effects, which are significantly different in males and females. Since the rest of the estimates were nearly identical across genders, the clinical relevance of the sex differences found in the current study should be interpreted with caution and needs further research.

Research Question 6: Does general cognitive ability affect these results?

These results did not differ as a function of general cognitive ability. Thus, although general cognitive ability is related to behavior problems, it does not modify the association between parental negativity and behavior problems over time. Difficulties in the cognitive domain may be independent from behavior difficulties at least in relation to parental negativity over time.

General discussion

In order to interpret these findings, especially regarding the role of genetic factors in the bidirectional association between parental negativity and behavior problems from early childhood to adolescence, from a developmental perspective, here we discuss the results in the light of the self-regulatory framework (Calkins & Keane, 2009). Although self-regulation was not measured per se, behavior problems, as defined in the current study, included different domains of adaptative functioning that are highly intercorrelated (Bornstein, Hahn, & Haynes, 2010; Masten, Burt, & Coatsworth, 2006; Mesman, Bongers, & Koot, 2001). Therefore, behavior problems may be reflecting difficulties in behavioral adjustment that may be underlined by deficits in self-regulatory processes. In this context, failures in the acquisition of basic processes such as emotion regulation and cognitive control early in life would ultimately lead to the expression of behavior problems. Applying a cross-lagged model design, we observed that behavior problems at age 4 predict behavior problems 8 years later. Moreover, also consistent with the self-regulation theory, the bidirectional relationship between parental negativity and behavior problems was significant even when the stability of the two phenotypes was also considered in the model. This supports the role of parenting in the early origins and maintenance of behavior problems from early childhood to adolescence. In the light of our findings, this cascade of effects may be underlined by genetic factors. Biological foundations related to the physiological and neurobiological mechanisms related to self-regulation process may well include genetic influences, therefore adding plausibility to our results (Calkins & Keane, 2009; Posner & Rothbart, 2009).

Finally, since our findings indicate that the association between parenting and adolescent behavior problems seems to be mainly accounted by genetic factors, the current study may have potential implications for molecular genetic studies. A burning issue nowadays is the fact that despite high heritabilities, molecular genetic studies, including genome-wide association studies, have not been successful in identifying DNA variants responsible for this heritability (Manolio et al., 2009), the *missing heritability problem* (Maher, 2008). One of many possible directions for finding the missing heritability lies in the interplay between genes and environment. In the case of behavior problems, several exciting findings involve gene–environment correlation (Jaffee & Price, 2007; Neiderhiser et al., 2004; O'Connor, Deater-Deckard, Fulker, Rutter, & Plomin, 1998).

Clinical implications

Although it is not novel to show that both parent-driven and child-driven effects independently contribute to the association between parental negativity and children's behavior problems, it is an important message for clinicians and parents. Regardless of their etiology, these bidirectional effects suggest a need to increase awareness of the developmental downward spiral between child problems and parental actions and reactions. A more novel finding concerns etiology: the child-driven effects were mainly genetically mediated and the parent-driven effects were mediated by both genetic and shared environmental factors. Although heritability does not imply immutability, these results suggest that parental reactions might provide a better target for prevention of the downward spiral.

From a developmental point of view, our findings show that the association between parental negativity and behavior problems in childhood can extend until adolescence. The cross-lagged analysis shows significant directional effects from parental negativity in childhood and adolescent behavior problems. Therefore, early interventions can potentially prevent the later consolidation of emotional and behavioral problems in the adolescence stage.

Limitations

The current results should be interpreted considering the following specific limitations, in addition to general limitations of the twin design (Plomin et al., 2008). First, one limitation is that parents reported both parental negativity and child behavior problems. Therefore, some of the overlap between parental negativity and behavior problems could be due to shared rater effects (Rutter, Pickles, Murray, & Eaves, 2001). Unfortunately, information regarding behavior problems at early childhood was only available from parents. Nevertheless, the pattern of our results is in general in agreement with previous research using different informants or combined informant approaches (Burt et al., 2005; Moberg et al., 2011; Neiderhiser et al., 1999). Furthermore, the validity and reliability of the par-

ent-reported SDQ scores has been shown in several studies (Hawes & Dadds, 2004; Muris, Meesters, & van den Berg, 2003; Rothenberger, Becker, Erhart, Wille, & Ravens-Sieberer, 2008). Second, the behavior problems composite used in the current study included emotional, hyperactivity, conduct, and peer problems in children. It is possible that each of these types of problems may have different etiological pathways. However, as mentioned before, these types of symptoms are highly comorbid (Angold et al., 1999) and may share etiological risk factors (Timmermans et al., 2010). Third, sex differences were explored in relation to twins, but we made no distinction between fathers' and mothers' negativity, which can also affect the analyzed association. Several studies provide evidence for different effects of parenting on child behavior depending on the gender of the parent (Blatt-Eisengart et al., 2009; Lifford, Harold, & Thapar, 2009; Vieno, Nation, Pastore, & Santinello, 2009). This information was not available for the current study, thus we cannot warrant that mother–son, mother–daughter, father–son, or father–daughter relationships differ between each other. Fourth, the parental measure represents the negative feelings that the parent reports experiencing toward the child rather than parenting practice per se. This can limit the comparability of our study to others using more behavior-based measures of parenting. Fifth, causal pathways were not decomposed per se into genetic and environmental contributions as is done in the model proposed by Luo et al. (2010). Thus, we track and decompose transmitted variance to understand how genetic and environmental factors shape the longitudinal association between parental negativity and behavior problems.

Despite the limitations, these findings contribute to the better understanding of the genetic and environmental contributions to childhood and adolescent behavior problems and, specifically, its relationship with parental negativity.

Conclusions

The current study provides evidence for the presence of both parent-driven and child-driven effects in the relationship between parental negativity and behavior problems even between two different developmental stages, early childhood and adolescence. Furthermore, this bidirectional association seems to be primarily of genetic origin. Future research may benefit from including a third time of assessment, to further explore the continuity of this association and possible shifts on the contribution and mediation of genetic and environmental factors to the phenotypes, its stability, and its relationship. Such studies would be of great interest especially when examining different developmental stages where relevant cognitive, psychological, neurobiological, and physiological changes involved in behavioral adjustment are taking place.

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

Dr. Lourdes Fañanás Saura, Associate Professor (Profesora Titular) at the Department of Animal Biology of the Faculty of Biology, University of Barcelona and supervisor of the present doctoral thesis by Silvia Alemany, hereby certifies that the participation of the PhD applicant in the article "Genetic origin of the relationship between parental negativity and behaviour problems from early childhood to adolescence: a longitudinal genetically informative design" included the following tasks:

- Participation in the conception and design of the study
- Statistical analysis and interpretation of data
- First drafting of the manuscript
- Critical revision of the arcticle for intellectual content

Dr. Lourdes Fañanás

Barcelona, February 11th 2013

7.2. Childhood abuse and the BDNF-Val66Met polymorphism: Evidence for gene-environment interaction in the development of adult psychosis-like experiences. Alemany S, Arias B, Aguilera M, Villa H, Moya J, Ibáñez MI, Vossen H, Gastó C, Ortet G, Fañanás L. 2011. *The British Journal of Psychiatry*, 2011.199: 38-42.

Resumen en Castellano

Abuso infantil y el polimorfismo BDNF-Val66Met: Evidencia de efectos de interacción gen-ambiente en el desarrollo de experiencias psicóticas positivas adultas.

Alemany S, Arias B, Aguilera M, Villa H, Moya J, Ibáñez MI, Vossen H, Gastó C, Ortet G, Fañanás L. 2011.

The British Journal of Psychiatry, 2011.199: 38-42.

Diversos estudios han puesto de manifiesto una asociación entre adversidad temprana y psicosis. Aunque los mecanismos biológicos que subyacen a esta asociación nose han esclarecido aun, es probable los factores genéticos estén involucrados y puedan contribuir a explicar porqué no todos los individuos expuestos a adversidad infantil desarrollan síntomas psicóticos más tarde en la vida.

En el presente estuido se exploró si el maltraro infantil (abuso y negligencia infantil) tenía un impacto diferencial sobre la presencia de las experiencias psicóticas (EPs) positivas y negativas en una muestra española extraída de la población general (n=533). Por otra parte, se investigó la existencia de un posible efecto de moderador por parte del polimorfismo BDNF-Val66Met sobre la relación entre maltrato infantil y el desarrollo de EPs mediante análisis de regresión lineal múltiple. Los resultados indicaron que las personas expuestas a eventos adversos en la infancia tienen más probabilidades de desarrollar experiencias psicóticas (EPs) posteriormente. Específicamente, el abuso infantil pero no la negligncia se asoció con el desarrollo de EPs positivas, indicando cierta especifidad entre el maltrato infantil y el desarrollo de EPs positivas siendo las experiencis caracterizadas por abuso de especial relevancia en esta asociación. Por otra parte, se observó un papel moderador por parte del polimorfismo BDNF-Val66Met en la relación entre abuso infantil y el desarrollo de EPs positivas. Este efecto de GxA indicaba que los portadores del alelo Met del gen BDNF eran más vulnerables a los efectos negativos del abuso infantil comparados con los portadores del genotipo Val/Val. Es decir, los portadores del alelo Met del gen BDNF podrían ser neurobiológicamente más vulnerables a los efectos del abuso infantil en relación con la expresión de EPs positivas.

BJPsych | The British Journal of Psychiatry (2011) 199, 38–42. doi: 10.1192/bjp.bp.110.083808

Childhood abuse, the BDNF-Val66Met polymorphism and adult psychotic-like experiences

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Background
The well-established relationship between childhood adversity and psychosis is likely to involve other factors such as genetic variants that can help us to understand why not everyone exposed to adverse events develops psychotic symptoms later in life.

Aims
We investigated the influence of childhood abuse and neglect on positive and negative psychotic-like experiences in adulthood and the potential moderating effect of the BDNF-Val66Met polymorphism.

Method
Psychotic-like experiences and childhood adversity were assessed in 533 individuals from the general population.

Results
Childhood abuse showed a strong independent effect on the positive dimension of psychotic-like experiences (β = 0.16, s.e. = 0.05, $P = 0.002$). Furthermore, this association was moderated by the BDNF-Val66Met polymorphism (β = 0.27, s.e. = 0.10, $P = 0.004$).

Conclusions
Individuals exposed to childhood abuse are more likely to report positive psychotic-like experiences. *Met* carriers reported more positive psychotic-like experiences when exposed to childhood abuse than did individuals carrying the *Val/Val* genotype. Therefore, the observed gene–environment interaction effect may be partially responsible for individual variation in response to childhood abuse.

Declaration of interest
None.

Psychological stress occurring during either childhood or adulthood has been related to psychosis.[1] Childhood adversity as a form of psychological stress has been shown to be a risk factor for the development of psychotic symptoms in clinical samples[2,3] and psychotic-like experiences in individuals from the general population.[4,5] Despite this established relationship, it is necessary to consider the type and severity of any environmental exposure, together with a specific individual genetic background of risk, in order to understand the development of psychosis in adulthood. The term childhood adversity usually refers to a wide range of severe adverse experiences occurring early in life (before 16 years of age) and includes sexual, physical and emotional abuse and neglect. In this regard, several lines of research have suggested a strong relationship between childhood abuse and positive psychotic symptoms.[6] However, less attention has been paid to the effect of neglect, and studies examining the impact of both types of childhood adversity are still relatively scarce. Genetic factors are also likely to be involved in the individual variation in response to stress. Genes involved in regulating the adaptive behavioural response to stress represent plausible candidates to explore putative gene–environment interaction effects in the association between childhood adversity and psychotic symptoms. In this context, the BDNF-Val66Met polymorphism has been related not only to psychosis but has also been shown to moderate the impact of childhood adversity on the later expression of affective symptoms.[7,8] Brain-derived neurotrophic factor (BDNF) is a neurotrophin that promotes the growth and differentiation of developing neurons in central and peripheral nervous systems.[9] It has been shown that early stress can influence BDNF expression and produce long-lasting effects on neurotrophic processes, thereby having an impact on neuronal maturation and plasticity in later life.[1] However, studies of the relationship between the functional BDNF-Val66Met polymorphism and schizophrenia have produced mixed results,[9] which may be because of the underlying gene–environment interaction.[10] One recent study found that *Met* carriers (negatively affecting intracellular processing and secretion of the mature protein) report more paranoid feelings in the presence of social stress than do *Val/Val* carriers.[11] Thus, it is plausible that the BDNF-Val66Met polymorphism might play a moderating role in the association between childhood adversity and psychosis. The present study aimed to explore whether childhood adversity (childhood abuse and childhood neglect) have a differential impact on the presence of psychotic-like experiences. Furthermore, a possible moderating effect of the BDNF-Val66Met polymorphism on the relationship between childhood adversity and psychotic-like experiences was also investigated.

Method

Sample

The sample consisted of 533 individuals who were recruited from the campus of the Jaume I University in Castelló (Spain), as well as from university offices and community technical schools in the metropolitan area of Barcelona (Spain). All the participants were adults (mean age 22.9 years, s.d. = 5.4) and 45.4% were males. At the time of the assessment 77% of the participants were students. Exclusion criteria were the presence of any major medical illness affecting brain function, neurological conditions and a history of head injury. All participants were of Spanish (White) ancestry, thereby reducing the possibility of confounding genetic differences by population stratification.[12] Ethical approval was obtained from

local research ethics committees. All participants provided written informed consent before inclusion in the study.

Measures

The Community Assessment of Psychic Experiences (CAPE)[13] was used to assess positive and negative psychotic-like experiences in the sample. This validated self-report questionnaire measures the lifetime prevalence of psychotic-like experiences on a frequency scale ranging from 'never' to 'nearly always'. Examples of the items assessing the positive and negative dimension are, respectively, 'do you ever feel as if things in magazines or TV were written especially for you?' and 'do you ever feel that you experience few or no emotions at important events?'. The CAPE provides a total score per dimension by adding up the scores on the frequency items.

Childhood adversity was assessed by the shortened version of the Childhood Trauma Questionnaire (CTQ).[14] This questionnaire consists of 28 items measuring five types of childhood trauma: emotional abuse, physical abuse, sexual abuse, emotional neglect and physical neglect. Childhood adversity was grouped into two main types: childhood abuse (including emotional, physical and sexual abuse) and childhood neglect events (including emotional and physical neglect). This was done in order to explore the putative specificity of the impact of these two types of childhood adversity. Childhood abuse and childhood neglect were calculated by summing the items included for each type of childhood adversity. Example items of childhood abuse and childhood neglect are, respectively, 'people in my family hit me so hard that it left me with bruises or marks' and 'my parents were too drunk or high to take care of the family'. The score for each item ranges from 1 to 5 ('never true' to 'very often true'), depending on the extent to which individuals agree with the statement. Reliability and validity of the CTQ have both been demonstrated.[15]

Because schizotypal personality, cannabis use and anxiety levels have all been related to both childhood adversity and psychotic-like experiences, and given the relationship between them,[16–18] the analyses were corrected for these variables, along with age and gender as other potential confounders. Schizotypal personality was measured with the Schizotypy Personality Questionnaire-Brief (SPQ-B).[19] Cannabis use was assessed with one question regarding the frequency of consumption: 'never', 'once', 'monthly', 'weekly' or 'daily' (this variable was then dichotomised into two categories: 'not exposed to cannabis': never, once; and 'exposed to cannabis': monthly, weekly, daily). Anxiety as a behavioural trait was assessed using the State–Trait Anxiety Inventory (STAI-T).[20]

Laboratory methods

Genomic DNA was extracted from peripheral blood cells using the Real Extraction DNA Kit (Durviz SLU, Valencia, Spain), or from buccal mucosa on a cotton swab using the BuccalAmp DNA Extraction Kit (Epicentre Biotechnologies, Madison, Wisconsin, USA). The rs6265 SNP (Val66Met) of the *BDNF* gene was determined using the Taqman 5′ exonuclease assay (Applied Biosystems) and genotyped using Applied Biosystems (AB) TaqMan technology. The probe for genotyping the rs6265 was ordered through the TaqMan SNP Genotyping assays (code C_11592758_10) AB assay-on-demand service. The final volume of the polymerase chain reaction was 5 ml, which contained 10 ng of genomic DNA, 2.5 ml of TaqMan Master Mix, and 0.125 ml of 40x genotyping assay. The cycling parameters were as follows: 95°C for 10 min followed by 40 cycles of denaturation at 92°C for 15 s and annealing/extension at 60°C for 1 min. Polymerase chain reaction plates were read on an ABI PRISM 7900HT instrument with SDS v2.1 software (Applied Biosystems).

Statistical analyses

Multiple linear regressions were conducted using STATA 10.0 for Windows. Separate models were tested for CAPE positive and CAPE negative as dependent variables. For the first hypothesis the independent variables of interest were childhood abuse, childhood neglect and the BDNF-Val66Met polymorphism. Schizotypal personality, cannabis use, trait anxiety, gender and age were included in the model as covariates. For the second hypothesis, two-way interaction effects between childhood abuse and the BDNF-Val66Met polymorphism and childhood neglect and the BDNF-Val66Met polymorphism were added to the model, as described for the first hypothesis. Since the *Met/Met* genotype ($n = 29$) has a much lower frequency than the *Val/Met* and *Val/Val* genotypes, the genotypes for this polymorphism were included in the analyses as a binary variable (*Met* allele carriers and *Val* homozygotes).

Results

Descriptive statistics

In order to obtain the prevalence of psychotic-like experiences in the current sample, CAPE scores were recoded to 0 (never, sometimes) and 1 (often, almost always). The resulting prevalence rate indicated that psychotic-like experiences were quite frequent. Specifically, 40.7% of the sample often or almost always experienced at least one positive psychotic-like experience; similarly, 47.6% reported experiencing at least one negative psychotic-like experience often or almost always.

The prevalence of childhood adversity was evaluated by recoding the answers to 0 (never true) and 1 (rarely true, sometimes true, often true and very often true). Thus, 1 indicates that the individual was exposed at least once to the adverse event. In the current sample, 25.5% of the individuals were exposed to childhood abuse and 32.2% to childhood neglect. More details of the distribution of dimensions in the sample can be found elsewhere.[8]

Genotype information was available for 470 individuals. The genotype frequencies for the BDNF-Val66Met polymorphism were: *Val/Val*: 60% ($n = 282$); *Val/Met*: 33.8% ($n = 159$); and *Met/Met*: 6.2% ($n = 29$). These frequencies did not differ from others described in previous studies conducted in White individuals.[21] Hardy–Weinberg equilibrium was verified for the present population ($\chi^2 = 1.05$, d.f. = 2, $P = 0.59$).

The final sample consisted of 411 individuals for whom all the variables included in the models were available.

Specificity of the impact of childhood adversity on psychotic-like experiences

We found a main effect of childhood abuse on positive psychotic-like experiences ($\beta = 0.16$, s.e. = 0.05, $P = 0.002$) and a marginally significant effect of childhood abuse on negative psychotic-like experiences ($\beta = 0.11$, s.e. = 0.06, $P = 0.055$) (Table 1). Childhood neglect did not have a direct influence on either positive or negative psychotic-like experiences. Furthermore, no main effect was found for the BDNF-Val66Met polymorphism on either dimension of psychotic-like experiences.

Gene–environment interaction between the BDNF-Val66Met polymorphism and childhood adversity with respect to subsequent psychotic-like experiences

A significant gene–environment interaction was detected between *BDNF Met* carriers and childhood abuse with regard to positive psychotic-like experiences ($\beta = 0.27$, s.e. = 0.10, $P = 0.004$). In this

Table 1 Main effects of childhood abuse, childhood neglect and the BDNF-Val66Met polymorphism (*Val/Val v. Met* carriers) on positive and negative psychotic-like experiences, correcting for age, gender, schizotypal personality, cannabis use and trait anxiety

	Positive psychotic-like experiences[a]			Negative psychotic-like experiences[b]		
	β	s.e.	*P*	β	s.e.	*P*
BDNF	−0.385	0.358	0.282	0.338	0.409	0.409
Childhood abuse	**0.155**	**0.049**	**0.002**	0.107	0.056	0.055
Childhood neglect	−0.085	0.053	0.110	−0.032	0.060	0.591

a. $R^2 = 0.31$.
b. $R^2 = 0.32$.
Values in bold are significant.

sample, individuals carrying the *Met* allele had higher scores on adult positive psychotic-like experiences when childhood abuse was present, as compared with participants carrying *Val/Val* homozygotes (Fig. 1). No significant gene–environment interaction was detected with respect to childhood neglect (β = −0.09, s.e. = 0.05, $P = 0.110$).

Discussion

This study shows that childhood adversity has a strong independent effect on positive psychotic-like experiences and a marginally significant effect on negative psychotic-like experiences, whereas childhood neglect was not associated with either dimension of psychotic-like experiences. The BDNF-Val66Met polymorphism shows a moderating effect between childhood abuse and the later development of positive psychotic-like experiences. These results are not confounded by the effect of gender, age, schizotypal personality, cannabis use or trait anxiety.

Childhood adversity and psychotic-like experiences

Several years ago it was postulated that light might be shed on the aetiology of psychosis by studying individuals who have psychotic symptoms without being in need of treatment.[22] Broadly, there are two potential approaches to the measurement of psychotic symptoms in non-clinical samples: one would be to measure schizotypal traits as an attenuated form of psychotic symptoms, whereas the other would involve measuring in the general population the occurrence of those symptoms that are seen in individuals with psychosis. The latter approach assumes that experiencing 'symptoms' of psychosis is not inevitably linked with the clinical disorder. Thus, even though the prevalence of the clinical disorder is low, the prevalence of these 'milder forms' of psychosis, namely psychotic-like experiences, may be much higher.[13,22] The rate of psychotic-like experiences in the present sample is in line with previous reports.[22] For example, Barrett & Etheridge found that 30–40% of individuals from the general population reported the experience of hearing voices.[23] Similarly, in a sample of college students, 71% reported at least brief, occasional hallucinated voices during periods of wakefulness, whereas 39% reported hearing their thoughts spoken aloud.[24]

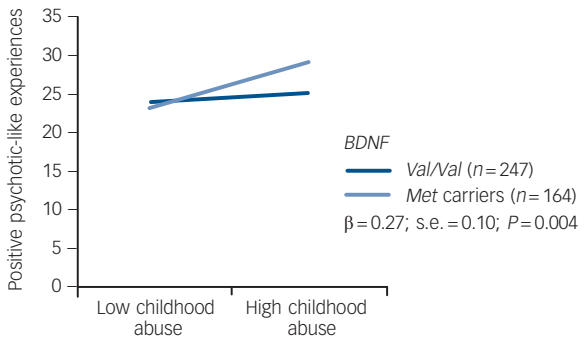

Fig. 1 Graphic respresentation of the interaction effect between childhood abuse and the BDNF-Val66Met polymorphism on positive psychotic-like experiences.

Corrected for age, gender, schizotypal personality, cannabis use and trait anxiety. Exposure to childhood abuse is moderated by the *BDNF* gene. *Met* carriers exposed to childhood abuse have significantly higher scores on positive psychotic-like experiences.

Regarding the aetiology of psychotic-like experiences, according to previous research, our findings support the role of childhood adversity as a risk factor underlying the development of psychotic-like experiences in the general population.[4–6] Specifically, there was a strong association between childhood abuse and positive psychotic-like experiences and a trend towards an association between childhood abuse and negative psychotic-like experiences. These results fit well with recent models suggesting that adverse events, especially those characterised by abuse, may produce a psychological and/or biological vulnerability for the development of positive psychotic symptoms, including subclinical forms such as psychotic-like experiences.[22,25,26] It has been suggested that early abusive experiences may create an enduring cognitive vulnerability characterised by negative schematic models of the self and the world (for example beliefs about the self as vulnerable to threat, or about others as dangerous) that facilitate external attributions, which may ultimately lead to paranoid ideation.[25] In this regard, current ideas about the biological consequences of childhood adversity lend even more credibility to the notion of an enduring psychological vulnerability. When exposure to stressors persists, the stress-induced glucocorticoid release can become chronic, leading to permanent changes in the hypothalamic–pituitary–adrenal (HPA) axis. This alteration of HPA functioning can lead to dysregulation of the dopaminergic system, which is generally thought to be involved in psychosis.[1,27] Specifically, it has been suggested that stress-induced dysregulation of the HPA axis causes increased dopamine receptor densities and greater dopamine release. The dopaminergic system is important as regards the interpretation of stress and threat-related stimuli, and therefore, relevant to the development of positive psychotic symptoms such as paranoid ideation.[26]

In our sample, childhood neglect was not significantly associated with psychotic-like experiences. Although this contrasts with some previous reports,[28,29] a recent study by Fisher and colleagues[30] also found no impact of neglect on the expression of psychosis when controlling for the impact of abuse. Conversely, events characterised by abuse have shown the most robust association with psychotic symptoms.[6,31,32] Moreover, it has been postulated that abusive experiences could have an aetiological significance in psychosis,[33] and research has described higher rates of abusive maltreatment than neglect among individuals with psychosis.[34] Hence, it may be that previous associations between psychosis and childhood adversity, where the latter included both abuse and neglect events, were inflated by the effect of abuse.

BDNF-Val66Met polymorphism, childhood adversity and psychotic-like experiences

Gene–environment interaction studies have shown exciting findings that made them appear to be promising mechanisms to understanding the joint effect between environmental and genetic factors in the aetiology of complex traits such as psychiatric symptoms.[35] However, dismissal of gene–environment interaction studies has recently arisen mainly as a result of the failure to replicate; as has happened before with genetic association studies.[36] It has been argued that the lack of replication may be related to the greater number of potential statistical tests that are possible when interaction effects are included in any analysis, which greatly increases the risk of false positives that can be nominally significant but do not represent true insight.[36] To prevent this, the present study was developed with *a priori* hypothesis that guides the choice of the gene and the polymorphism and the environmental risk factor. Furthermore, several reasons were considered to explain why gene–environment interactions might be expected in the relationship between childhood adversity and psychosis. Human development is an environmentally dependent process in which individuals need to adapt to environmental hazards. However, it is implausible that genetic variants do not contribute to individual variation in response to the environment, since this response is associated with pre-existing individual differences in temperament, personality and psychophysiology, all of which are known to be under a certain degree of genetic influence.[35] In this context, one genetic variant that is a candidate for moderating the association between childhood adversity and psychosis is the BDNF-Val66Met polymorphism. This polymorphism consists of a Val/Met single nucleotide polymorphism at position 66 in the *BDNF* gene, and it has been identified as a functional polymorphism.[37] The Val variant is associated with higher neuronal BDNF secretory activity than is the Met variant. Additionally, the coexpression of *Val* and *Met* alleles in heterozygotes results in less efficient intracellular trafficking and processing, leading to decreased BDNF secretion.[37] The secretion of BDNF is crucial for the growth and differentiation of developing neurons in both central and peripheral nervous systems, and BDNF is also implicated in the survival of neuronal cells in response to stress.[1,7,37] Evidence from animal studies suggests that individuals carrying the *Met/Met* genotype are more likely to develop anxiety-related behaviours in response to stressful events.[7] In humans, it has been shown that *Met* homozygotes and heterozygotes who have experienced childhood adversity could also be more genetically vulnerable to the development of affective symptoms, in comparison to *Val* homozygotes.[8] However, the potential moderating effect of the BDNF-Val66Met polymorphism on the relationship between psychosocial stress and psychosis has not been widely explored. To the best of our knowledge, only Simons and colleagues[11] have studied the relationship between minor stressful daily events, the BDNF-Val66Met polymorphism and paranoid experiences. These authors found that *BDNF-Met* allele carriers showed more social stress-induced paranoia than did individuals with the *Val/Val* genotype. The present results are in line with these findings. Specifically, we found that the impact of childhood abuse on the development of positive psychotic-like experiences was higher in those individuals carrying the *Met* allele. This provides evidence of a gene–environment interaction effect, whereby *Met* carriers would, genetically, be more vulnerable to the effects of childhood abuse than would *Val* homozygotes.

We believe that these findings are consistent with the hypothesised affective pathway to psychosis, which has been suggested to be preferentially underlying the positive symptoms of psychosis.[1] As mentioned earlier, childhood adversity has been shown to alter the functioning of the HPA axis, which is one of the most important brain circuits involved in regulating adaptive responses to stress. In this context, the intrusive nature of abusive experiences may indicate that they are especially likely to dysregulate the HPA axis. This dysregulation would, in turn, result in increased dopamine release in mesolimbic brain areas, which has been frequently related to the expression of positive psychotic symptoms.[1,2]

In summary, our results indicate that individuals carrying the *Met* allele, the variant associated with less BDNF secretion, would be more vulnerable, neurobiologically speaking, to the negative effects of early abusive experiences.

Strengths and limitations

Among the strengths of the present study it is worth noting that the results were not confounded by the effect of schizotypal personality traits, trait anxiety or cannabis use. Thus, the findings indicate that exposure to childhood abuse increases the risk of reporting adult psychotic-like experiences independently of any pre-existing schizotypal traits, which have also been shown to increase the likelihood of experiencing psychotic symptoms.[18] Similarly, although the use of cannabis is a well-known environmental risk factor for psychotic-like experiences,[38] this did not confound the present results as the frequency of cannabis use was controlled for. As regards the inclusion of trait anxiety as a confounder, it has been found that the strong emotions associated with childhood adversity, such as anxiety and memories of the earlier experience, contribute to an increased risk of later psychotic symptoms.[39] However, as trait anxiety was controlled for, we can rule out the possibility that the occurrence of psychotic-like experiences was linked to the anxiety associated with abusive events experienced in childhood. Overall, the present research design follows the recommendations of a systematic and critical review by Bendall *et al*[2] in that it includes confounders based on previous research into childhood adversity and psychosis.

Despite these strengths, the present study does have a number of limitations. First, the cross-sectional design prevents a robust test of causal associations, although *a priori* hypotheses were clearly defined and guided all the subsequent analyses as mentioned earlier. Second, the retrospective measure of childhood adversity may constitute an inherent source of bias. That said, the CTQ has been validated and is considered a reliable measure of childhood adversity,[15] as well as being recommended in the critical review by Bendall and colleagues[2] as a reliable tool for measuring childhood abuse. Third, although the current findings are in line with those reported by Simons *et al*,[11] the studies are not directly comparable since the outcomes and environmental risk factors analysed were different. Studies examining similar hypothesis but that differ in the exact variables analysed or the instruments used to measure such variables can also account for inconsistencies in the results and therefore, failure to replicate. Fourth, although the sample size is similar to that used in previous and similar studies,[4,11] it can be still considered relatively small. In the light of these limitations, our findings should be considered with caution and need replication in larger samples.

Implications

Our findings suggest a specific relationship between childhood abuse and positive psychotic-like experiences in the general population. The results also provide evidence for a gene–environment interaction effect underlying individual behavioural differences in response to childhood abuse; specifically, *Met* carriers are more likely to report positive psychotic-like experiences in the presence of childhood abuse compared with *Val* homozygotes. These results now require replication as they may have important implications for future research into the

aetiological mechanisms operating between childhood adversity and later psychosis.

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First received 21 Dec 2010, final revision 31 Jan 2011, accepted 21 Mar 2011

Funding

This study was supported through research projects funded by the Ministry of Science and Innovation (SAF2008-05674-C03-00/03 and PSI2008-05988) and the Institute of Health Carlos III, CIBER of Mental Health (CIBERSAM) and also by the Comissionat per a Universitats i Recerca del DIUE of the Generalitat de Catalunya (2009SGR827). S.A. thanks the Institute of Health Carlos III for her PhD grant (FI00272).

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

Dr. Lourdes Fañanás Saura, Associate Professor (Profesora Titular) at the Department of Animal Biology of the Faculty of Biology, University of Barcelona and supervisor of the present doctoral thesis by Silvia Alemany, hereby certifies that the participation of the PhD applicant in the article "Childhood abuse and the BDNF-Val66Met polymorphism: Evidence for gene-environment interaction in the development of adult psychosis-like experiences" included the following tasks:

- Participation in the conception and design of the study
- Analysis and interpretation of data
- First drafting of the manuscript
- Critical revision of the arcticle for intellectual content

Dr. Lourdes Fañanás

Barcelona, February 11th 2013

7.3. Psychosis-inducing effects of cannabis are related to both childhood abuse and COMT genotypes. Alemany S, Arias B, Fatjó-Vilas M, Aguilera M, Villa H, Moya J, Ibáñez MI, Ortet G, Fañanás L. *Acta Psychiatrica Scandinavica* (In press).

Resumen en Castellano

Los efectos inductores de psicosis del cannabis están relacionados con el abuso infantil y los genotipos del gen COMT.

Alemany S, Arias B, Fatjó-Vilas M, Aguilera M, Villa H, Moya J, Ibáñez MI, Ortet G, Fañanás L.

Acta Psychiatrica Scandinavica (En prensa).

El objetivo del presente estudio fue investigar si el impacto del maltrato infantil y el consumo de cannabis en el desarrollo de EPs varía en función de los genotipos del polimorfismo COMT-Val158Met en una muestra extraída de la población en general (n=533) mediante análisis de regresión lineal múltiple.

Se encontró una triple interacción GxAxA entre el abuso infantil, el consumo de cannabis y el polimorfismo COMT-Val158Met en el desarrollo de las EPs positivas. Concretamente, el consumo de cannabis en individuos expuestos a abuso infantil tiene efectos opuestos dependiendo de los genotipos del gent COMT.

En individuos no expuestos o expuestos a niveles bajos de abuso infantil, el consumo de cannabis y el polimorfismo Val158Met del gen COMT no tenían ningún efecto en cuanto al desarrollo de EPs positivas. Sin embargo, entre los individuos expuestos a niveles altos de abuso infantil y que consumen cannabis, la puntuación en EPs positivas aumentaban en función de las dosis del alelo Val del gen COMT. El efecto opuesto se observó en individuos expuestos a abuso infantil pero que no consumían cannabis, sus puntuaciones en EPs positivas aumentaban en función de las dosis del alelo Met del gen COMT. Este patrón de resultados coincide con la definición epidemiológica de interacción GxA cualitativa.

Acta Psychiatrica Scandinavica

Acta Psychiatr Scand 2013: 1–9

DOI: 10.1111/acps.12108

Psychosis-inducing effects of cannabis are related to both childhood abuse and COMT genotypes

Alemany S, Arias B, Fatjó-Vilas M, Villa H, Moya J, Ibáñez MI, Ortet G, Gastó C, Fañanás L. Psychosis-inducing effects of cannabis are related to both childhood abuse and COMT genotypes.

Objective: To test whether the association between childhood abuse, cannabis use and psychotic experiences (PEs) was moderated by the *COMT* (catechol-*O*-methyltransferase) gene.
Method: Psychotic experiences (PEs), childhood abuse, cannabis use and *COMT* Val158Met genotypes were assessed in 533 individuals from the general population. Data were analysed hierarchically by means of multiple linear regression models.
Results: Childhood abuse showed a significant main effect on both positive (β = 0.09; SE = 0.04; $P = 0.047$) and negative PEs (β = 0.11; SE = 0.05; $P = 0.038$). A significant three-way interaction effect was found among childhood abuse, cannabis use and the *COMT* gene on positive PEs (β = −0.30; SE = 0.11; $P = 0.006$). This result suggests that *COMT* genotypes and cannabis use only influenced PE scores among individuals exposed to childhood abuse. Furthermore, exposure to childhood abuse and cannabis use increased PE scores in Val carriers. However, in individuals exposed to childhood abuse but who did not use cannabis, PEs increased as a function of the Met allele copies of the *COMT* gene.
Conclusion: Cannabis use after exposure to childhood abuse may have opposite effects on the risk of PEs, depending on the *COMT* genotypes providing evidence for a qualitative interaction. Val carriers exposed to childhood abuse are vulnerable to the psychosis-inducing effects of cannabis.

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Key words: psychoses; trauma; cannabis; genetics

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Accepted for publication January 28, 2013

Significant outcomes

- The psychosis-inducing effect of cannabis use is related to exposure to childhood abuse and genetic variability in *COMT* gene.
- Cannabis use increased the likelihood to report positive psychotic experiences in Val carriers only when they were exposed to childhood abuse.
- Sensitization processes involving dopaminergic signalling may be underlying this gene–environment–environment interaction.

Limitations

- The sample size was modest.
- Childhood abuse was measured retrospectively.
- Age of onset, potency or duration of cannabis use were not assessed in the current sample.

Introduction

It is well established that attenuated psychotic symptoms occur in some individuals from the general population (1–3). In the absence of illness or the need for treatment, these milder forms of psychotic symptoms are referred to as psychotic experiences (PEs) (4). It has been suggested that clinical and subclinical expression of psychosis share genetic and/or environmental factors in their aetiology (4). Therefore, the study of the risk factors for PEs would ultimately contribute to the understanding of the aetiology of psychotic disorders.

In this context, both cannabis use (5–7) and childhood adversity (8–10) have been associated with an increased risk of developing psychosis in clinical and non-clinical samples. However, not everyone exposed to childhood adversity develops psychotic symptoms later in life. Similarly, only a minority of cannabis users develop psychotic symptoms suggesting the implication of other factors in this association (11).

In this regard, several studies have shown that the joint exposure to these two environmental factors, cannabis use and childhood adversity, may increase the likelihood of developing psychotic symptoms to a greater extent than the risk expected for each factor working independently (12–15).

These results are neurobiologically plausible, as both stressful experiences and delta-9-tetrahydrocannabinol (THC), the main psycho-active constituent of cannabis, have been found to increase dopaminergic signalling in the mesolimbic system (16), which is hypothesized to result in an increased risk of delusions and hallucinations (17). However, a recent study of a large sample drawn from the general population failed to replicate the interaction effect reported between cannabis and childhood trauma on the risk of developing psychotic symptoms (18). Individual differences in neurobiological susceptibility to the impact of childhood abuse and cannabis use might help to explain this failure to replicate. Indeed, recent evidence suggest that differential sensitivity to environmental stress occasioned by the Val158Met polymorphism of the catechol-*O*-methyltransferase (*COMT*) gene, probably in interaction with other factors, might be underlying psychosis risk (19–21).

The *COMT* gene encodes the enzyme catechol-*O*-methyltransferase, which plays an important role in the degradation of dopamine in the brain, and contains a functional polymorphism (*COMT*-Val158Met) that results in two common variants of the enzyme (Val and Met) (22). The Val variant is associated with increased *COMT* activity, which results in a combination of reduced dopamine neurotransmission in the prefrontal cortex and increased levels of dopamine in mesolimbic areas (23). Individuals carrying the Met/Met genotype have the lowest *COMT* activity and heterozygotes are considered to be of intermediate activity, as the two alleles are codominant (24).

In this regard, gene–environment interaction studies have shown that the Val158Met polymorphism of the catechol-*O*-methyltransferase (*COMT*) gene moderates i) the association between cannabis use and psychosis (25–27), although some studies failed to replicate the original findings from Caspi and colleagues [For review see: (28) and (11)] and ii) the association between childhood trauma and schizotypal traits (29). However, to our knowledge, no study to date has investigated whether the impact of the joint effect of exposure to childhood adversity and cannabis use on the subsequent development of PEs might be influenced by the *COMT*-Val158-Met polymorphism.

Aims of the study

This study aimed to investigate whether the impact of the childhood adversity and cannabis effects on the development of psychotic experiences varies according to *COMT*-Val158Met polymorphism genotypes.

Material and methods

Sample

The sample consisted of 533 individuals who were recruited from the campus of the Jaume I University in Castelló (Spain), as well as from university offices and community technical schools in the metropolitan area of Barcelona (Spain). Recruiting was conducted mainly through advertisements in the university offices and schools. All the participants were adults (mean age: 22.9 years; SD = 5.4) and 45.4% were males. At assessment, 77% of the participants were students. Further details of this sample can be found elsewhere (30, 31).

Exclusion criteria were the presence of any major medical illness affecting brain function, neurological conditions, current substance abuse (alcohol or any illicit drug), neurological conditions, history of head injury and personal history of psychiatric medical treatment. These areas were screened by means of a short interview designed ad hoc for this study. The design of the short interview was based on selected items from structured scales such as the *Structural Clinical Interview for*

DSM-IV disorders [SCID-I; (32)] and *Family Interview for Genetic Studies* [FIGS; (33)]. Specific questions about psychiatric assistance, psychotropic medication, hospital admissions and suicide attempts were asked to the participants.

All participants were of Spanish (Caucasian) ancestry, thereby reducing the possibility of confounding genetic differences by population stratification.

Ethical approval was obtained from local research ethics committees. All participants provided written informed consent before inclusion in the study. All procedures were carried out according to the Helsinki Declaration.

Measures

The Community Assessment of Psychic Experiences [CAPE; (34)] was used to assess positive and negative PEs in the sample. This self-report questionnaire measures the lifetime prevalence of PEs on a frequency scale ranging from 'never' to 'nearly always'. The positive dimension of the CAPE includes items mainly referring to subclinical expressions of positive psychotic symptoms (hallucinations and delusions) such as 'do you ever feel as if things in magazines or TV were written especially for you?'. Similarly, the negative dimension of CAPE includes items assessing subclinical expressions of negative psychotic symptoms such as alogia, avolition, anhedonia and lack of interest in social relationships. An example of item is 'do you ever feel that you experience few or no emotions at important events?'. The CAPE provides a total continuous score per dimension ranging from 20 to 80 in the positive dimension and from 14 to 56 in the negative dimension. To obtain the prevalence of PEs, CAPE scores were recoded as 0 (never, sometimes) and 1 (often, almost always). Self-report dimensions of psychotic experiences assessed by means of the CAPE have shown to be stable, reliable and valid (35); furthermore, this instrument has been validated in Spanish population (36).

Childhood abuse was assessed by the shortened version of the Childhood Trauma Questionnaire [CTQ; (37, 38)]. This questionnaire consists of 28 items that measure five types of childhood trauma: emotional abuse, physical abuse, sexual abuse, emotional neglect and physical neglect. In the current study, the subscales that assess abuse were combined to yield a total score of childhood abuse. Neglectful events were discarded, as only abusive events were shown to be associated with PEs in a previous study conducted in this sample (1). An example of an item on childhood abuse is 'people in my family hit me so hard that it left me with bruises or marks'. The score for each item ranges from 1 to 5 ('never true' – 'very often true'), depending on the extent to which individuals agree with the statement. The reliability and validity of the CTQ have been demonstrated (38). Childhood abuse was recoded as 0 (never true) and 1 (rarely true, sometimes true, often true and very often true) to calculate the prevalence. Reliability and validity of the CTQ have both been demonstrated (38).

Cannabis use was assessed with one question regarding the frequency of consumption: 'never', 'once', 'monthly', 'weekly' or 'daily' (this variable was then dichotomized into two categories: 'not exposed to cannabis': never, once; and 'exposed to cannabis': monthly, weekly, daily).

All analyses were corrected by gender, age, schizotypal personality and anxiety levels as in a previous study conducted in this sample (1). Schizotypal personality was measured with the Schizotypy Personality Questionnaire-Brief [SPQ-B; (39)]. Anxiety as a behavioural trait was assessed using the State-Trait Anxiety Inventory [STAI-T; (40)].

Laboratory methods

Genomic DNA was extracted from saliva samples using the Collection Kit BuccalAmp DNA extraction kit (Epicentre, ECOGEN, Barcelona, Spain). The SNP rs4680 (Val158Met) of the *COMT* gene was genotyped using Applied Byosystems (AB) TaqMan technology. The AB assay-on-demand service was used to order the probes. Genotype determinations were performed blind to the clinical condition. Randomized individuals were retested for their genotypes to confirm the pattern reproducibility.

Statistical analysis

Multiple linear regressions were conducted using STATA 10.0 for Windows. Separate models were tested for positive and negative PEs (continuous variables) as dependent variables. The independent variables for main and interaction effects were childhood abuse, cannabis use and the Val158Met polymorphism of the *COMT* gene (continuous childhood abuse, dichotomous cannabis use and three categories in the *COMT* gene: Val/Val, Val/Met and Met/Met). Data were analysed hierarchically. In the first step, the main effects of childhood abuse, cannabis use and the Val158Met polymorphism of the *COMT* gene on positive PEs were tested in the same model on positive and negative

PEs separately. Two-way interaction terms (childhood abuse*cannabis use; childhood abuse*-*COMT* gene and *COMT* gene*cannabis use) were added in a second step. In the third step, a three-way interaction term (childhood abuse*cannabis use**COMT* gene) was entered.

Age, gender, schizotypy and trait anxiety were included as covariates in all analyses.

Additional analyses were carried out using logistic regression analysis to investigate whether childhood abuse increased the risk of cannabis use and whether the *COMT*-Val158Met polymorphism was associated with cannabis use.

The log-likelihood ratio test was used to assess the difference between nested models. In our case, if a significant interaction effect was detected, the log-likelihood ratio test was used to examine whether the addition of the interaction term (either two-way or three-way) significantly improved the model fit compared to the main effects model.

A power analysis was performed using the QUANTO V.1.2 program (41). The sample of 419 individuals had 0.85 power to detect a gene–environment interaction effect, accounting for at least 2% of the variance of the studied outcome at an α level of 0.05. If a gene–environment interaction was detected, the effect size was calculated using eta squared (η^2). This parameter can be used to estimate the proportion of variance in the outcome that is accounted for by the predictor.

In addition, $P < 0.05$ was considered to indicate statistical significance, but we used a more stringent P-value, based on the Bonferroni correction, for the interactions tested. We conducted three tests (main effects, two-way interaction effects and three-way interaction effects) for two outcomes (positive and negative PEs). Therefore, for a Bonferroni correction on the P-values for interactions, we used $P = 0.05/6 = 0.0083$ as a threshold for significance.

Results

In the current sample, 40.7% of the individuals reported that often or almost always experienced at least one positive PE. For the negative dimension, 47.6% of the sample often or almost always experienced at least one negative PE. Of note, prevalences for some items addressing more severe psychotic experiences were lower. For example, 4.8% of the sample often or almost always felt that they were 'under the control of some force or power other than themselves'; similarly, 1.8% of the sample often or almost always 'heard voices talking to each other' [CAPE; (34)].

With regard to childhood abuse, 25.5% of the individuals were exposed to at least one abusive event during childhood. Nevertheless, regarding to specific and severe forms of childhood abuse and neglect, only the 9.2% and 10.3% of the sample reported being exposed to sexual abuse and physical neglect respectively.

For cannabis use, 29.1% of the sample used cannabis monthly, weekly or daily.

All the variables included in the model were available for 419 individuals from the total sample. In this final sample, the genotype frequencies for the Val158Met polymorphism of the *COMT* gene were as follows: Val/Val: 30.3% ($n = 127$); Val/Met: 48.0% ($n = 201$); and Met/Met: 21.7% ($n = 91$). These frequencies did not differ from others described in Caucasian individuals (25). The Hardy–Weinberg equilibrium was verified for the present population ($\chi^2 = 0.47$; d$f = 2$; $P = 0.49$).

A main effect of childhood abuse was found in both positive ($\beta = 0.09$; SE = 0.04; 95% CI .01–0.17; $P = 0.047$) and negative PEs ($\beta = 0.11$; SE = 0.05; 95% CI .01–0.21; $P = 0.038$). Cannabis use showed a main effect on negative PEs ($\beta = 0.88$; SE = 0.44; 95% CI .01–1.75; $P = 0.047$) but not on positive PEs. However, these main effects did not remain significant after correcting for multiple testing. No main effect was found for the Val158Met polymorphism of the *COMT* gene on either dimension of PEs.

None of the two-way interactions tested (childhood abuse*cannabis use; childhood abuse*-*COMT* gene or cannabis use**COMT* gene) were significant.

However, a significant three-way interaction among childhood abuse, cannabis use and the *COMT* gene was found in positive PEs [$\beta = -0.30$; SE = 0.11; 95% CI (−0.51)–(−0.09); $P = 0.006$] (Table 1; Fig. 1). This result was significant even after correction for multiple testing. It accounted for 2% of the variance of positive PEs ($\eta^2 = 0.2$).

In individuals exposed to childhood abuse who used cannabis, positive PEs score increased as a function of the Val allele dose of the *COMT* gene. However, among individuals exposed to childhood abuse who did not use cannabis, the positive PEs score increased as a function of the Met allele copies of the *COMT* gene. When individuals were exposed to low rates of childhood abuse, cannabis use and the Val158Met polymorphism of the *COMT* gene had a negligible effect on the presence of positive PEs scores.

The log-likelihood ratio test indicated that addition of the three-way interaction term in the third step resulted in a statistically significant

Table 1. 1) Main effects, 2) two-way interaction effects and 3) three-way interaction effects of childhood abuse, cannabis use and the *COMT* Val158Met polymorphism are presented for positive psychotic experiences (PEs) and negative PEs. All the models were corrected by age, gender, schizotypal personality and trait anxiety. Adjusted R^2 values (Adj-R^2) are presented for each step for positive and negative PEs. Significant results are indicated in bold

	Positive PEs			Negative PEs		
	β	SE	*P*	β	SE	*P*
1) Main Effects						
Childhood abuse	**0.088**	**0.044**	**0.047***	**0.107**	**0.051**	**0.038***
Cannabis use	0.378	0.384	0.325	**0.883**	**0.443**	**0.047***
COMT	0.148	0.241	0.541	−0.157	0.278	0.573
2) Two-way interaction effects						
Childhood abuse* Cannabis use	0.058	0.089	0.516	−0.063	0.103	0.539
Childhood abuse**COMT*	0.098	0.053	0.065	0.068	0.061	0.269
Cannabis use**COMT*	−0.452	0.519	0.384	−0.231	0.602	0.702
3) Three-way interaction effects						
Childhood abuse* Cannabis use**COMT*	**−0.303**	**0.110**	**0.006***	−0.156	0.129	0.228

Positive PEs: i) Adj-R^2 = 0.29, ii) Adj-R^2 = 0.29 and iii) Adj-R^2 = 0.30.
Negative PEs: i) Adj-R^2 = 0.33, ii) Adj-R^2 = 0.33 and iii) Adj-R^2 = 0.33.
β, regression coefficient; SE, standard error.
*$P < 0.05$.

improvement in model fit compared to the main effects (χ^2 = 12.7; d*f* = 2; *P* = 0.013).

Additional logistic regression analyses revealed that neither childhood abuse (OR = 1.01; 95% CI .96–1.07; *P* = 0.671) nor the COMT-Val158Met polymorphism (OR = 1.19; 95% CI .71–1.98; *P* = 0.513) was associated with cannabis use.

Discussion

Rates for PEs and childhood trauma in the current sample were consistent with previous reports in European and North American samples (4, 37, 42) [further details can be found elsewhere (1, 30)]. Also, the rate of individuals using cannabis (monthly, weekly or daily) was 29.1%, which is similar to the rates reported in other European countries (43).

As previously shown in this sample, childhood abuse was associated with both positive and negative PEs (1). These findings support the role of childhood abuse in the development of PEs in the general population, as reported in previous research (8–10). Furthermore, the fact that cannabis use did not show a main effect on positive PEs in the current study may be related to the inclusion of childhood abuse in the model [in univariate analyses cannabis was significantly associated with positive PEs (β = 1.20; SE = 0.44; *P* = 0.007)]. As previous studies have suggested, explorations of the association between cannabis and psychosis need to consider the effects of childhood trauma as an important potential effect modifier (12, 14). Nevertheless, both childhood abuse and cannabis use were associated independently with negative PEs. The association between cannabis and negative PEs has been reported previously (7, 44).

The term gene–environment correlation refers to the fact that exposure to an environmental risk factor is not random but is influenced by the individual's genotype. Similarly, environment–

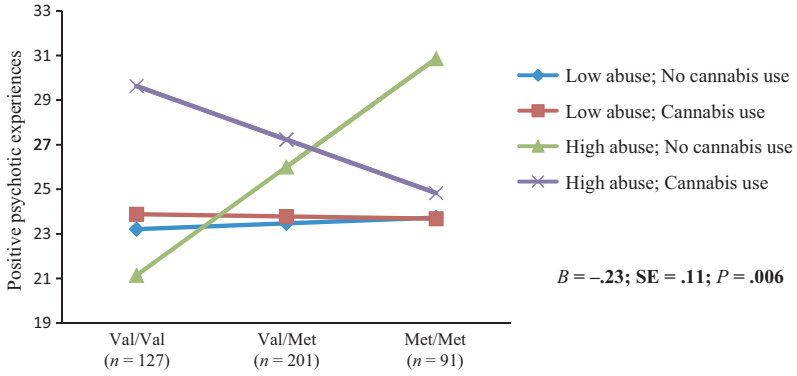

Fig. 1. Graphic representation of the interaction effect among childhood abuse, cannabis use and the Val158Met polymorphism of the COMT gene on positive psychotic experiences (PEs) corrected for age, gender, schizotypal personality and trait anxiety. Cannabis use and the Val158Met polymorphism of the *COMT* gene have a negligible effect on positive PEs when individuals are not exposed to childhood abuse or exposed to low rates of such events (red and blue lines). The use of cannabis in individuals exposed to childhood abuse has opposite effects depending on their genotype (purple and green lines). Positive PEs score increases as a function of the number of copies of the Met allele of the *COMT* gene in those individuals exposed to childhood abuse who do not use cannabis (green line). Thus, Met carriers seem to be especially vulnerable to the effect of childhood abuse on their later development of PEs and cannabis use may have a protective effect. However, in individuals exposed to childhood abuse who use cannabis, a positive PEs score increases as a function of the Val allele copies of the *COMT* gene (purple line).

environment correlation occurs when the exposure to a given environmental factor is influenced by the previous exposure to another environmental factor (45, 46). With regard to these mechanisms, additional analyses enabled us to rule out the possibility that childhood abuse increased the likelihood of using cannabis (environment–environment correlation). A gene–environment correlation can also be discarded, as *COMT* genotypes were not associated with cannabis use.

In accordance with recent evidence, we did not find an interaction between the effect of childhood abuse and cannabis use on PEs (18). However, we believe that this may be related to the inclusion of *COMT* genotypes in the analyses, as a significant gene–environment–environment interaction effect was detected. This finding is consistent with previous studies indicating that environmental exposures, in interaction with genetic factors, may induce psychological or physiological alterations that can be traced to a final common pathway of altered dopamine neurotransmission. This pathway facilitates the onset and persistence of psychotic symptoms (47).

Therefore, our main findings suggest that the psychosis-inducing effects of childhood abuse and cannabis use are moderated by the Val158Met polymorphism of the *COMT* gene, which supports a gene–environment–environment interaction effect.

This three-way interaction effect indicated that positive PEs showed almost no variation for individuals exposed to low rates of childhood abuse, regardless of their cannabis use frequency or their genotype for the Val158Met polymorphism of the *COMT* gene. However, among individuals exposed to childhood abuse, cannabis use only increased the likelihood of reporting positive PEs if individuals were carriers of the Val allele of the *COMT* gene. Furthermore, Met carriers exposed to childhood abuse were more likely to report positive PEs without cannabis use. Thus, our findings suggest that use of cannabis after exposure to childhood abuse may have opposite effects on the development of positive PEs depending on the *COMT* genotypes.

Although the effect size of this finding is modest (2% of the variance of positive PEs) and requires replication, these results may partially account for previous discrepancies found when examining the possible moderator role of *COMT* genotypes in the association between cannabis and clinical and non-clinical expression of psychosis. For example, as abovementioned, Kuepper (18) and colleagues failed to replicate the interaction shown between childhood trauma and cannabis use (12–15). This discrepancy could be owing to sampling variation or different time of follow-up (11) but it might be also possible that *COMT* genotypes play a role in this association considering our results. With regard to the interaction between cannabis and *COMT*-Val158Met polymorphism, several studies examining different aspects of the psychosis phenotype (psychotic symptoms, psychotic disorders, age of onset or duration of untreated psychosis) have yielded inconsistent results (11, 25–27). Also, a failure to find such interaction effect has been reported (48). As our findings suggest that psychosis-inducing effects of cannabis have opposite effects depending on the *COMT* genotypes but only among those exposed to childhood abuse; future studies testing this gene–environment interaction effect may consider including childhood trauma in this association if the measure is available.

The fact that exposure to both childhood abuse and cannabis was associated with higher scores of positive PEs in Val carriers may be explained by sensitization involving dopaminergic signalling. Evidence from animal studies suggests a possible interaction (exposure to one factor increases sensibility to the effects of the other factor) between stress and THC. Rats living under normal conditions (i.e. access to water and food), that were exposed to THC, showed only minor behavioural changes and no change in dopaminergic transmission (49). In contrast, under stressful conditions (i.e. isolation and food deprivation), THC administration had marked behavioural consequences and was associated with a significant increase in dopamine uptake (49). Similarly, it has been shown in humans that the psychosis-inducing effect of cannabis may be stronger in subjects exposed to early stress (15). Our results indicate that variability in the *COMT* gene confers different neurobiological vulnerability to cannabis use in the risk of developing PEs. In accordance with previous studies, Val carriers are more vulnerable to the psychosis-inducing effects of cannabis than Met/Met individuals (25–27), but only when exposed to childhood abuse. Consistent with previous studies indicating that Met carriers were more vulnerable to stress than carriers of the Val/Val genotype (21), Met carriers were vulnerable to the psychosis-inducing effects of childhood abuse, but only when they did not use cannabis. Previous evidence indicates that the risk of psychosis did not increase in Met carriers of the *COMT* gene who used cannabis (25). However, in the current study, individuals exposed to childhood abuse who are homozygous for the Met allele appeared to be able to use cannabis without any increase in risk of developing PEs. It might be possible that cannabis may exert some

benefit effect in certain individuals. Indeed, it has been suggested that cannabis use alleviate the stress associated with childhood traumatic events and the experience of PEs (50). However, such conclusions cannot be drawn from our results, thus this result needs replication and must be interpreted with caution.

In this regard, although the findings of gene–environment interaction studies have been exciting, there is increasing concern about the reliability and contribution of such results to the understanding of complex traits such as PEs (51). Dismissal of gene–environment interaction studies arises mainly as a result of the failure to replicate (52, 53). As there are powerful reasons to expect that gene–environment interaction effects are involved in the aetiology of complex traits and psychiatric disorders (54), the debate is more focused on the reliability and clinical relevance of such findings (51). To prevent false positive results or statistically significant results that may not represent true insights, the current study was developed with an a priori hypothesis that guided the choice of the gene, the polymorphism and the environmental risk factors that were explored. Moreover, as abovementioned, power analyses are specified and correction for multiple testing was applied. Furthermore, the use of cannabis after exposure to childhood abuse had opposite effects on positive PEs depending on the *COMT* genotypes. This pattern of results coincides with the epidemiological definition of *qualitative interaction*. A qualitative interaction refers to an inverse or crossover effect from a given variable (e.g. cannabis exposure) according to differences in another variable (e.g. *COMT* genotypes) (51, 55). Although these type of interactions have only rarely been observed in medicine, the implications of qualitative or crossover interactions are believed to have a clear biological meaning and be more helpful than the ones derived from quantitative or non-crossover interactions (51).

The results of this study should be interpreted in the context of its limitations. First, we used a relatively small sample size to detect a three-way interaction, replication in larger samples with higher statistical power are needed to confirm these findings. Second, the characteristics of the sample – young age, educational level, no history of psychiatric treatment – need to be considered when generalizing the present findings. Also, as substance abuse constituted an exclusion criterion, heavy cannabis users, who experience problems in their daily life because of their cannabis consumption, were not included in the study. Therefore, although the sample is drawn from the general population, the representativeness of the sample is limited by these characteristics. Third, no main genetic effects for *COMT*-Val156Met polymorphism on PEs were found in the current study. As the power to detect interactions is typically lower than the power to detect main effects (56), well-powered studies should be able to detect statistically significant main genetic effects unless a qualitative interaction effect is detected as is the case for this study. In qualitative interactions, main effects are cancelled out; therefore, the lack of significant main genetic effects in this study should not compromise the reliability of the reported results. Fourth, the cross-sectional nature of the design does not allow causal inference. Fifth, childhood abuse was measured retrospectively, which may constitute an inherent source of bias. Furthermore, this instrument has not been yet validated in Spanish population. That said, the Childhood Trauma Questionnaire has been validated in several European countries including Dutch and Swedish populations (57, 58) and is considered a reliable measure of childhood adversity (38). Finally, frequency of cannabis use was dichotomously defined in this study, and other parameters that have been related to the expression of psychotic symptoms such as onset, duration or potency of cannabis consumed (7, 34, 59, 60), were not specified. Furthermore, biological samples for confirming drug use by means of laboratory techniques were not available in this study.

Of note, we would like to stress the fact that consistent evidence indicates that cannabis may induce psychosis and/or worse psychotic symptoms (5–7). Therefore, public health message about the potential risk of cannabis use should not be modified by results indicating that its use may not be harmful for a subgroup of the population.

To conclude, our findings suggest that the psychosis-inducing effects of childhood abuse and cannabis use are moderated by the Val158Met polymorphism of the *COMT* gene, which supports a gene–environment–environment interaction effect. Cannabis use after exposure to childhood abuse may have opposite effects on the risk of PEs development, depending on the *COMT* genotypes.

Acknowledgements

We thank all participants of the study. This work was supported by research projects funded by the Ministry of Science and Innovation (grant numbers SAF2008-05674-C03-00 and 03; PNSD2008-I090; PNSD2009-I019), the Institute of Health Carlos III, CIBER of Mental Health (CIBERSAM), the Comissionat per a Universitats i Recerca, DIUE, Generalitat de Catalunya (grant number 2009SGR827) and Fundació Caixa Castelló-Bancaixa (grant numbers P1·1B2010-40 and

P1·1B2011-47). Silvia Alemany would like to thank the Institute of Health Carlos III for her PhD grant (FI00272).

Declaration of interest

None of the authors have anything to declare.

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

Dr. Lourdes Fañanás Saura, Associate Professor (Profesora Titular) at the Department of Animal Biology of the Faculty of Biology, University of Barcelona and supervisor of the present doctoral thesis by Silvia Alemany, hereby certifies that the participation of the PhD applicant in the article "Psychosis-inducing effects of cannabis are related to both childhood abuse and COMT genotypes" included the following tasks:

- Participation in the conception and design of the study
- Analysis and interpretation of data
- First drafting of the manuscript
- Critical revision of the arcticle for intellectual content

Dr. Lourdes Fañanás

Barcelona, February 11th 2013

7.4. Childhood adversity and psychosis: examining whether the association is due to genetic confounding using a monozygotic twin difference approach. Alemany S, Goldberg X, Van Winkel R, Gastó C, Peralta V, Fañanás L. *European Psychiatry*, 2012 (In press)

Resumen en Castellano

Adversidad infantile y psicosis: Eaminando si la relación se debe a factores genéticos confusores aplicando un diseño de diferencias en gemelos monozigóticos.

Alemany S, Goldberg X, Van Winkel R, Gastó C, Peralta V, Fañanás L.

European Psychiatry, 2012 (En prensa)

El própisto del presente estudio fue examinar en una muestra de gemelos provenientes de la población general (n=230): i) si el maltrato infantil se asocia con el desarrollo de EPs positivas y negativas, ii) en qué medida los gemelos monozigóticos (MZ) son similares en cuanto a la exposición a maltrato infantil y a la presencia de EPS y iii) si las diferencias en la exposición a maltrato infantil se asocian con diferencias en la expresión de EPs en una submuestra de gemelos MZ (n=85 pares de gemelos) utilizando un diseño de diferencias en gemelos MZ.

Se observó un efecto ambiental significativo por parte del maltrato infantil en el desarrollo de EPs positivas y negativas. Este hallazgo sugiere que, si bien algunas personas pueden ser genéticamente vulnerables al impacto del maltrato infantil como se mencionaba anteriormente, los eventos adversos tempranos, en ciertas circumstancias, pueden contribuir al desarrollo de EPs positivas independientemente de la carga genética del invididuo.

European Psychiatry xxx (2012) xxx–xxx

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Original article

Childhood adversity and psychosis: Examining whether the association is due to genetic confounding using a monozygotic twin differences approach

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ARTICLE INFO

Article history:
Received 2 March 2012
Accepted 4 March 2012
Available online xxx

Keywords:
Schizophrenia and psychosis
Genetics
Stress
Child abuse

ABSTRACT

Purpose: To test whether the association between childhood adversity and positive and negative psychotic experiences is due to genetic confounding.
Method: Childhood adversity and psychotic experiences were assessed in an ongoing sample of 226 twins from the general population. A monozygotic (MZ) twin differences approach was used to assess possible genetic confounding.
Results: In the whole sample, childhood adversity was significantly associated with positive ($\beta = 45$; SE = 0.16; $P = 0.008$) and negative psychotic experiences ($\beta = 0.77$; SE = 0.18; $P < 0.01$). Within-pair MZ twin differences in exposure to childhood adversity were significantly associated with differences in positive ($\beta = 71$; SE = 0.29; $P = 0.016$) and negative psychotic experiences ($\beta = 98$; SE = 0.38; $P = 0.014$) in a subsample of 85 MZ twin pairs.
Conclusions: Individuals exposed to childhood adversity are more likely to report psychotic experiences. Furthermore, our findings indicate that this association is not due to genetic confounding.

1. Introduction

A growing body of research indicates that attenuated psychotic experiences are present in a substantial proportion of healthy individuals [26,44,6]. This evidence supports the conceptualization of psychosis as a continous trait, the distribution of which extends into the general population [25,42]. In the absence of illness and need of treatment, these milder forms of psychotic symptoms are referred to as psychotic experiences [25]. The study of the risk factors underlying the expression of psychotic experiences can greatly contribute to the understanding of psychotic disorders because it has been shown that: psychotic experiences precede the onset of psychosis, thus psychotic experiences can help to identify subjects at risk [26,14] and; clinical and subclinical psychotic symptoms are likely to involve common risk factors in their etiology [26,44,25]. In this context, childhood adversity constitutes an environmental risk factor which has been frequently related to the expression of both clinical [7,8] and subclinical psychotic symptoms or psychotic experiences [27,38].

Interestingly, despite the efforts made in the genetics of psychotic disorders in the last decades, a growing body of research points toward a contribution of environmental factors, including childhood adversity, to their etiology [45,43,49]. Furthermore, Van Os et al. [45] have recently pointed out that genetic factors involved in these disorders are likely to operate via environmental factors by making individuals more sensitive (gene-environment interaction) or prone (gene-environment correlation) to certain environments [41]. These mechanisms of gene-environment interplay may underlie previously reported associations between environmental risk factors such as childhood adversity and psychotic outcomes. For instance, a twin-based study suggested that higher level of genetic risk associated with psychosis may moderate the impact of childhood adversity on the risk of adult psychotic symptom formation [31]. Furthermore, two recent studies provide evidence for gene-environment interaction effects in the association between psychosocial stress factors and

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doi:10.1016/j.eurpsy.2012.03.001

psychotic experiences in samples drawn from the general population [1,37]. However, it would be also important to clarify whether environmental factors *per se* have an impact on the expression of psychosis. So far associations between environmental risk factors and psychotic outcomes have been explored without controlling for genetic confounding [45], that is, individuals at increased genetic risk for psychosis may be more vulnerable to be victimised because of traits associated with psychosis, such as cognitive impairments, impaired social functioning, oddness or others. To the best of our knowledge, only one previous study provided evidence for an association between childhood trauma and risk to develop psychotic symptoms after controlling for genetic liability for psychosis [4]. Therefore, although childhood adversity as an environmental risk factor for psychosis has been extensively studied and the neurobiological impact of early adverse events in the brain is well-established [20,30,46], whether the association between childhood adversity and psychosis is likely causal or merely reflects gene-environment correlation remains to be examined.

In this context, twin designs offer a unique opportunity to disentangle genetic and environmental effects on complex phenotypes such as psychotic experiences [9]. Specifically, the monozygotic (MZ) twin differences approach has been referred to as a strong test of the unique environmental experiences that make family members different from each other (also called non-shared environment) independently of genetics [12,32,47]. Since MZ twins are, nearly always, identical at the DNA sequence level [9]; phenotypic differences observed between MZ twins must be explained by differential exposure to environmental factors. In other words, if differences in the expression of subclinical psychotic experiences in MZ twins are associated with exposure to childhood adversity, this would provide strong evidence that the observed association between childhood adversity and psychosis is not due to genetic confounding.

Therefore, the present study aimed to examine:

- whether childhood adversity was associated with positive and negative psychotic experiences in a twin sample from the general population;
- to what extent MZ twins were similar for their exposure to childhood adversity and presence of psychotic experiences;
- whether differences in exposure to childhood adversity were associated with differences in the expression of psychotic experiences in a subsample of MZ twins.

2. Subjects and methods

2.1. Participants

The sample consisted in 230 Spanish adult twins (115 twin pairs) from the general population, including 86 MZ twin pairs. The mean age was 34 years (SD = 13.28) and 34.2% of the subjects were males. Recruiting was conducted from the University of Barcelona Twin Register and media advertisements. The University of Barcelona Twin Register consists of a list of twin pairs from Catalonia who gave permission to be contacted for research purposes. Identified twin pairs were first contacted by telephone and invited to participate. A battery of psychological and neurocognitive tests was administered to the twins by trained psychologists (S.A. and X.G.). Of note, twins were requested to fill self-report questionnaires in separate rooms in order to avoid sharing of responses between twins and to ensure confidentiality. Twins were interviewed face-to-face for personal medical records (S.A. and X.G.). Also, lifetime DSM-IV-TR [2] Axis-I diagnosis was assessed in a face-to-face interview with a clinical trained clinical psychologist (X.G.) using the Structural Clinical Interview for DSM-IV disorders (SCID-I; [17]). Exclusion criteria applied were age under 17 and over 65 years, a medical history of neurological disturbance, presence of sensory or motor alterations and current substance misuse or dependence. All subjects were from Caucasian origin. Written informed consent was obtained from all participants after a detailed description of the study aims and design, approved by the local Ethics Committee.

2.2. Measures

2.2.1. Childhood adversity

To assess childhood adversity, we used an adapted version of the Adverse Childhood Experiences Questionnaire (ACEQ; [16]). This questionnaire assesses the exposure to events of childhood abuse, childhood neglect and household dysfunction. Three items regarding bullying and parental loss were added to the original version. Our adapted version consists on 19 items. Each item assesses the exposure to a particular adverse event. Participants are requested to answer "yes" or "no" to each item which indicates whether they were exposed or not to each adverse event. Items are detailed in the Appendix. All the positive answers are added up to obtain a total childhood adversity score which ranges from 0 to 19.

2.2.2. Psychotic experiences

The Community Assessment of Psychic Experiences (CAPE; [40]) was used to assess positive and negative psychotic experiences. This validated self-report questionnaire measures the lifetime prevalence of psychotic experiences in a frequency scale ranging from "never" to "nearly always". The positive dimension of the CAPE includes 20 items mainly referring to hallucinations and delusions such as "do you ever feel as if things in magazines or TV were written especially for you?". The negative dimension, which consists of 14 items, mainly assesses alogia, avolition, anhedonia and lack of interest in social relationships. An example of item is "do you ever feel that you experience few or no emotions at important events?". Participants are asked to indicate the frequency of ocurrence of psychotic experiences on a four-point scale (ranging from "never" to "nearly always"). The instrument provides a total continuous score per dimension ranging from 20 to 80 in the positive dimension and from 14 to 56 in the negative dimension.

2.2.3. Zygosity

Zygosity was established genotyping 16 loci: 15 short tandem repeat (STR) loci and amelogenin, the gender determining marker. Genomic DNA was extracted from peripheral blood cells using the Real Extraction DNA Kit (Durviz S.L.U., Valencia, Spain). The PowerPlex® 16 System (Promega Corporation) allowed the co-amplification and three-color detection of 16 loci. Twins with only one divergent allele were genotyped a second time to limit the scope for genotyping error. Identity on all the markers can be used to assign monozygosity with greater than 99% accuracy [33].

2.2.4. Further characteristics of the sample

As data derived from this twin sample has not been published yet, further characteristics of the sample are reported for descriptive purposes. Apart from age and sex, sociodemographic characteristics include estimated Intelligence Quotient (IQ) assessed by four subtests (Block design, matrix reasoning, information and vocabulary) from the Wechsler Adult Intelligence Scale (WAIS-III; [48,36]); level of education (elementary school, high school and university), birth of place ("urban" when the twins were born in the city of Barcelona and "non-urban" when they were born at other Spanish towns with lower number of habitants compared to Barcelona city) and socioeconomic status (SES). A continous score representing SES was obtained using four-factor

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index of social status developed by Hollingshead [15,23]. SES scores ranging from 8 to 30 were defined as "Low SES" and scores between 31 and 66 were classified as "Average SES" [13]. Of note, some of these measures were not available for all subjects.

2.3. Statistical analysis

Data was analysed in three phases. First, multiple regression models were conducted to map the association between childhood adversity and positive and negative psychotic experiences. In these models, childhood adversity was the variable of interest, sex and age were included as covariates and positive and negative psychotic experiences were used as the outcome measures. Separate models were conducted for each outcome measure. The non-independence of clustered twin data was corrected for by using tests based on the sandwhich or Huber/White variance estimator [51]. In these analyses, the individual was the unit of analysis.

Second, MZ intrapair correlations were calculated for childhood adversity and positive and negative psychotic experiences. These analyses let us confirm that MZ twins differed in their exposure to childhood adversity and their scores for positive and negative psychotic experiences. The proportion of the variance of the phenotype which can be directly attributable to unique environment (which includes measurement error) can be obtained by this formula: 1 – rMZ, where r represents the within-pair correlation [34].

Third, associations between intrapair differences in childhood adversity, positive and negative psychotic experiences were analysed by linear regression analysis. Intrapair scores were calculated for childhood adversity, positive and negative psychotic experiences by substracting the score of the Twin 2 from the score of Twin 1 (Twin 1–Twin 2). Twins were randomly assigned to be 1 or 2. Associations between intrapair differences in CA and intrapair differences in positive and negative PEs scores were conducted in a subsample of 85 MZ twin pairs. Because intrapair analyses in MZ twins fully control for genetic influences, any association between the abovementioned variables would be attributable to environmental factors [32,35] and thus, reject the hypothesis that the association is due to genetic confounding. In the last two analyses each MZ twin pair was the unit of analysis.

Statistical analyses were carried out in STATA 10.0 [39] following the procedures described in Carlin et al. [11].

3. Results

Most of the sample was composed of females (66.1%), the average age was 33.8 years (SD = 13.3) and more than half of the sample had completed university educational level (59.3%). Average IQ scores were within the normal range for non-clinical samples (103.3; SD = 11.5). Around half of the sample was born in non-urban areas (58.7%). Most of the twins were of average SES level (65.8%). The MZ twin subsample showed very similar sociodemographic characteristics compared to the whole twin sample (Table 1).

In the whole sample, positive CAPE score ranged from 13 to 39 (mean = 25.3; SD = 4.0) and negative CAPE score ranged from 12 to 49 (Mean = 22.1; SD = 4.8). CAPE scores were very similar in the MZ twins subsample (CAPE positive: Mean = 25.7; SD = 4.24; CAPE Negative: Mean = 22.5; SD = 5.05). In order to obtain the prevalence of psychotic experiences in the current sample, CAPE scores were recoded to 0 (never, sometimes) and 1 (often, almost always). Specifically, 37.1 to 38.8% of the sample often, or almost always, experienced at least one positive or negative psychotic experience. Similarly, in the MZ twin subsample, 41% of the sample often, or almost always, experienced at least one positive or negative psychotic experience.

With regard to childhood adversity (CA) score, the mean was 2.0 (SD = 2.2) and it ranged from 0 to 14. In the MZ twin subsample, CA score also ranged from 0 to 14 and the mean was 2.0 (SD = 2.4). In the whole sample, 26.3% of the individuals did not experience any adverse childhood event and 26.3% reported one adverse childhood event; the rest of the sample reported two or more adverse childhood events. Similarly, in the MZ twin subsample, 27.3% of the individuals did not report any adverse childhood event, 26% reported one adverse childhood event and the rest reported two or more adverse childhood events.

First, regarding the association between CA and psychotic experiences, analyses based on the whole sample showed that CA was significantly associated with both positive ($\beta = 0.45$; SE = 0.16; $P = 0.008$) and negative psychotic experiences ($\beta = 0.77$; SE = 0.18; $P < 0.01$) (Table 1). These analyses were adjusted for clustering.

Second, we conducted within-pair correlations to index the similarity for the outcome measures and the variable of interest between twin 1 and twin 2 in the subsample of MZ twin pairs. The within-pair correlations for positive and negative psychotic experiences were $r = 0.48$ ($P < 0.01$) and $r = 0.44$ ($P < 0.01$) respectively. Therefore, around 52 to 56% of the variance of CAPE could be attributed to unique environmental factors not shared by twins. In regard to CA score, the within-pair correlation was $r = 0.79$ ($P < 0.01$). Thus, although most of the childhood adverse events experienced by the twins are common, some of them are specific (21% of the variance of childhood adversity).

Table 1
Sociodemographic characteristics of the final sample included in the analysis by zygosity: 85 MZ twin pairs ($n = 170$); 28 DZ twin pairs ($n = 56$) and the whole sample ($n = 226$). Number of individuals varies in function of the measure.

	MZ-twins subsample	DZ-twins subsample	Whole sample
Male sex	31.8% ($n = 27$)	40.7% ($n = 22$)	34.5% ($n = 78$)
Age in years, mean (SD)	33.7 (12.8) ($n = 170$)	32.5 (11.9) ($n = 56$)	33.7 (12.7) ($n = 226$)
Education level			
Elementary school	16.9% ($n = 28$)	10.4% ($n = 5$)	15.3% ($n = 33$)
High school	31.3% ($n = 52$)	12.5% ($n = 6$)	25.5% ($n = 55$)
University	51.8% ($n = 86$)	77.1% ($n = 37$)	59.3% ($n = 128$)
IQ, mean (SD)	102.9 (12.5) ($n = 166$)	104.2 (11.6) ($n = 49$)	103.3 (11.5) ($n = 218$)
SES			
Low	39.0% ($n = 46$)	20.5% ($n = 8$)	34.2% ($n = 53$)
Average	61.0% ($n = 72$)	79.5% ($n = 31$)	65.8% ($n = 102$)
Birth place			
Urban	41.2% ($n = 70$)	46.4% ($n = 26$)	42% ($n = 95$)
Rural	58.8% ($n = 100$)	53.6% ($n = 30$)	58% ($n = 131$)

SD: standard deviation; IQ: Intelligence Quotient; SES: sociodemographic status.

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Table 2
In the left side of the table, association between Childhood Adversity (CA) score and positive and negative psychotic experiences (PEs) in the whole sample ($n = 226$) adjusting for the non-independence nature of the data. In the right side of the table, association between intrapair scores (twin 1- twin 2) for CA and intrapair scores for positive and negative PEs in a subsample of MZ twin pairs ($n = 85$ pairs). All analyses were adjusted by sex and age.

	Positive PEs				Intrapair positive PEs		
	β	SE	*P*		β	SE	*P*
CA Score	0.45	0.16	0.008*	Intrapair CA Score	0.71	0.29	0.016*
	Negative PEs				Intrapair negative PEs		
	β	SE	*P*		β	SE	*P*
CA Score	0.77	0.18	0.000**	Intrapair CA Score	0.95	0.38	0.014*

β: unstandardized coefficient; SE: standard error; *$P < 0.05$; **$P < 0.01$.

Finally, associations between intrapair differences in childhood adversity, positive and negative psychotic experiences were analysed. The mean score of within-pair differences for childhood adversity, positive and negative psychotic experiences was 0.13 (SD = 1.6; range = –4 – 4), –0.17 (SD = 4.1; range = –15 – 10) and–0.41 (SD = 5.3; range = –30–12) respectively. Regression analyses using within-pair MZ differences showed that MZ differential exposure to childhood adversity was significantly related to phenotypic differences in both positive (β = 66; SE = 0.28; $P = 0.026$) and negative dimensions of psychotic experiences (β = 93; SE = 0.37; $P = 0.014$) (Table 2).

4. Discussion

To our knowledge, this is the first study adding evidence to the growing literature on the relationship between childhood adversity and psychotic experiences using an MZ-twin differences approach. The MZ-twin differences design ensures that pure unique or non-shared environmental effects, rather than gene-environment interaction or evocative gene-environment correlation, are quantified [47].

Firstly, the present twin sample from the general population showed similar means and prevalences of psychotic experiences to those reported previously in singleton samples [6,1]. A recent study demonstrated a significant impact of the type of instrument used on the rate of psychotic experiences that was found [29]. Therefore, it is worth mentioning that we are comparing our means and prevalences obtained for CAPE scores with studies which used the same instrument [6,1].

With respect to childhood adversity, in a large community-based study using the original version of the ACE questionnaire, 36.1% of the sample reported 0 adverse childhood experiences, 26.0% reported one adverse childhood experience and the rest reported two or more adverse childhood experiences [3]. These prevalences are very similar to those reported in the current sample.

Secondly, in agreement with previous studies [27,38,24,50], our findings provide support for the association between childhood adversity and psychotic experiences in the general population.

Thirdly, the fact that within-pair MZ correlations were not equal to 1 for any of the measured phenotypes indicated that we could test for unique environmental effects of childhood adversity on psychotic experiences.

Fourthly, regarding the primary goal of the current study, within-pair MZ differences in exposure to childhood adversity were significantly related to phenotypic differences for both positive and negative dimensions of psychotic experiences. Because the members of the MZ twin pair are genetically identical to each other, any environmental effects operate upon genotype effects that do not differ between the members of the MZ twin pair [47]. These findings indicate that the association between childhood adversity and psychosis cannot be solely attributed to genetic confounding and thus, that childhood adversity may represent a true risk factor for the development of psychotic experiences.

These results are in agreement with those reported by Arseneault et al. [4], who reported that childhood adversity may constitute a risk factor for the development of psychotic symptoms independently of the genetic background of the individual.

Proposed neurobiological and psychological mechanisms of risk underlying this association also add plausibility to these findings. Converging evidence from neurobiology and epidemiology suggests that early adverse events cause enduring brain dysfunction [20,10,21]. Persistent exposure or impact of stressors in the developing brain has been proposed to lead to chronically heightened stress-induced glucocorticoid release which, in turn, may impact on the hypothalamic-pituitary-adrenal (HPA) axis. Dysregulation of the HPA axis has been suggested to contribute to the dopaminergic abnormalities that are generally thought to be involved in the expression of psychotic phenotype [46,28]. At the psychological level, exposure to early adversity may create, also, a cognitive vulnerability, characterized by a tendency to perceive the self as powerless and others as malevolent, which in combination with an externalizing attribution style may ultimately lead to paranoid interpretation of anomalous experiences [5,19]. These risk mechanisms could be moderated by genetic variants, making some individuals more sensitive to psychosocial stress factors than others [1,37].

The results of the present study should be interpreted in the context of its limitations. First, due to the limited sample size our findings require replication and have to be interpreted with caution. Also, most of the sample consists of women and this may limit generalization of our findings. However, the sample showed to be representative of the general population regarding the sociodemographic characteristics and the prevalences of the variables studied and all the analyses were adjusted by sex. Second, the cross-sectional nature of our design did not allow inference of causal associations. Third, the retrospective measure of CA may be influenced by recall bias. Nevertheless, it is worth it to mention that retrospective self-reports of childhood trauma are more likely to be an underestimation of the true prevalence of childhood maltreatment than an overestimation [22]. Fourth, it has been shown that the impact of childhood adversity on psychosis may depend on the type or frequency of such events [18]. However, there was insufficient power to investigate the impact of unique environmental effects of specific types of childhood adversity or reporting one versus reporting multiple childhood adverse events in the present sample of MZ twins.

Finally, as other studies using an MZ-twin differences design [12,47], we cannot rule out the possibility that some unmeasured non-genetic factor could have contributed to our findings.

Please cite this article in press as: Alemany S, et al. Childhood adversity and psychosis: Examining whether the association is due to genetic confounding using a monozygotic twin differences approach. European Psychiatry (2012), doi:10.1016/j.eurpsy.2012.03.001

Therefore, further research in larger samples is needed to better understand under which circumstances childhood adversity environmentally increases the risk or frequency of psychotic experiences.

5. Conclusion

Our findings shed new light regarding the role of childhood adversity as an environmental risk factor involved in the development of psychotic experiences. We found a significant environmental effect of childhood adversity on the development of positive and negative psychotic experiences using an MZ-twin differences approach, suggesting that the association cannot be solely attributed to genetic confounding. Therefore, although some individuals may be genetically vulnerable to the impact of childhood adversity [1], our findings indicate that childhood adversity can independently contribute to the development of psychotic experiences. Further research is needed to better understand under which circumstances childhood adversity environmentally increases the risk or frequency of psychotic experiences.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgements

We gratefully acknowledge the collaboration of the participants. We also thank Nadia Vilahur, Sergi Papiol, Mar Fatjó-Vilas, Bárbara Arias and Araceli Rosa for their contribution in sample collection. This study was supported by the Ministry of Science and Innovation (SAF2008-05674-C03-00; 02 and 03), the Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), European Twins Study Network on Schizophrenia Research Training Network (grant number EUTwinsS; MRTN-CT-2006-035987) and by the Comissionat per a Universitats i Recerca del DIUE of the Generalitat de Catalunya (2009SGR827). Goldberg X. was supported by a Marie Curie grant (grant number EUTwinsS; MRTN-CT-2006-035987). Alemany S. thanks the Institute of Health Carlos III for her PhD grant (FI00272).

Appendix. Childhood Adversity Questionnaire

While you were growing up, during your first 18 years of life:

1. Did a parent or other adult in the household swear at you, insult you, put you down or humiliate you?

2. Did a parent or other adult in the household act in a way that made you feel that you might be physically hurt?

3. Did a parent or other adult in the household push, slap or throw something at you?

4. Did a parent or other adult in the household hit you so hard that you had marks or were injured?

5. Have your mother or father ever left home for a long period of time for any reason?

6. Did a parent or other adult in the household touch your body or fondle you in a sexual way?

7. Did a parent or other adult in the household attempt or had any sexual activity with you (oral, anal or vaginal)?

8. Did you often or very often feel that no one in your family loved you or thought you were special or important?

9. Did you often or very often feel that your family look out for each other, feel close to each other, or support each other?

10. Did you often or very often feel that you didn't have enough to eat, had to wear dirty clothes, and had no one to protect you?

11. Did you often or very often feel that your parents were too drunk or high to take you to the doctor if you needed it?

12. Were your parents ever separated or divorced?

13. Was your mother or stepmother ever pushed, grabbed, slapped, or had something thrown at her?

14. Was your mother or stepmother ever kicked, bitten, hit with a fist, or hit with something hard?

15. Did you live with anyone who was a problem drinker or alcoholic or who used to use street drugs?

16. Was a household member depressed or mentally ill, or did a household member attempt to suicide?

17. Did a household member go to prison?

18. At the school, did one or more peers make fun of you, call you by nicknames or bully you?

19. At the school, did one or more peers insult, threat, steal or hit you?

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Please cite this article in press as: Alemany S, et al. Childhood adversity and psychosis: Examining whether the association is due to genetic confounding using a monozygotic twin differences approach. European Psychiatry (2012), doi:10.1016/j.eurpsy.2012.03.001

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Please cite this article in press as: Alemany S, et al. Childhood adversity and psychosis: Examining whether the association is due to genetic confounding using a monozygotic twin differences approach. European Psychiatry (2012), doi:10.1016/j.eurpsy.2012.03.001

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

Dr. Lourdes Fañanás Saura, Associate Professor (Profesora Titular) at the Department of Animal Biology of the Faculty of Biology, University of Barcelona and supervisor of the present doctoral thesis by Silvia Alemany, hereby certifies that the participation of the PhD applicant in the article "Childhood adversity and psychosis: examining whether the association is due to genetic confounding using a monozygotic twin difference approach" included the following tasks:

- Participation in the conception and design of the study
- Twin recruitment and collection of data
- Analysis and interpretation of data
- First drafting of the manuscript
- Critical revision of the arcticle for intellectual content

Dr. Lourdes Fañanás

Barcelona, February 11th 2013

7.5. Regional gray matter reductions are associated with genetic liability for anxiety and depression: a MRI Twin Study. Alemany S, Mas A, Goldberg X, Falcón C, Fatjó-Vilas M, Arias B, Nenadic I, Bargalló N, Gastó C, Fañanás L. *Journal of Affective Disorders* (In press).

Resumen en Castellano

Reducciones en sustancia gris se asocian a la vulnerabilidad genética para ansiedad y depresión: Un estudio de neuroimagen basado en gemelos.

Alemany S, Mas A, Goldberg X, Falcón C, Fatjó-Vilas M, Arias B, Nenadic I, Bargalló N, Gastó C, Fañanás L.

Journal of Affective Disorders (In press).

La influencia de los factores genéticos y/o ambientales sobre los cambios volumétricos cerebrales observados en individuos afectados por trastornos de ansiedad y depresión sigue siendo en general poco conocida. El presente estudio tuvo como objetivo investigar en una muestra de gemelos MZ (n=53) provenientes de la población general (incluyenco pares concordantes afectados de ansiedad y depresión, pares discordantes para ansiedad y depresión y gemelos sanos controles) si la vulnerabilidad genética y ambiental para el desarrollo de ansiedad y depresión se asocía de forma diferencial a anormalidades en el volumen de sustancia gris en estructurales cerebrales.

Este objetivo se testó utilizando el diseño de gemelos concordantes y discordantes MZ. Deacuerdo con este diseño, los gemelos MZ concordantes para ansiedad y depresión representarían un grupo con una vulnerabilidad genética para estos trastornos particularmente alta.

Cuando comparamos las imágenes de resonancia magnética cerebral (RMs) de los gemelos MZ concordantes con los gemelos MZ sanos, observamos que el primer grupo presentaba un volumen de sustancia gris significativamente menor a nivel bilateral del giro fusiforme y de la amígdala. Este hallazgo sugiere que la reducción de sustancia gris en el giro fusiforme y en la amígdala en ansiedad y depresión estaría asociado al riesgo genético para estos trastornos.

No se observaron diferencias significativas a nivel intrapair cuando se examinaron las RMs del grupo de gemelos MZ discordante para ansiedad y depresión. Por lo tanto, nuestro estudio no proporciona evidencias de la posible contribución de factores ambientales únicos en las anormalidades cerebrales asociadas a la ansiedad y la depresión.

Journal of Affective Disorders ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at SciVerse ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research report

Regional gray matter reductions are associated with genetic liability for anxiety and depression: An MRI twin study

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ARTICLE INFO

Article history:
Received 18 May 2012
Received in revised form
23 December 2012
Accepted 24 January 2013

Keywords:
Twins
Gray matter volume
Depression
Anxiety
Amygdala
Fusiform gyrus

ABSTRACT

Background: The influence of genetic and/or environmental factors on the volumetric brain changes observed in subjects affected by anxiety and depression disorders remains unclear. The current study aimed to investigate whether genetic and environmental liabilities make different contributions to abnormalities in gray matter volume (GMV) in anxiety and depression using a concordant and discordant MZ twin pairs design.
Methods: Fifty-three magnetic resonance imaging (3T) brain scans were obtained from monozygotic (MZ) twins concordant (6 pairs) and discordant (10 pairs) for lifetime anxiety and depression disorders and from healthy twins (21 subjects). We applied voxel-based morphometry to analyse GMV differences. Concordant affected twins were compared to healthy twins and within-pairs comparisons were performed in the discordant group.
Results: GMV reductions in bilateral fusiform gyrus and amygdala were observed in concordant affected twins for anxiety and depression compared to healthy twins. No intrapair differences were found in GMV between discordant affected twins and their healthy co-twins.
Limitations: The sample size was modest. This might explain why no intrapair differences were found in the discordant MZ twin group.
Conclusions: As concordant affected MZ twins are believed to have a particularly high genetic liability for the disorder, our findings suggest that fusiform gyrus and amygdala gray matter reductions are related to a genetic risk for anxiety and depression. Discrepancies in regard to brain abnormalities in anxiety and depression may be related to the admixture of patients with GMV abnormalities mainly accounted for by genetic factors with patients presenting GMV mainly accounted for by environmental factors.

1. Introduction

Major depressive disorder (MDD) ranks among the top causes of worldwide disease burden and disability, with a lifetime risk of 7–12% in men and 20–25% in women (Kessler et al., 2005). The various anxiety disorders, including panic disorder and phobias, are also extremely common, with lifetime prevalences of 19.2% in men and 30.5% in women (Kessler et al., 1994). Anxiety disorders can seriously interfere with daily life and, overall, have rates of failure to respond similar to those of MDD (Ressler and Mayberg, 2007). Furthermore, a number of reasons have lead some authors to argue that anxiety and depression may share common etiological pathways (Ressler and Mayberg, 2007). First, it is well established that symptoms of anxiety and depression commonly co-occur, with estimations of the comorbidity ranging from 10% to more than 50% (Gorman, 1996; Ressler and Mayberg, 2007; Roy-Byrne et al., 2000). More than half of all individuals with MDD also

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http://dx.doi.org/10.1016/j.jad.2013.01.019

develop an anxiety disorder during their lifetime (Kessler et al., 1996). Similarly, 10–65% of the individuals diagnosed with panic disorder (PD) experience comorbid MDD (Mosing et al., 2009; Wittchen et al., 2008). Second, there is an overlap of symptoms associated with both anxiety and depression which makes diagnosis classification particularly difficult (Gorman, 1996; Ressler and Mayberg, 2007). Third, the most powerful treatments for both disorders are the same, including antidepressants and cognitive behavioural therapy (Ressler and Mayberg, 2007). Fourth, several lines of evidence suggest that affective and anxious symptoms arise from dysregulation of the limbic–cortical system that mediate stress-responsiveness (Ressler and Mayberg, 2007).

In this context, from a neuroimaging perspective, several studies of anxiety and depression have identified gray matter alterations in brain structures related to the hypothalamus–pituitary–adrenal axis function, emotion perception, and regulation such as the amygdala, anterior cingulate cortex, orbitofrontal cortex, hippocampus and superior temporal gyrus (Bora et al., 2011; Hamilton et al., 2008; Lange and Irle, 2004; Massana et al., 2003; Macqueen and Frodl, 2011; Sheline et al., 2003, 1998; Brambilla et al., 2002; Van Tol et al., 2010). However, different studies tend to implicate these brain regions to varying degrees, and both increases and decreases in gray matter volume (GMV) have been observed (Bora et al., 2011; Hamilton et al., 2008).

Although several reasons have been put forward to explain the heterogeneity of these results – mainly referring to clinical variables (Bora et al., 2011) – a relevant issue is the possibility that genetic and environmental risk factors have different impacts on the neuroanatomic abnormalities observed in anxiety and depression. It is not clear yet whether genetic and environmental risk factors for anxiety and depression act along the same neurobiological pathways. Therefore, we cannot exclude the possibility that some brain regions are more affected by genetic factors and others by environmental ones (De Geus et al., 2007). In this context, twin studies offer a unique opportunity to address this issue.

To separate the effects of genetic and environmental risk factors on brain structure, the concordant and discordant monozygotic (MZ) twin pair design has been applied in neuroimaging research (Borgwardt et al., 2010; De Geus et al., 2007; Ettinger et al., 2010; Wolfensberger et al., 2008).

This design assumes that the comparison between concordant affected monozygotic (MZ) twin pairs and healthy MZ twins is likely to reflect a contrast in genetic liability for the phenotype of interest. In this regard, concordant MZ twin pairs (i.e., genetically identical pairs in which both members have the disorder) would be subject to a greater genetic liability for the disorder studied than discordant pairs (i.e., genetically identical pairs in which only one member has the disorder) (Borgwardt et al., 2010; De Geus et al., 2007; Ettinger et al., 2007, 2010; Wolfensberger et al., 2008). In schizophrenia research, MZ twins concordant for schizophrenia are believed to carry a particularly high genetic load for the disorder – and, specifically, greater than discordant pairs – reflected in an earlier age of onset, a more severe clinical course, and a less marked association with putative environmental risk factors (Borgwardt et al., 2010). Although the literature on this issue in anxiety and depression disorders is still scarce, De Geus et al. (2007) provided support for the notion that MZ twins concordant for anxiety and depression may be subject to a greater genetic risk; they observed higher levels of anxiety, depression and neuroticism among parents of concordant twins than in parents of healthy twins (De Geus et al., 2007).

The concordant and discordant monozygotic (MZ) twin pair design also assumes that any within-pair differences in GMV between MZ twin pairs who are discordant for anxiety and depression may be attributable to *unique* environmental influences (Plomin et al., 2008). In summary, according to this twin design, GMV differences between concordant MZ twins and healthy MZ twins may be related to the genetic risk for anxiety and depression, while intrapair GMV differences in discordant MZ twins may highlight brain regions particularly susceptible to the impact of environmental factors. Therefore, the current study aimed to explore whether (i) concordant affected twins presented GMV changes compared to healthy MZ twins and (ii) in discordant pairs the affected MZ twins presented GMV changes compared to their healthy co-twins.

2. Methods

2.1. Participants

Twins were selected and invited to participate from an ongoing sample consisting of 120 Spanish twin pairs from the general population. Further information about this sample can be found elsewhere (Alemany et al., 2012). The selection strategy is detailed below, and it was carried out on the basis of data collected in the 2007–2010 period.

Concordant and discordant twin pairs were considered eligible by applying the following inclusion criteria: (1) a monozygotic (MZ) twin pair with an age at scan between 18 and 55 years; (2) both twins right-handed; and (3) at least one twin with a lifetime (current or past) *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV-TR) (American Psychiatric Association) diagnosis of major depressive disorder (MDD) or any anxiety disorder. The control group consisted of healthy twins meeting the same criteria as concordant and discordant twins, except that neither twin had a personal lifetime history of a DSM-IV-TR Axis I diagnosis.

Exclusion criteria for the three groups were: (1) Neurological or major medical illness; (2) pregnancy (temporary exclusion); and (3) metallic implants in the body incompatible with MR scan. The exclusion criteria were designed to eliminate most known causes of changes in brain structure and conditions contraindicated for MRI.

From the total of 67 twin pairs eligible for the study, 58 twin pairs agreed to participate. Two pairs of twins and one subject ($n=5$) were excluded from the final sample due to image artifacts. Thus, the final sample included 12 twins concordant for anxiety and/or depression disorders, 20 twins discordant for anxiety and/or depression disorders, and 21 healthy twins, 53 individuals in total. Mean age of the sample was 36.7 years ($SD=13.4$), and 37.7% ($n=20$) were males.

Written informed consent was obtained from all participants after a detailed description of the study aims and design, approved by the local Ethics Committee. All procedures were carried out according to the Declaration of Helsinki.

2.2. Clinical, cognitive and environmental measures

Lifetime DSM-IV-TR Axis-I diagnosis was assessed in a face-to-face interview with a clinical trained clinical psychologist (XG) using the Structural Clinical Interview for DSM-IV disorders (SCID-I) (First, 1997). Anxiety and depression levels were assessed by means of the Beck Anxiety Inventory (BAI) (Magan et al., 2008) and the beck Beck Depression Inventory (BDI-II) (Sanz et al., 2003). In this interview, twins were asked whether they had ever been treated by a psychologist or psychiatrist. In the total sample included in the present study, three subjects had been under pharmacological treatment, five subjects had been under psychotherapeutic treatment and three subjects had been under a combination of pharmacological and psychotherapeutic treatment. Of note, none of the

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twins presenting a lifetime diagnosis of MDD had suffered more than one depressive episode.

Family history of any psychiatric disorders was assessed by means of the Family Interview for Genetic Studies (FIGS) (Nimh, 1992).

Additionally, twins were asked to report whether they were under medication or psychological treatment and whether they had consulted a psychiatrist or psychologist since they first participated in the study (there was a span time of minimum one year and maximum two years since twins were assessed for the first time). Only three individuals were under pharmacological and/or psychotherapeutic treatment at scan time, one individual from the concordant group (under pharmacological treatment with selective serotonin re-uptake inhibitors (SSRIs) and benzodiazepines) and two individuals from the discordant group (one under pharmacological treatment with SSRIs and one under both psychotherapeutic and pharmacological treatment also with SSRIs).

Estimated intelligence quotient (IQ) was assessed using four subtests (block design, matrix reasoning, information and vocabulary) from the Wechsler Adult Intelligence Scale (WAIS-III) (Wechsler, 1997; Sattler, 2008).

2.3. Zygosity

Genomic DNA was extracted from peripheral blood cells using the Real Extraction DNA Kit (Durviz S.L.U., Valencia, Spain), or from buccal mucosa on a cotton swab using the BuccalAmp DNA Extraction Kit (Epicentre® Biotechnologies, Madison, WI). Biological samples were collected when twins first participated in the study.

Zygosity was established by genotyping 16 loci: 15 short tandem repeat (STR) loci and amelogenin, the gender determining marker. Twins with only one divergent allele were genotyped a second time to limit the scope for genotyping error. Identity on all the markers ensures monozygosity with $>99\%$ accuracy (Price et al., 2000).

2.4. Brain MRI procedures

2.4.1. Image acquisition

Subjects were scanned in the the MRI Unit of the Image Platform of IDIBAPS located at Hospital Clínic de Barcelona. A high resolution 3D structural dataset using a T1-weighted magnetization prepared rapid gradient echo was acquired on a TIM TRIO 3 T scanner (Siemens, Erlangen, Germany) with the following parameters: 3D T1-weighted MPRAGE sequence, $TR=2300$ ms, $TE=3.03$ ms, $TI=900$ ms, Flip Angle $=9^{\circ}$, 192 slices in the sagittal plane, matrix size $=256\times256$, 1 mm isometric voxel, using a 8-channel coil.

2.4.2. Image preprocessing

Imaging data were analysed with SPM8 (Wellcome Trust Centre for Neuroimaging, UCL, United Kingdom). Images were visually inspected for eventual artifacts and centered to the anterior commissure. The 3D structural dataset images were segmented into gray matter, white matter, and cerebrospinal fluid. The deformations that best aligned the images together were estimated by iteratively registering the imported images with their average through the Diffeomorphic Anatomical Registration Through Exponential Lie Algebra (DARTEL) algorithm (Ashburner, 2007). Subsequently, the images were normalized to the standard Montreal Neurological Institute (MNI) brain template using the parameters obtained in the DARTEL's template normalization to MNI template. To preserve initial volumes, Jacobian scaled warped tissue images were generated through Jacobian modulation, and the warped images smoothed with isotropic Gaussian Kernels (12 mm). Spatial smoothing has the effect of rendering the data more normally distributed and reduces the influence of inaccuracies in spatial normalization of individual brains on the morphometric comparisons.

Total intracranial volume (TIV) was calculated from SPM8 segmentation maps for use as a covariate in the statistical tests.

A whole-brain analysis was performed since no a priori regions of interest (ROI) were defined in this study, due to the large number of brain areas that have been related to anxiety and depression (Bora et al., 2011; Lorenzetti et al., 2009).

2.5. Statistical analysis

Analyses of demographic, clinical, cognitive, genetic and environmental measures were carried out in STATA 10.0 (Statacorp, 2007). The non-independence of clustered twin data was corrected for by using tests based on the sandwich or Huber/White variance estimator (Williams, 2000). Group differences (concordant, discordant and control twins groups) in continuous variables (age, IQ, BAI, BDI-II) were examined by means of linear regression models. Group differences in sex were examined by means of a chi-square test selecting one member of each pair. Group differences between the concordant and the discordant twin groups in lifetime DSM-IV-TR diagnoses (anxiety disorders, major depressive disorder or comorbid anxiety and major depressive disorders) were also examined using a chi-square test selecting only affected individuals (concordant twins and discordant affected twins). In these analyses tests were considered to be significant if $p<0.05$.

A voxel-by-voxel two-sample t-test on modulated gray matter maps was used to assess differences in GMV between affected concordant twins and healthy twins. Of note, healthy control twins are used as individuals in the present study. For this reason, although brain images of one control twin pair had to be discarded due to artefacts, we were able to use the brain images of the co-twin in order to increase the statistical power of the analyses. A paired t-test was used to explore within-pair GMV differences between the affected twins and their healthy co-twins in the discordant twin group. TIV was included as a covariate in all the tests in order to reject variability related to head size differences. TIV has been shown to produce best results when used as a single covariate in gray matter volumetric analysis (Pell et al., 2008). The level for the absolute threshold masking tissue map was set at 0.2. Results were considered significant at $p<.05$ family-wise error (FWE) voxel corrected at peak-level with an extent threshold of 50 voxels.

For informative reasons, when not significant results at $p<.05$ FWE were found, significant results applying a more relaxed statistical criterion ($p<.001$ uncorrected with an extent threshold of 50 voxels) are also reported. This also allows the comparison between our study and a previous similar one (De Geus et al., 2007).

Coordinates of peak significant voxels were assigned to anatomic regions by means of automated anatomic labeling (Tzourio-Mazoyer et al., 2002).

3. Results

3.1. Demographic data and clinical characteristics of the subjects

From the 22 affected individuals, six had a lifetime history of anxiety disorders including specific phobia, social phobia, panic disorder, agoraphobia and obsessive–compulsive disorder; 10 had a lifetime history of major depressive disorder (MDD) and six presented comorbid anxiety and MDD. The number of subjects affected by anxiety disorders, MDD or both for concordant and discordant MZ twin groups is detailed in Table 1.

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Table 1
Demographic, cognitive, clinical and environmental data for concordant, discordant and healthy control MZ twin pairs. Means and standard deviations are indicated for continuous measures. Number of individuals with depression, anxiety and comorbid depression and anxiety and percentage of the total affected individuals in each group is indicated. Significant differences between groups are indicated in bold.

	MZ concordant ($n=12$)	**MZ discordant** ($n=20$)	**MZ control** ($n=21$)	**Group comparison**	
				F* (*df*) or χ^2 (*df*); *p	**Post-hoc tests**
Total pairs	6	10	10 (plus 1 subject)	–	–
M/F	2/10	6/14	12/9	3.5 (2);.177	–
Age	41.6 (13.4)	33.7 (10.9)	34.9 (8.0)	0.7; (2);.501	–
Lifetime DSM-IV-TR diagnosis				3.7 (2);.161	–
Depression	3 (25%)	6 (60%)	0		
Anxiety	5 (41.7%)	1 (10%)	0		
Comorbid	4 (33.3%)	3 (30%)	0		
IQ	98.6 (14.1)	104.0 (9.7)	105.2 (6.5)	0.9 (2);.439	–
BAI	**13.3 (9.5)**	**7.2 (4.8)**	**3.7 (4.1)**	**5.3 (2);.011**	[a] **(.009);** [b] **(.047)**
BDI-II	10.2 (5.9)	5.3 (4.6)	5.5 (10.4)	1.9 (2);.159	–

M=males; *F*=females; DSM-IV-TR=Diagnostic and Statistical Manual of Mental Disorders; IQ=intelligence quotient; BAI=Beck Anxiety Inventory; BDI-II=Beck Depression Inventory.
[c]Significant differences between concordant and discordant twins.
[a] Significant differences between concordant and control twins.
[b] Significant differences between discordant and control twins.

Table 2
Clinical, cognitive and environmental data for the affected twin and his/her healthy co-twin from the MZ discordant twins group for anxiety and depression. Means and standard deviations are indicated.

	MZ discordant		***t*(*df*); *p***
	Affected twin ($n=10$)	**Healthy twin ($n=10$)**	
BAI	7.2 (5.1)	7.2 (4.8)	1.6 (9);.114
BDI-II	5.3 (3.5)	5.2 (5.8)	−.1 (9);.961
IQ	106.9 (11.8)	104.0 (9.7)	−.9 (7);.386

BAI=Beck Anxiety Inventory; BDI-II=Beck Depression Inventory; IQ=intelligence quotient.

All pair twins included in the concordant and discordant group presented a positive family history of psychiatric disorders. Three pairs from the control group presented a positive family history of psychiatric disorders.

Table 1 also displays demographical, clinical and cognitive data for the three MZ twin groups. Groups did not significantly differ in sex, age, IQ, BDI-II scores or child abuse. There were no significant differences with respect to lifetime DSM-IV-TR diagnosis categories (depression disorder, anxiety disorder, and comorbid depressive and anxious disorder) between affected twins from concordant and discordant groups. Concordant and discordant twins had significantly higher BAI scores than control twins.

No significant within-pair differences were detected when comparing clinical, cognitive and environmental data of the affected twin with his/her healthy co-twin in the discordant twin group (Table 2).

3.2. Regional morphometry

Total intracranial volume (TIV) did not significantly differ between groups ($F=1.5$; $df=2$; $p=.241$). Paired *t*-tests revealed no significant differences for TIV within each twin pair in the discordant group ($t=-1.2$; $df=9$; $p=.281$).

Compared to healthy twins, concordant twins showed a significant decrease in gray matter mainly in bilateral fusiform gyrus and bilateral amygdala (the left amygdala approaching significance) compared to healthy twins ($p<0.05$ FWE) (Table 3, Fig. 1).

GMV in right temporal inferior gyrus, bilateral temporal superior pole and cerebellum was also reduced in concordant affected twins with minor cluster percentages (Table 3).

The comparison analysis within discordant twins showed no significant differences in regional GM volumes ($p<.05$ FWE). At uncorrected $p<0.001$ value, affected twins showed decreased gray matter volumes in left precuneus (MNI Coordinates: $x=-3$, $y=-54$, $z=48$; $T=6.63$; $k=140$ voxels; 100% of the cluster) and right parahippocampal gyrus and hippocampus (MNI coordinates: $x=35$, $y=-38$, $z=-9$; $T=6.05$; $k=100$ voxels; 73% and 23% of the cluster, respectively).

4. Discussion

In the present study we sought to discriminate between GMV correlates of genetic risk for anxiety and depression disorders and the GMV correlates of environmental risk for these disorders by comparing MZ twins with varying concordance for anxiety and depression to concordant healthy twins. We found that concordant twins had significantly lower GMV mainly in bilateral fusiform gyrus and bilateral amygdala compared to healthy control twins, suggesting that a genetic risk for anxiety and depression may underlie GMV changes in these regions. No intrapair significant differences in whole brain GMV were detected in discordant twins, thus, our study does not provide evidence for *unique* environmental factors accounting for GMV changes in anxiety and depression.

First, most of the twins were not under pharmacological or psychological treatment. Furthermore, according to the BAI and BDI-II scoring guidelines (Magan et al., 2008; Sanz et al., 2003), the concordant affected twin group presented moderate levels of anxiety and depression. Thus, patients included in this sample are mainly asymptomatic or in remission. In this regard, our results indicate brain changes that constitute persistent brain abnormalities or neurobiological markers of vulnerability to depression and anxiety rather than brain correlates of these symptoms.

Second, although most lifetime affected individuals were not severely depressed at scan time, the present study replicates previous reports of associations between brain areas such as the fusiform gyrus and amygdala and depressive and anxiety disorders (Hamilton et al., 2008; Lai et al., 2010; Lee et al., 2011).

Reductions in fusiform gyrus have been reported in depressed patients (Lee et al., 2011). The fusiform gyrus, a region in the inferotemporal cortex, has been consistently associated with the perception of human faces (Haxby et al., 2000; Kanwisher et al., 1997) and is thought to act as a feedforward modulator of amygdala activation (Fairhall and Ishai, 2007). It has been shown

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Table 3
Areas of reduced GM in concordant affected twins compared to concordant control twins. Percentages in brackets indicate percentage of the cluster.

Region	R/L	Peak					T	Z	p (unc)	p (FWE)	Cluster p (FWE)
		k	k (%)	MNI coordinates							
				x	y	z					
Fusiform gyrus	R	2246	56	−37	−34	−31	6.0	4.8	$P<.001$	**0.008**	0.013
Temporal inferior gyrus			16								
Cerebellum 6			10								
Amygdala	R	574	30	−28	3	−18	5.3	4.4	$P<.001$	**0.042**	0.262
Temporal superior pole			15								
Fusiform gyrus	L	645	76	37	−31	−30	5.2	4.4	$P<.001$	**0.051**	0.226
Amygdala	L	373	27	34	5	−16	5.0	4.2	$P<.001$	0.080	0.405
Temporal superior pole			12								

R, right; L, left; k, number of significant voxels; k%, percentage of significant voxels in the anatomical region; MINI, Montreal Neurological Institute; unc, uncorrected; FWE, family wise error correction.

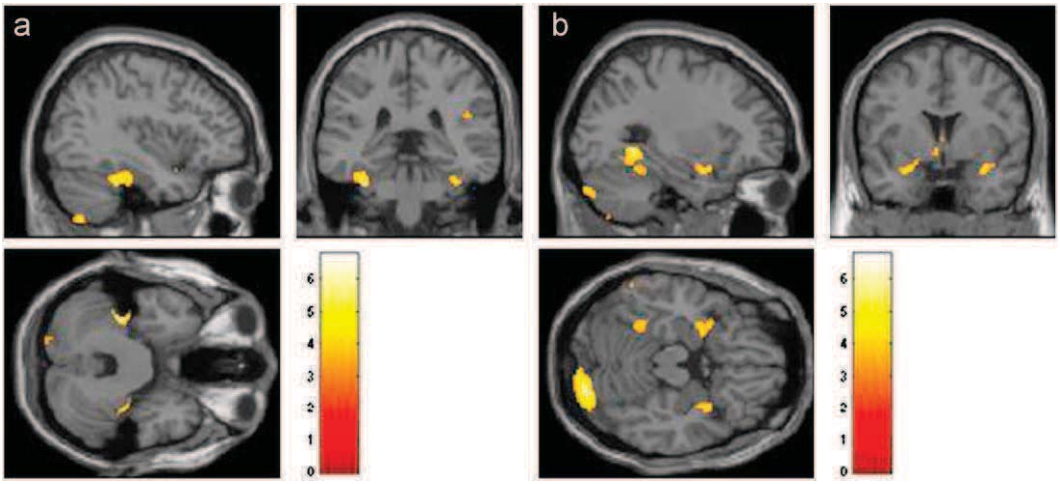

Fig. 1. Areas of reduced GMV in MZ twin pairs concordant for anxiety and depression compared to control MZ twins ($p<0.05$ FWE). Relative to the healthy control twins, the MZ concordant twin pairs showed smaller GMV in bilateral fusiform gyrus and amygdala. Colored clusters show mapped *T* values. The *z* coordinate shows the position of each slice with respect to the MNI atlas. Images correspond to (A) $z=-31$ transversal, coronal and sagittal cuts with colored right and left fusiform clusters and the edge of the amygdalar clusters; (B) $z=-18$ transversal plane and associated coronal and sagittal cuts with colored amygdalar clusters and left and right fusiform clusters. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to be more active during the processing of expressive (e.g., fearful) faces than neutral faces (Vuilleumier et al., 2001, 2004).

Our finding of a reduction in the amygdala is in accordance with previous research in MDD (Hastings et al., 2004; Sheline et al., 1998), anxiety disorders (Hayano et al., 2009; Massana et al., 2003) and comorbid anxiety and depression (Lai et al., 2010). As part of the limbic system, the amygdala constitutes a crucial structure for the perception and memory of emotional material (Bear et al., 2002; Adolphs and Tranel, 2004, Cahill et al., 1995). Furthermore, together with other regions such as the anterior insula, it plays an important role in the generation of negative mood states and is associated with internal somatic changes (Mayberg et al., 1999). Afferents to the amygdala come from a large variety of sources, including the neocortex in all lobes of the brain as well as the hippocampal and cingulate gyrus (Bear et al., 2002). It has been shown in several species that bilateral ablation of the amygdala results in flattening emotion and can profoundly reduce fear (Bear et al., 2002).

Interestingly, a previous MRI-twin study suggested that familial factors, which include both genetic and common environmental factors, might influence amygdala volumes (Munn et al., 2007). Munn and colleagues found that MZ control twins had significant, high intrapair correlations for amygdala volumes. They concluded that familial or perhaps genetic influence may account for amygdala volume (Munn et al., 2007). Our findings agree with this conclusion but highlight especially the effects of genetic risk factors on amygdala volume, since in our study concordant affected twins, hypothesized to be subject to a particularly high genetic loading for anxiety and depression, had smaller amygdala volume than concordant healthy twins.

Genetic vulnerability for anxiety and depression is likely to involve genetic variants which affect the functional or structural integrity of neural circuits through molecular and cellular mechanisms (Meyer-Lindenberg and Weinberger, 2006). Among these genetic variants, could be of particular interest the role of serotonergic (SLC6A4, HTR1A, MAOA, TPH2) and neurotrophic (BDNF) genes (Scharinger et al., 2011). For example, the 5-HTTLPR polymorphism of the serotonin transporter gene (SLC6A4) has been associated to an increased risk for depression and anxiety-related behaviours but also to GMV reduction of amygdala and increased amygdala reactivity (Frodl et al., 2008; Lau et al., 2009; Pezawas et al., 2005; Schinka et al., 2004). Individuals at particularly high genetic risk for anxiety and depression, such as concordant affected MZ twins, may present an especially high frequency of risk alleles for these genes.

Of note, the fact concordant twins may have a greater genetic risk than discordant twins, and discordant twins a greater

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environmental risk than concordant twins does not rule out the possible involvement of gene-environment interaction effects underlying the development of anxiety and depression disorders and their putative neuroanatomical correlates.

Third, many studies have reported a reduced volume in the anterior cingulate cortex (ACC) in depression (Koolschijn et al., 2009; Van Tol et al., 2010; Bora et al., 2011). However, we found no significant differences in the ACC of concordant affected twins compared to healthy controls. In this regard, a recent meta-analysis of VBM studies in MDD found that longer illness duration was associated with greater gray matter reduction in this region (Bora et al., 2011). Furthermore, reduction in ACC was only observed in samples including multi-episode patients suggesting a possible progression of abnormalities in these regions over time. The authors also proposed the possibility that reduction in ACC might be related to recurring hypoactivity in depressive episodes in currently depressed samples included in the meta-analysis (Bora et al., 2011). The fact that, as abovementioned our sample mostly included individuals not currently severely ill with a history of only one depressive episode may help to explain why we did not found reduced GMV in ACC in concordant affected twins compared to healthy control twins.

Fourth, we did not detect statistically significant within-pair differences at peak-level ($p < 0.05$ FWE) when comparing the affected discordant twin to his/her healthy co-twin. However, when applying a more relaxed statistical threshold ($p < 0.001$ uncorrected at peak-level), as De Geus et al. (2007) applied, we found that the affected twins presented smaller GMV at precuneus, hippocampus and hippocampal gyrus compared to their healthy co-twins. Although these findings are partially in line with those reported by De Geus et al. (2007), who found intrapair differences but in left hippocampal regions, our findings did not reach the statistically significant criterion previously established and should be interpreted with caution.

Finally, the current study has to be considered in the context of its limitations. First, the sample size was modest, though similar to those used in previous studies using a concordant and discordant MZ twin design in anxiety and depression (De Geus et al., 2007; Wolfensberger et al., 2008). The limited sample size of discordant MZ twin pairs could partially explain the lack of significant differences between affected and healthy co-twins in this group. Second, our conclusions are based in the assumption of the concordant and discordant MZ twin pair design that states that the comparison between concordant affected MZ twin pairs and healthy MZ twins is likely to reflect a contrast in genetic liability for the phenotype of interest. However, this assumption needs further research to test its validity in the context of anxiety and depression studies. Third, among the lifetime affected subjects included in the present study, some had been treated and three of them were under pharmacological treatment at scan time. This might constitute a source of bias since antidepressant treatment has been suggested to reduce neuronal damage and the rate of neuronal death caused by corticosteroids (Haynes et al., 2004) or apoptosis (Kosten et al., 2008).

In conclusion, GMV abnormalities in bilateral fusiform gyrus and amygdala in anxiety and depression were observed in twin pairs concordant for these disorders compared to healthy twins, but not within discordant twin pairs. These two groups of MZ twins – concordant affected and discordant – ,may reflect a contrast in genetic liability for anxiety and depression. Therefore, our findings suggest that fusiform gyrus and amygdala reductions are related to genetic risk for anxiety and depression.

Role of funding source

Funding projects had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

Acknowledgments

We gratefully acknowledge the collaboration of the participants. We thank César Garrido and Santi Sotés (MRI technicians) for their collaboration. This study was supported by the Ministry of Science and Innovation (SAF2008-05674-C03-00; 02 and 03), the Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), European Twins Study Network on Schizophrenia Research Training Network (grant number EUTwinsS; MRTN-CT-2006-035987; local PIs: L.F. and I.N.) and by the Comissionat per a Universitats i Recerca del DIUE of the Generalitat de Catalunya (2009SGR827). Goldberg X was supported by a Marie Curie grant (grant number EUTwinsS; MRTN-CT-2006-035987). Alemany S thanks the Institute of Health Carlos III for her PhD grant (FI00272).

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

Dr. Lourdes Fañanás Saura, Associate Professor (Profesora Titular) at the Department of Animal Biology of the Faculty of Biology, University of Barcelona and supervisor of the present doctoral thesis by Silvia Alemany, hereby certifies that the participation of the PhD applicant in the article "Regional gray matter reductions are associated with genetic liability for anxiety and depression: a MRI Twin Study" included the following tasks:

- Participation in the conception and design of the study
- Twin recruitment and collection of data
- Analysis and interpretation of data
- First drafting of the manuscript
- Critical revision of the arcticle for intellectual content

Dr. Lourdes Fañanás

Barcelona, February 11th 2013

7.6. Psychotic experiences influence emotional processing in individuals affected by anxiety and depression: An fMRI community-based twin study. Alemany S, Goldberg X, Falcón C, Mas A, Bargalló N, Garrido C, Gastó C, Nenadic I, Fañanás L. (In preparation)

Resumen en Castellano

La presencia de experiencias psicóticas influencía el procesamiento emocional en individuos afectados de ansiedad y depresión: Un estudio de neuroimagen funcional basado en gemelos de la población general

Alemany S, Goldberg X, Falcón C, Mas A, Bargalló N, Garrido C, Gastó C, Nenadic I, Fañanás L.

(En preparación).

Existen evidencias a favor de que el funcionamiento emocional está alterado tanto en la esfera psicopatológica psicótica como en la afectiva. Sin embargo, los posibles correlatos cerebrales de la co-ocurrencia de estas características psicopatológicos han sido poco explorados en muestras de población general.

En el presente estudio se estudió, mediante neuroimagen funcional (fMRI): i) la relación entre las experiencias psicóticas (EPs) y la respuesta cerebral a la emoción facial; y ii) si la activación cerebral estaba moderada por la presencia de EPs en personas afectadas por la ansiedad y/o depresión.

La activación cerebral durante la exposición a caras humanas que expresaban diferentes emociones (paradigma de emoción facial), se evaluó en pares de gemelos monozigóticos (MZ) concordantes para ansiedad y/o depresión (n=6 pares), pares de gemelos MZ discordante para ansiedad y/o depresión (n=10 pares) y gemelos MZ sanos que constituían el grupo control (n=21).

La presencia de las EPs influyó en la respuesta cerebral a la emoción facial. Específicamente, la activación del cortex cingulado anterior durante la exposición de caras de enfado se asoció a las puntuaciones de EPs negativas mientras que la deactivación del cortex cingulado anterior durante la exposición de caras de miedo se asoció a las puntuaciones de EPs positivas. Estos resultados apoyan la hipótesis de que exisitiría una desregulación emocional presente en el *continuum* de la psicosis. Por otra parte, la dimensión positiva de las EPs moderaba la respuesta emocional fundamentalmente a nivel del cerebelo, en el grupo de gemelos MZ concordantes para ansiedad y depresión. Esto sugiere que estas dimensiones psicopatológicas pueden compartir procesamiento emocional alterado.

Psychotic experiences influence emotional processing in individuals affected by anxiety and depression: An fMRI community-based twin study

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Abstract

There is increasing evidence that emotional functioning is altered in both psychotic and affective psychopathology. However the brain correlates of the co-occurrence of these symptoms in non-clinical samples remain unclear. In the present study we used functional magnetic resonance imaging (fMRI) to examine i) the relationship between psychotic experiences (PEs) and the brain response to facial emotion and ii) whether PEs moderated brain activation to facial emotion in subjects affected by anxiety and/or depression. We assessed brain response to facial emotion during fMRI imaging in monozygotic (MZ) twin pairs concordant for anxiety and depression (n=6 pairs), discordant for anxiety and depression (n=10 pairs) and healthy control twins (n=21). Positive and negative PEs were assessed using the Community Assessment of Psychic Experiences (CAPE). Activation of the anterior cingulate cortex (ACC) to angry faces was associated with the negative dimension of CAPE ($p<0.05$ FWE corrected). A significant association was also found between activation of the ACC to fear faces and positive CAPE ($p<0.05$ FWE corrected). Furthermore, CAPE positive scores moderated the activation of cerebelum, hippocampus and fusiform gyrus among other areas to fear faces only in affected concordant MZ twins for anxiety and depression. Our findings indicate that dimensions of PEs are associated with anterior cingulated cortex (ACC) activation during emotion processing. Furthermore the presence of PEs influenced emotional processing in individuals affected by anxiety and depression indicating that these psychopathological dimensions may share altered emotional functioning.

Keywords: psychotic experiences, depression, anxiety, facial emotion, fMRI.

1.INTRODUCTION

Human social interactions involve recognizing other people's identities, actions, emotions and intentions. Much of this information is available from facial expressions (1). The ability to extract information from the expressions of others and make inferences about their mental states is essential to successfully engage in social interactions (2). A growing body of research indicates that these functions can be impaired in individuals affected by anxiety and depressive disorders (3-5) and also in schizophrenia (6-7).

Although it remains unclear whether emotion processing is already affected before the onset of the disorders, it has been shown that not only relatives such as healthy siblings of schizophrenic patients but also individuals from the general population reporting subclinical psychotic symptoms or psychotic experiences (PEs) present deficits in emotion processing (8-10). Similarly, deviant amygdala responses to emotional faces have been observed in subjects at risk for anxiety and depression (11). Two observations can be drawn from these findings, i) altered emotion processing seems to occur across the continuum of psychosis from full clinical diagnoses to isolated psychotic experiences (12); and ii) it is plausible that alterations in emotion processing might be shared by different psychopathological dimensions such as psychosis, depression and anxiety.

In this regard, epidemiological evidence indicates that subclinical psychotic symptoms and depression are associated along the continuum of psychosis in general population samples (13). Indeed, a population-based study concluded that both

individuals affected by psychotic disorders, and individuals affected by depression and anxiety were more likely to report psychotic symptoms compared to healthy individuals (14). Furthermore, there is evidence for the existence of shared underlying endophenotypes, mainly between psychosis and depression, such as alterations in cognitive, social and emotional functioning (15-16).

Considering the above evidence, in the present study we hypothesized that the presence of PEs would influence brain response to facial emotion; furthermore, it would interact with the presence of depressive and anxious symptoms when processing facial emotion information.

The specific aims of the current study were to explore i) brain activation to facial emotion in subjects reporting positive and negative PEs using a functional magnetic resonance imaging (fMRI) community-based twin study and ii) whether scores in psychotic experiences interact with anxiety and depression and genetic risk for these disorders.

2. Materials and Methods

2.1 Sample

Subjects were selected and invited to participate from an ongoing sample consisting of 120 Spanish twin pairs from the general population (further information about this sample can be found elsewhere (17)). Twins were originally selected to form a sample including concordant and discordant MZ twin pairs for anxiety and/or depression disorders.

All subjects had been interviewed face-to-face using the *Structural Clinical Interview for DSM-IV disorders* (SCID-I; (18) by a trained clinical psychologist (XG) during the 2007-2010 period. Concordant and discordant twin pairs were considered eligible applying the following inclusion criteria: 1) a monozygotic (MZ) twin pair with an age at scan between 18 and 55 years; 2) both twins right-handed; and 3) at least one twin with a lifetime DSM-IV-TR (19) diagnosis of Major Depressive Disorder (MDD) or any Anxiety Disorder. The control group consisted of healthy twins meeting the same criteria as concordant and discordant twins, except that neither twin had a personal lifetime history of a DSM-IV-TR Axis I diagnosis. Exclusion criteria for these three groups were: 1) neurological or major medical illness; 2) pregnancy (temporary exclusion); and 3) incompatibility with MRI scan.

From the total of 67 twin pairs eligible for the study, 58 twin pairs agreed to participate. Two pairs of twins and one subject were excluded from the final sample due to image artefacts. Thus, the final sample included four groups of twins according to their psychopathological status concordance: 12 twins concordant affected for anxiety and/or depression disorders (6 pairs), 10 affected twins discordant for anxiety and/or depression disorders, 10 healthy twins discordant for anxiety and/or depression disorders and 21 healthy control twins (10 pairs plus 1 individual), i.e. 53 individuals in total.

Written informed consent was obtained from all participants after a detailed description of the study aims and design, approved by the local Ethics Committee. All procedures were carried out according to the Declaration of Helsinki.

2.2 Measures of psychopathology

The Community Assessment of Psychic Experiences (CAPE; (20)) was used to assess positive and negative psychotic experiences. This validated self-report questionnaire measures the lifetime prevalence of psychotic experiences in a frequency scale ranging from 'never' to 'nearly always'. The positive dimension of the CAPE includes items mainly referring to hallucinations and delusions such as 'do you ever feel as if things in magazines or TV were written especially for you?'. The negative dimension mainly assesses alogia, avolition, anhedonia and lack of interest in social relationships. An example of item is 'do you ever feel that you experience few or no emotions at important events?'. The CAPE provides a total continuous score per dimension ranging from 20 to 80 in the positive dimension and from 14 to 56 in the negative dimension.

Anxiety level before the scan was assessed by means of the *State-Trait Anxiety Inventory* (STAI-S; (21)). To further clinically characterize the sample, twins completed the trait version of the STAI questionnaire and the Beck Depression Inventory (BDI-II; (22).

Additionally, twins were asked to report whether they were receiving medication or psychological treatment or had consulted a psychiatrist or psychologist since they first participated in the study. Only three individuals had life-time exposure to pharmacological treatment for anxiety or depression.

2.3 fMRI facial emotion paradigm

Based in a previous developed facial emotion paradigm (11), black and white photographs of angry, fearful, sad, happy, surprise and neutral facial expressions ((23), in addition to a control condition consisting of scrambled faces, were presented. These categories represented the face stimulus conditions. Each face stimulus condition consisted of 10 pictures, and each picture was presented three times using Presentation software (Neurobehavioral Systems, San Francisco, USA). Stimulus order was randomized once and then presented in the same fixed order to all subjects. Stimuli were displayed for 2500 ms with a variable interstimulus interval (400 - 600 ms) to decrease expectancy effects. Subjects were requested to make sex judgments during presentation of face stimuli to control for attentional level. Control condition stimuli, scrambled faces, were imbedded with two arrows in the center of the screen, and subjects were asked to indicate whether arrows

pointed to the left or right. Task was explained outside the scanner before fMRI was performed. The duration of the facial emotion paradigm was approximately 10 minutes.

2.4 Image acquisition

Subjects were scanned in the MRI Unit of the Image Platform of IDIBAPS located at Hospital Clínic de Barcelona. Imaging was performed on a 3-Tesla TIM TRIO scanner (Siemens, Erlanger, Germany) using an 8 channel head coil. fMRI data comprised 210 echo-planar (EPI) BOLD sensitive volumes (TR=3000 ms, TE=26 ms, 45 slices parallel to anterior-posterior commissure plane acquired in interleaved order and no gap, 3.0 mm slice thickness, field of view=224 mm). In addition, a 3D T1-weighted MPRAGE sequence was obtained for anatomical reference (TR=2300 ms TE=3.03 ms, 192 sagittal slices, matrix size=256 x 256, 1mm isometric voxel, TI = 900ms, Flip Angle = 9º).

2.5 Image processing

Imaging data were analysed with SPM8 (Wellcome Trust Centre for Neuroimaging, UCL, United Kingdom). Images were visually inspected for eventual artifacts and centered on the anterior commissure. Standard pre-processing was applied. The first step was realignment to the first volume to correct for interscan movements. After realignment, a mean EPI image was created and normalized to the standard stereotactic space defined by the Montreal Neurological Institute (MNI) using the EPI template from SPM as a target. Subsequently, fMRI images were spatially normalized to MNI by applying the mean image normalization parameters. Functional images were then smoothed with an 8-mm FWHM Gaussian kernel. Low-frequency noise was removed from the fMRI temporal series by applying a high-pass filter (cut-off of 128 s). Effects were modeled using an array of delta functions at the presentation times convolved with the canonical hemodynamic response function, separately for each kind of stimulus. Significant hemodynamic changes for each condition were examined using the General Linear Model. Statistical parametric maps for each predefined contrast were calculated on a voxel-by-voxel basis for each subject. Negative emotions (sadness, fear and anger) were contrasted individually against scrambled.

2.6 Statistical Analysis

2.6.1 Analysis of demographic and clinical data

Descriptive analyses of demographic and clinical measures (CAPE Positive and Negative and STAI-S) were carried out in STATA 10.0 (24). As the sample was based on twins, between-group differences in demographic and clinical measures were analyzed with regression models (and post hoc tests). Non-independence of clustered twin data was corrected for using tests based on the sandwich or Huber/White variance estimator (25). Also, since the sample was not clinically homogenous, differences in demographic and clinical variables among the four groups included (concordant affected, discordant affected, discordant healthy and healthy control twins), were explored. Group differences in continuous variables (age, STAI-S, STAI-T, BDI-II, CAPE Positive and CAPE negative) were examined using linear regression models. Group differences in categorical variables (gender and lifetime DSM-IV-TR diagnoses: anxiety disorders, major depressive disorder or comorbid anxiety and major depressive disorders) were examined by logistic regression models.

2.6.2 fMRI group analyses

A $P<0.001$ uncorrected threshold was set for whole brain observations but detected clusters had to meet $P<0.05$ family-wise error (FWE; corrected for multiple comparisons) voxel corrected to be considered as a significant result. Anxiety level score before MRI scan was included as nuisance variable in all analyses. The effect of viewing facial expressions versus the control condition stimuli was tested using a one sample t-test in the whole sample. To test the association between facial emotion processing and PEs we used two full factorial designs. Firstly, whether PEs dimensions were associated with brain response to facial emotion was tested in the whole sample. One factor with four levels (representing the four groups) was specified in the model indicating non-independence within the four levels to account for within-pair correlated observations. Secondly, to test whether brain responses to facial emotion differed among the four groups as a function of PE dimensions, an interaction between the group and PE dimensions was tested. In both cases, facial emotion processing and positive and negative PEs were assessed separately.

Table 1. Demographic and clinical data. Means and standard deviations are indicated for continuous measures. Number of individuals with depression, anxiety and comorbid depression and anxiety and percentage of the total affected individuals in each group is indicated.

	Whole Sample	Affected Concordant	Affected Discordant	Healthy Discordant	Control Healthy	Analysis F or χ^2 (df); p
Total Individuals	53	12	10	10	21	-
M/F	20/33	2/10	3/7	3/7	12/9	2.80 (2); .248
Age	35.5 (10.8)	40.8 (13.3)	33.2 (11.4)	33.3 (11.3)	34.7 (8.0)	.76 (3); .524
Lifetime DSM-IV-TR Diagnosis						
Depression	9 (41%)	3 (25%)	6 (60%)	-	-	
Anxiety	6 (27%)	5 (41.7%)	1 (10%)	-	-	3.70 (2); .161
Comorbid	7 (32%)	4 (33.3%)	3 (30%)	-	-	
STAI-State	14.5 (7.9)	19.9 (9.5)	11.9 (5.6)	11.8 (5.6)	13.9 (7.8)	1.79 (3); .174
STAI-Trait	15.8 (8.6)	20.4 (7.5)	17.5 (12.1)	18.7 (6.5)	11.0 (5.9)	4.77 (3); .009 (a; b)
BDI-II	6.5 (7.8)	10.2 (7.5)	5.3 (3.5)	5.2 (5.8)	5.5 (10.4)	1.33 (3); .067
CAPE Positive	25.2 (3.4)	25.7 (2.2)	26.5 (5.1)	26.5 (3.3)	23.6 (2.6)	1.43 (3); .256
CAPE Negative	22.5 (5.6)	24.8 (4.7)	21.2 (3.2)	24.8 (3.3)	20.8 (7.1)	2.3 (3); .100

M = males; F = females; DSM-IV-TR= Diagnostic and Statistical Manual of Mental Disorders; STAI= State-Trait Anxiety Inventory

a) Significant differences between concordant affected and control healthy twins
b) Significant differences between healthy discordant and control healthy twins

3. RESULTS

3.1 Demographic and clinical data

Mean age of the sample was 36.7 years (SD=13.4), and 37.7% (n=20) were males. From the 22 affected individuals, six had a lifetime history of anxiety disorders including specific phobia, social phobia, panic disorder, agoraphobia and obsessive-compulsive disorder; 10 individuals had a lifetime history of MDD and six presented comorbid anxiety and MDD.

Demographic and clinical data for the whole sample and for each group are listed in Table 1. Age, gender distribution, number of individuals affected by depression, anxiety or both disorders, STAI-S and positive and negative PEs scores did not significantly differ across the groups. Concordant affected and discordant healthy twins scored significantly higher in anxiety trait variable (STAI-T) compared to healthy control twins (Table 1).

3.2 Effect of emotional faces

At the brain level, viewing facial expressions (>scrambled faces) elicited significant activation of limbic (hippocampus, amygdala, parahippocampus, anterior cingulate cortex), occipital (occipital inferior gyrus, calcarine, lingual), parietal (postcentral gyrus, paracentral lobule, supplementary motor area), frontal (medial frontal gyrus, superior frontal gyrus, precentral gyrus, gyrus rectus) and temporal areas (temporal superior pole, middle temporal gyrus, temporal superior gyrus) and cerebellum in the whole sample (Table 2; Fig 1).

3.3 PEs and response to facial emotion

Testing the relationship between PEs scores and brain response to emotional stimuli, activation of the anterior cingulate cortex (ACC) to angry faces was positively associated with the negative dimension of PEs (Table 3; Fig 1). Also, a significant negative association was also found between activation of the ACC to fear faces and positive PEs (Table 3; Fig 1). No other clinically relevant associations were found for the rest of the stimuli categories.

3.4 PEs x Group interaction

For group vs PEs interactions, positive PEs moderated brain activation to fear faces in different cerebellum regions in affected concordant twins for anxiety and depression, but not in the other groups (Table 4; Fig 1). Another cluster including left parahipocampus, left fusiform and left hippocampus was marginally significant (Table 4; Fig 1). Again, no other clinically relevant associations were found for the rest of groups and stimuli categories.

4. DISCUSSION

Regarding the first aim of the study, our findings suggest that the presence of psychotic experiences (PEs) can be directly related to how individuals process negatively valenced emotional stimuli in the anterior cingulate cortex (ACC). Specifically, positive and negative dimensions of PEs were associated with hypoactivation and hyperactivation of the ACC to angry and fearful faces, respectively. The ACC is thought to play a key integrative role in emotion, performance monitoring, motivation and arousal (26-27). Both structural and functional studies have implicated this structure in schizophrenia (28-31). Similar fMRI studies have found activation changes in ACC to aversive stimuli when comparing schizophrenic patients to healthy subjects (29). As we used a non-clinical sample, our findings may indicate that activity changes in ACC when processing emotion could be related to vulnerability to psychosis.

Table 2. Effect of viewing faces with emotional content (sad, fear, angry, surprise, happy) versus scrambled face. Size does include voxels located at white matter (outside) or brain regions represented with less than 10 voxels.

Brain Region	Cluster - Level			Peak-Level				
	Cluster-Size	Size	p(FWE)	MNI Coordinates			T(max)	p(FWE)
				x	y	z		
Parahippocampal gyrus (R)	692	175	0.000	18	-10	-20	7.7	0.000
Amygdala (R)		166		20	-4	-28		
Hippocampus (R)		149		20	-10	-20		
Temporal Superior Pole (R)		46		28	4	-24		
Hippocampus (L)	430	251	0.003	-20	-10	-20	7.0	0.000
Amygdala (L)		118		-22	-8	-16		
Parahippocampal gyrus (L)		21		-18	-8	-24		
Occipital Inferior Gyrus (R)	237	93	0.044	24	-100	-10	6.7	0.001
Lingual (R)		71		22	-100	-10		
Calcarine (R)		61		22	-102	-6		
Postcentral gyrus (L)	661	200	0.000	-34	-26	72	6.5	0.001
Precentral gyrus (L)		146		-34	-24	72		
Paracentral lobule (L)		133		-14	-26	80		
Supp. Motor Area (L)		114		-6	28	66		
Frontal Sup. Medial (L)		18		-6	56	24		
Frontal Sup. (L)		11		-12	52	22		
Frontal Sup. Med. (L)	1192	614	0.000	6	56	24	6.3	0.002
Frontal Sup. Med. (R)		373		2	54	22		
Frontal Sup. (L)		95		-12	52	22		
Anterior Cingulate (R)		57		6	52	14		
Anterior Cingulate (L)		31		0	28	0		
Temporal Mid Gyrus (R)	533	276	0.001	56	-34	4	6.3	0.002
Temporal Sup Gyrus (R)		217		54	-34	4		
Cerebelum Crus 2 (L)	248	183	0.037	-20	-90	-32	6.0	0.006
Cerebelum Crus 1 (L)		65		-24	-90	-30		
Frontal Med Orb (L)	296	123	0.019	0	38	-18	5.6	0.020
Frontal Med Orb (R)		90		2	50	-12		
Gyrus rectus (R)		42		2	38	-18		
Gyrus rectus (L)		41		0	38	-18		

R, right; L, left; MINI, Montreal Neurological Institute; FWE, family wise error correction.

However, negative PEs were associated to activation in the ACC in response to angry faces in our study. This is contrast with a previous study in which activation of the ACC to aversive images was inversely related to severity of avolition and anhedonia symptoms in a group of schizophrenic patients (29). On the other hand, while a negative association was found between ACC activation to fear faces and positive PEs, there is evidence indicating that increased reactivity of limbic areas involved in emotion processing is correlated with positive symptoms (12, 32-33). These discrepancies between our findings and previous ones may be due to differences in methodology and analytic strategy. For instance, the study of Dichter and colleagues (29) used a different emotional task paradigm and their study included adults with schizophrenia and healthy control adults, while ours included individuals from the general population, some of them affected by anxiety and depression. Furthermore, although compensatory mechanisms have been proposed to account for impaired emotion processing in psychosis (34-35), the neurobiological mechanisms underlying hyperactivations and hypoactivations linked to positive and negative dimensions respectively remain unclear.

Table 3. Brain regions which deactivation was significantly correlated with positive PEs during fear faces presentation (A). Brain regions which activation was significantly correlated with negative PEs during angry faces presentation (B). Size does include voxels located at white matter (outside) or brain regions represented with less than 10 voxels.

Brain Region	Cluster - Level			Peak-Level				
	Cluster- Size	Size	p(FWE)	MNI Coordinates			T(max)	p(FWE)
				x	y	z		
A) Positive and fear								
ACC (R)	352	68	0.028	4	28	8	4.7	0.184
ACC (L)		128		-2	36	12		
B) Negative and anger								
ACC (R)	352	129	0.007	6	52	8	5.3	0.060
Frontal Sup. Medial Cortex (R)		124		6	56	6		
ACC (L)		87		2	50	8		

R, right; L, left; MINI, Montreal Neurological Institute; FWE, family wise error correction.

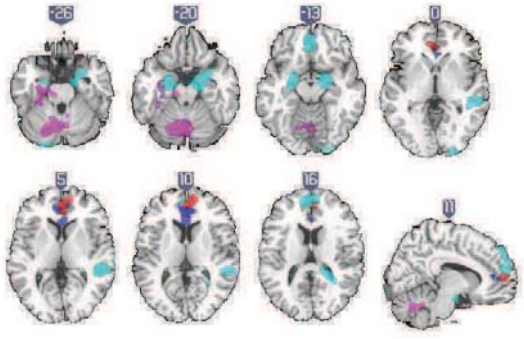

Fig. 1. Brain areas where activity changes to facial emotion were observed in relation to psychotic experiences are depicted in sagital (first row), coronal (second row) and axial slices (third row). Brain regions activated when viewing emotional faces are indicated in cyan. Effect of negative psychotic experiences on brain activation to angry faces is indicated in red. Effect of positive psychotic experiences on brain deactivation to fear faces is indicated in blue. Brain areas of concordant affected twins for anxiety and depression where brain activation to fear faces was moderated by positive psychotic experiences are indicated in violet. Orange colour indicates overlapping among brain areas.

Regarding the second aim of the study, positive PEs were found to moderate emotional processing in individuals affected by depression and anxiety disorders indicating that these psychopathological dimensions may share altered emotional functioning. The fact that positive PEs but not negative PEs moderated brain response in concordant depressed and anxious twins is in line with the consistently reported stronger relationship of affective symptoms with the positive than with the negative dimension of psychotic symptoms (36). Specifically, the positive dimension of PEs moderated emotional processing of fearful faces in individuals affected by depression and anxiety mainly in cerebellar areas. Although cerebellum has been traditionally argued to play a pivotal role in posture, balance and movement coordination, there is increasing evidence that this brain area is also involved in cognition and emotion (37-38). The cerebellum is connected with the limbic system and cortex. Different neural pathways involving cerebellum have been proposed in relation to abnormal emotion processing in schizophrenia, depression and anxiety (37). Indeed, the cerebellum has been found to be involved in pathophyisiology of depression in a meta-analysis of brain activation changes in depression (Fitzgerald *et al.,* 2008) and it has also been linked to vulnerability to psychosis (39). The experience of positive psychotic symptoms, depression or anxiety can be considered particularly associated with negative affect (negative psychotic symptoms reflect a relative deficit in affect that is inconsistent with the experience of anxiety and depression (36)). Changes in cerebellar blood flow have been reported during the experience of negative mood states (40). Taken together, our findings suggest that these psychopathological traits; psychotic experiences, depressive and anxious symptoms, may share altered emotional functioning mainly implicating cerebellar areas.

Interestingly, positive PEs moderated brain response to facial emotion only in the concordant affected MZ twins for anxiety and depression but not in the other groups. This group seemed to present a more severe expression of anxiety and depression disorders than the discordant affected twins based on the clinical measures. This is in agreement with the notion that MZ twins concordant for anxiety and depression carry a particularly high genetic load for these disorders, especially compared to discordant MZ twins. De Geus and colleagues (41) provided support for this notion observing higher levels of anxiety, depression and neuroticism among parents of concordant twins than in parents of healthy twins (41). Therefore, it might be possible that the occurrence of positive PEs only moderates brain response to facial emotion in individuals affected by anxiety and depression carrying a particularly high genetic load for these disorders which might be related to a more severe expression of these disorders.

The current study has to be considered in the context of its limitations. Firstly, the sample size is modest and the present findings need to be further explored and replicated in larges samples. Secondly, the clinical heterogeneity of the affected twins (depressive and anxious symptoms) may be a source of variability not controlled in the present study since diverse signs and symptoms may have distinct neurophysiological correlates (42). Nevertheless, the

extent to which differing symptom profiles across depressed samples have contributed to differences in the results across studies remains unclear. Furthermore, it has been argued that anxiety and depression may have common etiological pathways (43-44).

In conclusion, the occurrence of PEs is related to changes in brain response to facial emotion. These findings support the hypothesis of emotion dysregulation in the psychosis continuum and suggest that abnormalities in emotion regulation may be part of the vulnerability to psychosis. Futhermore, positive dimesion of PEs moderates emotional processing in individuals affected by depression and anxiety disorders. This suggests that these psychopathological dimensions may share altered emotional functioning.

Role of funding source

Funding sources listed in the Acknowledgement section had no role in the study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the paper for publication.

Contributors

Alemany, S contributed to design of the study, data collection, statistical analysis, interpretation of results and drafted the manuscript. Falcón, C supervised MRI data processing contributed to statistical analysis, interpretation of results and writing of the paper. Goldberg, X contributed to data collection, clinical part of the study and writing of the manuscript. Mas, A processed and analyzed MRI data and contributed to statistical analysis. Bargalló, N contributed to implementation of the MRI protocol and writing of the manuscript. Garrido, C contributed to implementation of the MRI protocol and implemented the emotional paradigm in the MRI protocol. Gastó, C and Nenadic, I contributed to writing of the manuscript. Fañanás, L supervised the design of the study, interpretation of results and writing of the manuscript.

Conflicts of interest

All authors report no conflict of interest.

Acknowledgements

We gratefully acknowledge the collaboration of the participants. This study was supported by the Ministry of Science and Inovation (SAF2008-05674-C03-00 and 03), the Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), European Twins Study Network on Schizophrenia Researcg Training Network (grant number EUTwinsS; MRTN-CT-2006-035987; PIs: L.F., I.N.) and by the Comissionat per a Universitats I recerca del DIUE of the Generalitat de Catalunya (2009SGR827). Goldberg X, was supported by a Marie Curie grant (grant number EUTwinsS; MRTN-CT-2006-035987). Alemany S, thanks to the Institute of Health Carlos III for her PhD grant (FI00272).

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

Dr. Lourdes Fañanás Saura, Associate Professor (Profesora Titular) at the Department of Animal Biology of the Faculty of Biology, University of Barcelona and supervisor of the present doctoral thesis by Silvia Alemany, hereby certifies that the participation of the PhD applicant in the article "Psychotic experiences influence emotional processing in individuals affected by anxiety and depression: An fMRI community-based twin study" included the following tasks:

- Participation in the conception and design of the study
- Twin recruitment and collection of data
- Analysis and interpretation of data
- First drafting of the manuscript
- Critical revision of the arcticle for intellectual content

Dr. Lourdes Fañanás

Barcelona, February 11th 2013

Cover Design

Salvador Alemany (2013) - www.salvadoralemany.net