UNIVERSITAT DE BARCELONA

Early Neurodevelopment, adult human cognition and depressive psychopathology: analysis of neuroimaging brain correlates and epigenetic mediators

Aldo Córdova Palomera

Early neurodevelopment, adult human cognition and depressive psychopathology: analysis of neuroimaging brain correlates and epigenetic mediators

Ph.D. thesis presented by

Aldo Córdova Palomera

Directed by Prof. Dr. Lourdes Fañanás Saura

Associate Professor, University of Barcelona

Doctoral program in Biomedicine

Anthropology Unit – Department of Animal Biology – Faculty of Biology

University of Barcelona

Lourdes Fañanás Saura — Advisor

Aldo Córdova Palomera — Ph.D. Candidate

The following funding sources have partly sponsored this doctoral thesis:

- Ministerio de Educación y Ciencia. Project: “Analysis of epigenetic mechanisms and DNA polymorphisms in candidate genes for schizophrenia and major affective disorders: an approach based on European MZ Twin samples and case-control family designs” (SAF2008-05674-C03-00).
- ERA-NET NEURON (Network of European Funding for Neuroscience Research). Project: “Genetic factors, brain dysfunction and clinical phenotypes in schizophrenia and autistic disorders” (PIM2010ERN-00642).
- Centro de Investigación Biomedica en Red de Salud Mental (CIBERSAM), Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación, Gobierno de España.
- The Institute of Biomedicine of the University of Barcelona (IBUB).
- Generalitat de Catalunya. Sponsorship to the research group "Genes and environment in the comprehension of diversity of human behaviour and the ethiopathogeny of mental illness" (2009SGR827 and 2014SGR1636).
- National Council for Science and Technology (CONACyT, Mexico). Ph.D. fellowship grant.

Part of this thesis was developed at the Dutch Connectome Lab, in the Brain Center Rudolf Magnus (University Medical Center Utrecht). This research stay was conducted under the supervision of Dr. Martijn van den Heuvel, and sponsored by the National Council for Science and Technology (CONACyT, Mexico) and ERA-NET NEURON (Network of European Funding for Neuroscience Research).

שבקתני למא אלהי אלהי

Matthew 27:46

CONTENTS

1. INTRODUCTION

1.1. Neurophysiological plasticity and (ab)normal psychology

One of the wide-sense definitions of *experience* is “an event or occurrence which leaves an impression on someone” (Simpson *et al.*, 1989). In contemporary Psychobiology, it is broadly accepted that human brain organization is fundamentally shaped by *experience*. This concept would embrace not only factors from outer events, but also “internal” incidents such as genetically encoded developmental changes, or merely psychological processes (Kolb, 1995).

In this sense, constantly fluctuating experiential challenges largely influence brain evolution at both phylogenetic and ontogenetic levels (Killackey, 1990). Normally, brain performance and human behavior are characterized by great flexibility in response to these context-specific experiences (Greenough *et al.*, 1987; Gunnar and Nelson, 1992). Biologically, this continuous flow of experiential interactions leading to brain evolution is fostered by brain plasticity, a process consisting of several organized and extremely dynamic steps, which are genetically determined, epigenetically directed and environmentally influenced (Gilbert *et al.*, 2005; Tau and Peterson, 2010). Of note, recent research indicates that brain plasticity is closely related to individual differences in experience, and that these experiential dissimilarities constitute the very basis of phenotypic individuality (Freund *et al.*, 2013).

The presence of enriching experiences, especially during specific developmental windows, may increase the adaptability of humans and other species through cerebral modifications (Rosenzweig and Bennett, 1996). However, human individuals may likewise lose the normal plasticity of their brains when exposed to unbearable patterns of stress during their lifespan (Harkness *et al.*, 2015). Highly stressful experiences during critical or circumscribed periods of brain development can impair, often permanently, the activity of major neuroregulatory systems, with profound and lasting neurobehavioral consequences as those observed in cognitive impairments and psychopathological disorders (de Kloet *et al.*, 2005).

As can be inferred from above, the notion of brain plasticity has enormous consequences for the understanding of behavior, cognition and psychopathology. Though outstanding conceptual and technically complex issues still exist in the empirical formulations of a link between the neurophysiology of the brain and both psychic and behavioral phenotypes (Schall, 2004), the contemporary tradition in brain science has largely built upon –and continues developing– this concept (Rolls, 2012). Accordingly, the experiential configuration of brain plasticity would mediate the plasticity of phenotypes such as those studied by Psychology, Psychiatry and related behavioral sciences.

1.1.1. Phenotypic plasticity in response to the experience

In the behavioral sciences, the concept of phenotypic plasticity can be roughly categorized into two classes: developmental and activational plasticity (Snell-Rood, 2013). In short, the concept of *developmental plasticity* denotes the capacity of an individual carrying a specific genetic background to adopt different developmental trajectories under distinct settings. This kind of plasticity has largely been studied in the literature. It parallels –and is sometimes equivalent to– the concept of phenotypic plasticity in evolutionary biology. Phenotypic plasticity was initially popularized in the early 1960s as the mechanism whereby the expression of a genotype is altered by its environment (Bradshaw, 1965). Despite its early origin, research on phenotypic plasticity is still under development (Walsh *et al.*, 2015). For instance, recent studies on the epigenetics of disease highlight the role of phenotypic plasticity to understand the biological disruptions leading to pathological conditions (Feinberg, 2007; Petronis, 2010).

Classical studies in evolutionary biology recognize phenotypic plasticity as a potentially important element in evolution, which is under genetic control and, remarkably, may or may not be adaptive (Caswell, 1983). Though the idea of an “adaptive” plasticity contrasts with the notion of *homeostasis* as an evolutionary goal, theoretical formulations have demonstrated how these two

concepts can converge: in order to promote adaptation, plasticity in “response variables” should shelter “essential variables” from environmental disturbances (Ashby, 1956; Caswell, 1983).

Thus, *developmental plasticity* is thought to promote integrated adult phenotypes (at the ontogenetic level) and to be one –though not the sole– mechanism prompting phylogenetic evolution. A schematic representation of this concept using neural networks has been proposed by Snell-Rood (2013), and has been adapted here in Figure 1. As shown in sections A and B, if there were two genetically-identical individuals, differences in their experiential inputs (E1 and E2) may sensitize distinct afferent, processing and efferent network layers to change the probabilities of reaching particular psychobiological phenotypes (P1 or P2). For instance, the individual in section A, after continuous exposure to a given experience E2, may have increased its synaptic weights to favor a particular phenotype (i.e., P2). In contrast, the final phenotype outcome should be different had this individual been exposed to E1. In that case, other neural circuits could have been sensitized to give rise to a different phenotype (i.e., P1). Thus, during adulthood, the individual in section A may be relatively insensitive to stimulus E1, whereas the individual in section B may lack an appropriate biobehavioral response to stimulus E2. Though this may seem a somehow abstract model, its biological feasibility is clearly observed in phenomena such as synaptic potentiation and depression (Linden, 1994; Royer and Pare, 2003).

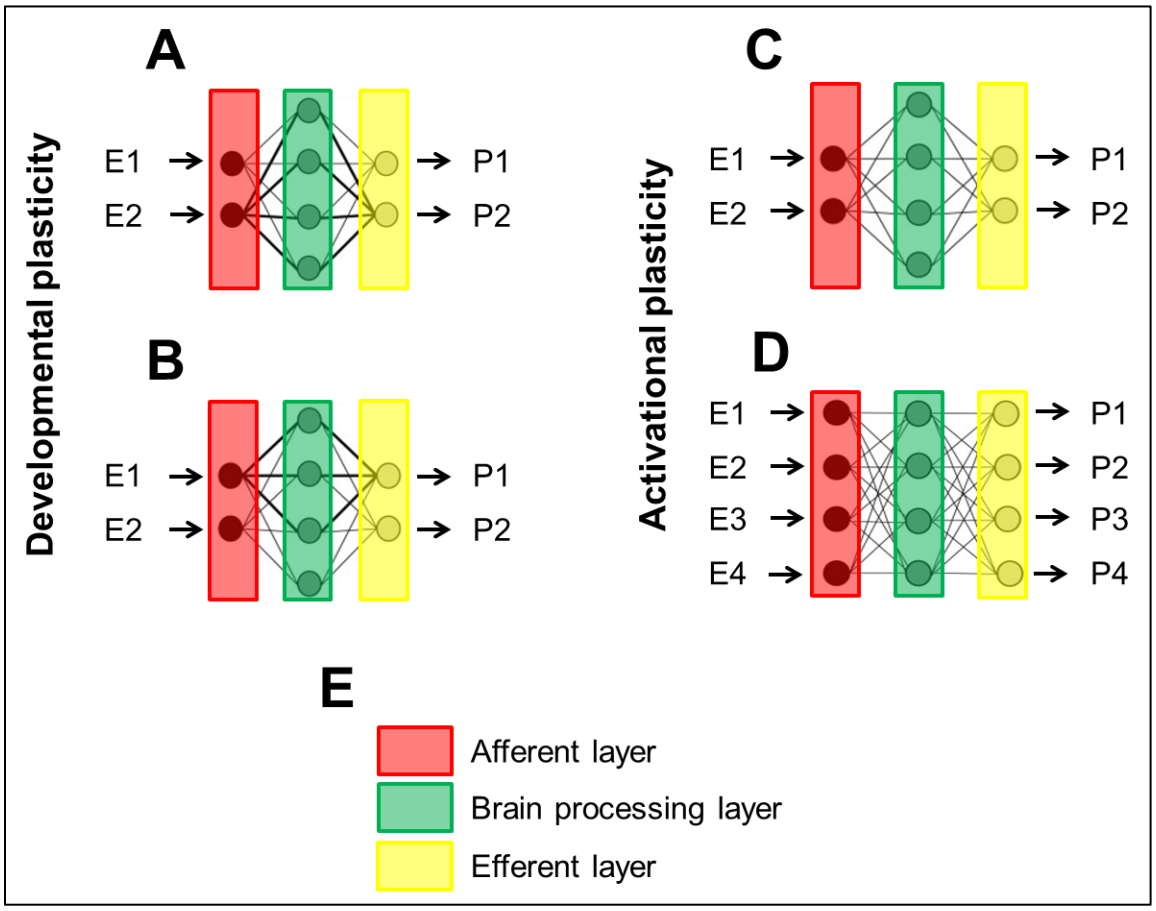


Figure 1. Neural network diagrams illustrating behavioral plasticity. A: Activational plasticity refers to differential activation of an underlying network by different environments. B: Developmental plasticity refers to the differential development of neural networks in different environments such as a change in synaptic weights as a result of experience. Adapted from Snell-Rood (2013).

Complementarily, *activational plasticity* refers to the differential activation of underlying networks (Snell-Rood, 2013). In the present context, there may be some overlap between this concept and the notion of *phenotypic flexibility* (Forsman, 2014). An individual with high activational plasticity would be able to detect a wide range of environments (E1-E4), and to respond to it using a psychobiological phenotype from a relatively large catalogue (P1-P4), as depicted in section D of Figure 1. In contrast, individuals with low activational plasticity would be insensitive to some

environmental clues, and would have only a small set of responses in front of their experiences (Figure 1, section C).

As mentioned by Snell-Rood (2013), this type of plasticity has likewise been referred to as “behavior as plasticity” or “innate behavioral plasticity” (Dukas, 1998; Mery and Burns, 2010). Innate behavioural responses lead to behavioral modifications in response to the environment (Mery and Burns, 2010). High activational plasticity may be enormously advantageous when an individual is required to show rapid and reversible behavioral changes in response to extremely unstable temporally fluctuating environments (Van Buskirk, 2002). Nevertheless, issues such as environmental differences between generations of individuals may prompt other kinds of flexibility (Moran, 1992) such as the aforementioned developmental plasticity.

1.1.2. Developmental plasticity: the brain and the early origins of mental disorders

Human brain development occurs across several phases, conferring a scenario of potential vulnerability windows. Early brain insults can often have severe consequences on the ensuing growth stages (Andersen, 2003).

A conventional schema portraying normal neurodevelopmental windows can be found in Figure 2. As depicted therein, the earliest maturational stages typically imply a large number of modifications (birth, migration, synaptic production, and others) to the majority of neurons. These biological processes provide the basis of the long-lasting impact of early insults. Immature brains adapt by integrating information to generate a stable biological structure and appropriate functioning mechanisms, whereas grown-up organisms normally adapt to changing stimuli by compensatory mechanisms (Andersen, 2003). Paralleling the notions deployed in the previous section of this document, information coming to immature brains would help sensitize different structures and functions to rise a relatively complete adult organism (developmental plasticity?), whereas

accommodation of the adult brain to rapidly changing environments would depend on timely mechanisms (activational plasticity?).

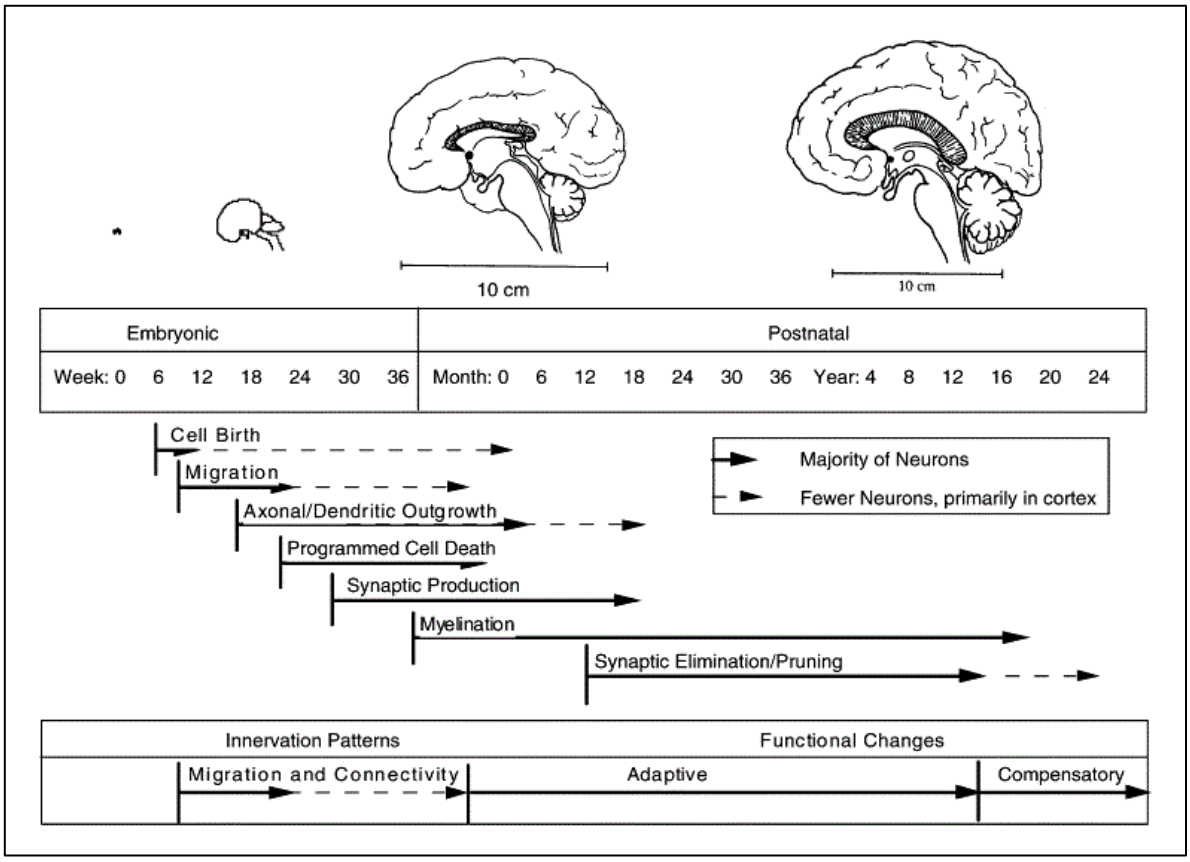


Figure 2. Stages of brain development and windows of vulnerability. Developmental periods occurring in phases set a stage for putative vulnerability periods. Early life insults (bottom) would be integrated as innervation patterns, and a later pre-pubertal insult may cause more adaptive functional changes. Adapted from Andersen (2003).

Theories of early neurodevelopmental origins of adult diseases have been robustly validated for psychiatric conditions with clinical manifestations as diverse as schizophrenia, autism, bipolar disorder, depression and even personality disorders (Raine, 2006; Ansorge *et al.*, 2007; Gliga *et al.*, 2014; Bavamian *et al.*, 2015). In addition, cognitive phenotypes closely related to

neuropsychiatric disorders are extremely sensitive to neurodevelopmental stages of maturation (Bunge and Wright, 2007).

Among various hypotheses, these theories of early developmental disruptions propose that specific genetic and environmental vulnerability factors may alter the maturation of brain circuits to increase the risk for psychopathology and cognitive deficits later in life (Ansorge *et al.*, 2007). Namely, these neuropsychiatric conditions would be the final state of an abnormal neurodevelopmental trajectory started several years before the disease onset (Rapoport *et al.*, 2012).

Complementarily, further research suggests that other neurodevelopment-based mechanism underlies the clinical manifestation of several neuropsychiatric disorders. Specifically, it has likewise been proposed that, though these diseases may have a peak in symptom manifestation at certain points in life, different symptom courses may be present both before and after the "clinical onset" (Andersen, 2003; Paus *et al.*, 2008). This is depicted by epidemiologically-derived data in Figure 3. For instance, though the classical hypotheses on the neurodevelopmental roots of schizophrenia would suggest a severe prenatal disruption of brain development due to (intra-uterine) environmental factors which manifests during adulthood (Fananas *et al.*, 1990; Fananas *et al.*, 1996; Rapoport *et al.*, 2012), Figure 3 indicates that some (soft?) clinical signs can be observed even before the age of fifteen. Likewise, though the clinical onset (and the symptom peak) of mood disorders would be at around 25 years of age, some signs should appear before the age of ten. These evidences may somehow indicate that some brain sensitization mechanisms may gradually evolve to sustain the clinical expression of the disease. Perhaps this process may be related to developmentally-induced neural facilitation and depression, one of the very bases of plasticity.

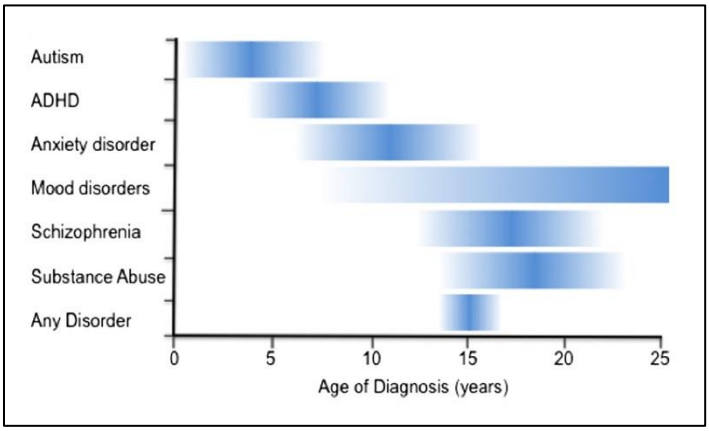


Figure 3. Age of onset and peak of mental disorders. Adapted from Casey *et al.* (2014) and Paus *et al.* (2008).

1.1.3. Is there also room for activational plasticity in psychopathology?

The classical ethological framework proposes that when there is no sensitivity to fluctuations, a behavioral pattern is repetitive despite contextual differences, and thus an organism lacks plasticity (Japyassú and Caires, 2008). Stereotyped behavior may thus represent a state of low activational plasticity (Japyassu and Malange, 2014). The extent to which this type of behavior may be maladaptive and lead to (psycho)pathological conditions has largely been studied within the subject of psychological flexibility (Kashdan and Rottenberg, 2010; Aldao and Nolen-Hoeksema, 2012). In short, psychological flexibility –as opposed to stereotypy– is thought to promote mental health by spanning a wide range of capabilities such as i) adaptation to several situational demands, ii) mindset and behavioral shifting to promote personal functioning, iii) maintenance of balance across life domains and iv) awareness and commitment to behavior promoting specific values (Kashdan and Rottenberg, 2010). A lack of psychological flexibility –corresponding to low activational plasticity– has been suggested as a key factor behind several psychopathological conditions such as depression and anxiety (Fresco *et al.*, 2006; Kashdan and Steger, 2006; Bylsma *et al.*, 2008). This lack of adaptability seems at least partly rooted on cognitive profiles, *default* mental states and personality (Kashdan and Rottenberg, 2010), three factors that have likewise been related to a

broad range of psychiatric outcomes (Halligan and David, 2001; Widiger, 2011; Whitfield-Gabrieli and Ford, 2012).

Taken together, these evidences provide a basis to understand a large extent of the etiology and the clinical manifestation of severe psychiatric disorders. They somehow argue for a model of psychopathology as disrupted ways of integrating the experience through brain plasticity mechanisms.

1.2. An empirical approach to psychopathology and plasticity: genetically informative designs

The previous ideas support a theoretical framework to understand the concept of psychopathology through the dynamics of brain plasticity. Remarkably, these ideas have been outlined on the basis of experimentally derived (scientific) evidences. Namely, the empirical support to the concept of plasticity as a key element in mental health and disease comes from research spanning a huge amount of diverse techniques (Cicchetti and Cohen, 2006; Turecki *et al.*, 2014). The current section expands on some of the most popular approaches to this subject.

1.2.1. Gene × Environment interactions

A recent review by Belsky and Pluess (2013) has highlighted the historical role that genetically informative designs has had to approach the subject when investigating human populations. These authors emphasize that the study of Gene × Environment (G×E) interactions (Caspi *et al.*, 2002) has enriched the understanding of how some *susceptible* individuals have an increased risk for developmental alterations when exposed to adverse circumstances, whereas other subjects do not have this liability and can thus be considered *resilient*.

Until recent years, almost all the Psychiatry-related literature on (G×E) interactions had been conducted using the framework of "vulnerability genes" or "risk alleles" (Belsky *et al.*, 2009; Zammit *et al.*, 2010). A number of mechanisms have been proposed within this framework of "vulnerability genes". For instance, Figure 4 (sections A and B) exemplifies some of these paradigms. In section A, a genetic risk factor increases the risk for psychopathology in the broad population, although its effect is higher when individuals are exposed to environmental risk factors (in this case, cannabis use). Complementarily, section B shows another mechanism of vulnerability induced by the genetic background. Briefly, it shows how the average effect of the genetic risk factor on psychopathology in the population would be small if the people were not exposed to cannabis; however, cannabis users would have differential psychosis risk depending on their genotype.

Despite the usefulness of these classical G×E approaches built upon the notion of genetic vulnerability, the concept of "plasticity genes" has gained popularity in Psychiatry during the last decade (Belsky *et al.*, 2009). A "plasticity gene" would make individuals more responsive to both enriched/positive and deprived/negative environments. For example, section C of Figure 4 depicts how the effect of the genotype would depend on the (lack of) exposure to cannabis: valine allele carriers with no cannabis consumption would have low risk for psychosis, whereas those consuming cannabis would be at increased risk. Likewise, individuals lacking the valine allele may or may not display psychosis depending on whether or not they consumed cannabis. The valine allele would thus be a risk factor for psychosis in cannabis users and a protective factor in non-users. In this scenario, the valine allele would have (on average) no effect on psychosis risk in the population if there were equivalent amounts of cannabis users and non-users. But perhaps the clearest "plasticity gene" scenario is shown in section D. Therein, individuals who lack the valine allele have the same risk for psychosis, regardless of their cannabis consumption status. In contrast, valine allele carriers who do not consume cannabis have lower risk for psychopathology

than the rest of the population, and valine carriers who are also consumers have actually an increased risk for psychosis.

The mechanism shown in section D of Figure 4 may also be illustrative of the concepts of *developmental* and *activational* plasticity. For instance, let the horizontal axis span from *resilient* to *susceptible* organismic background. For the case of developmental plasticity, assume that the environmental exposure consists of prenatal insults, and let the vertical axis be the risk for schizophrenia during adulthood. Thus, broadly speaking, individuals who are not exposed to obstetric complications (dashed line in the plot) have similar risks for adult schizophrenia, regardless of their initial biological background. On the contrary, individuals who are genetically *susceptible* to schizophrenia (perhaps due to genetic alterations) would have an increased risk when exposed to environmental insults. Likewise, there may be a compensatory genetic load making other subjects *resilient* and thus reducing their risk to develop adult psychosis even in the presence of obstetric complications. Additional examples of G×E can be found across the literature (Alemany *et al.*, 2011; Estrada *et al.*, 2011; Goldberg *et al.*, 2013; Alemany *et al.*, 2014).

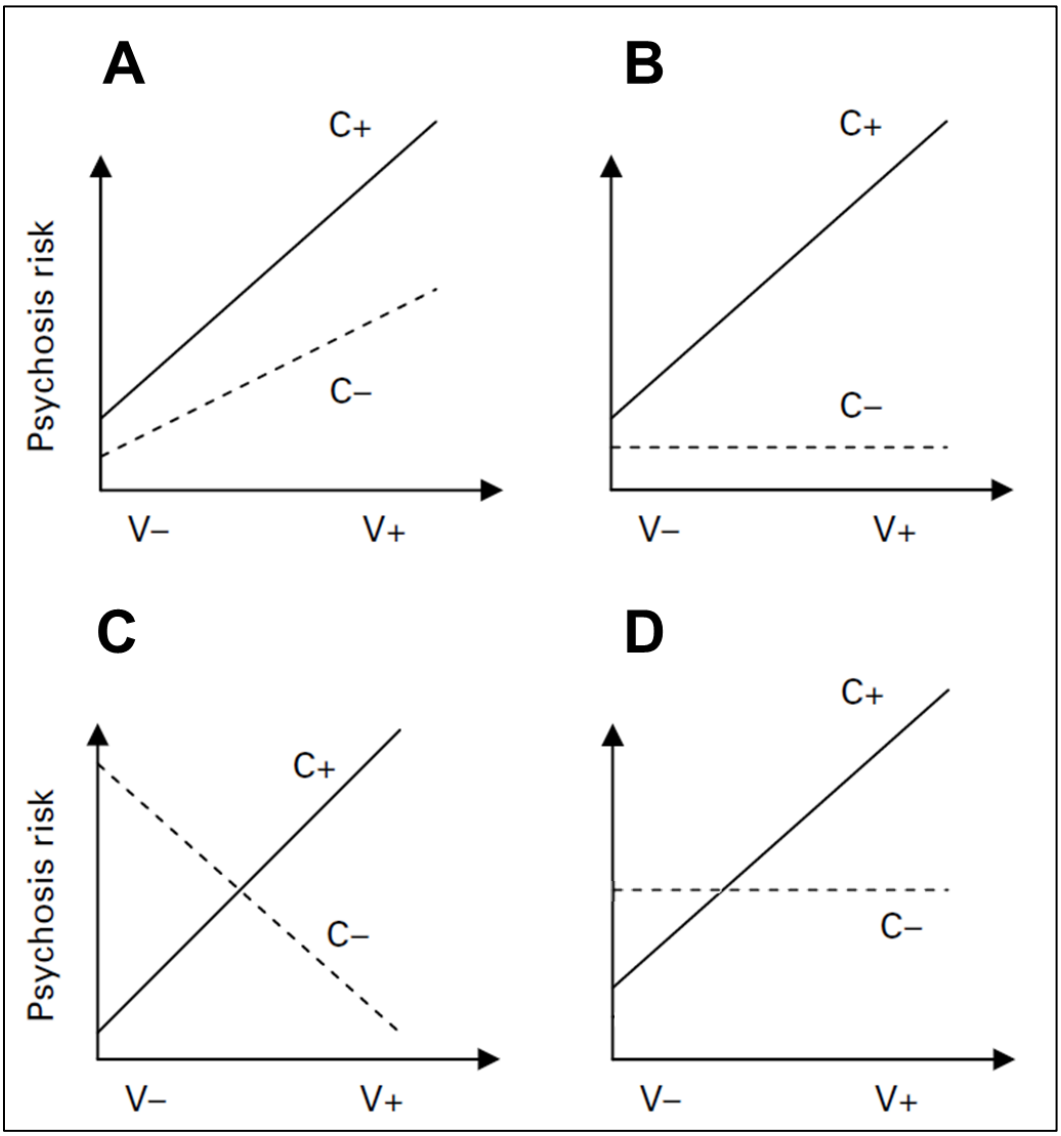


Figure 4. Four hypothesized mechanisms of G×E interaction between *COMT*'s rs4680 valine allele (V+: presence of valine allele, V-: absence of valine allele) and cannabis use (C+: presence of cannabis exposure; C-: no cannabis exposure) on risk of psychosis. Sections A and B represent classical "vulnerability gene" approaches, whereas sections C and D correspond to a "plasticity gene" effect. Adapted from Zammit *et al.* (2010).

Activational plasticity may be exemplified by considering short-term phenotypic responses. In the previous case, obstetric complications differentially affected *resilient* and *susceptible* individuals and, perhaps decades later, schizophrenic disorders would appear. In contrast, one could consider two variables with immediate connections: a particular stressful situation and an outcome of anxiety. In the absence of stressful situations, the majority of the individuals would have an average risk for an anxious disorder (dashed line). However, the emotional coping style of the individuals –which makes them more or less *susceptible* and *resilient*– could determine whether a particular person has low or high risk for anxiety following a concrete life event.

1.2.2. Monozygotic twin designs: classical approaches

Far-reaching psychiatric research has been conducted using human twins throughout the history. Perhaps the first studies on this topic using scientific methodologies were conducted in the 19th century by Savage (1883) and Gill (1883), by examining a couple of cases of mania and melancholia.

There are two types of twins: monozygotic (MZ) and dizygotic (DZ). MZ twin pregnancies result from a single fertilized egg (a "zygote") that suddenly splits producing two individuals. MZ twins have almost completely identical DNA sequences (Mastroeni *et al.*, 2009; van Dongen *et al.*, 2012). In contrast, DZ (also known as "fraternal") twins are produced when two zygotes are separately fertilized; as other pairs of siblings, DZ twins have an average of 50% of genetic resemblance. Some of the explanations about the appearance of this type of twinning include the maturation of more than one dominant ovarian follicle during the same menstrual cycle, or increased concentrations of follicle-stimulating hormone in the mother (Hall, 2003). Further information on the origins and biological features of twinning can be found in Figure 5 and elsewhere (Hall, 2003; Machin, 2009).

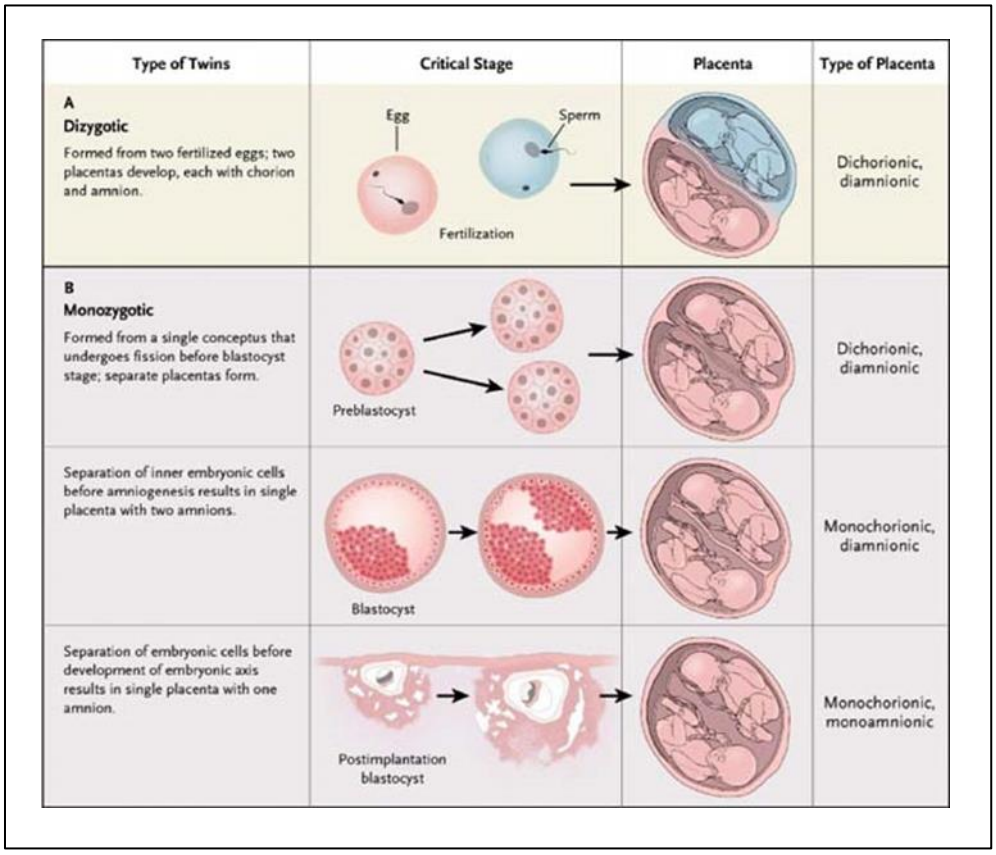


Figure 5. Origin of MZ and DZ twins. A: DZ twins are typically considered dichorionic diamniotic. B: MZ twins may be dichorionic diamniotic, monochorionic diamniotic or monochorionic monoamniotic. Adapted from Machin (2009).

Conventional approaches to study data from both MZ and DZ twins typically come from Quantitative Genetics (Posthuma *et al.*, 2003). Though there are several techniques to analyze this information, some principles of biometrical genetic theory classical twin studies have been largely employed in Behavior Genetics (Rijsdijk and Sham, 2002; Posthuma *et al.*, 2003). Typically, the methodologies used in this field are based on examining the correlations in phenotypic traits between MZ and DZ twins. From this, the relative importance of *latent* factors is derived as follows (Rijsdijk and Sham, 2002). Briefly, the phenotypic variance (P) can be decomposed into an additive equation of the next four terms: i) additive genetic influences (A: the sum of the effects of individual

alleles at all loci that influence the trait), ii) non-additive genetic influences (D: interactions between alleles), iii) environmental influences shared by family members (C: common environmental variation) and iv) differences among family members (E: unique environment) (Figure 6). Thus, the phenotypic variance is expressed as P = A + D + C + E, and the four elements contributing to the phenotype are disentangled by examining the patterns of correlation between MZ and DZ twins.

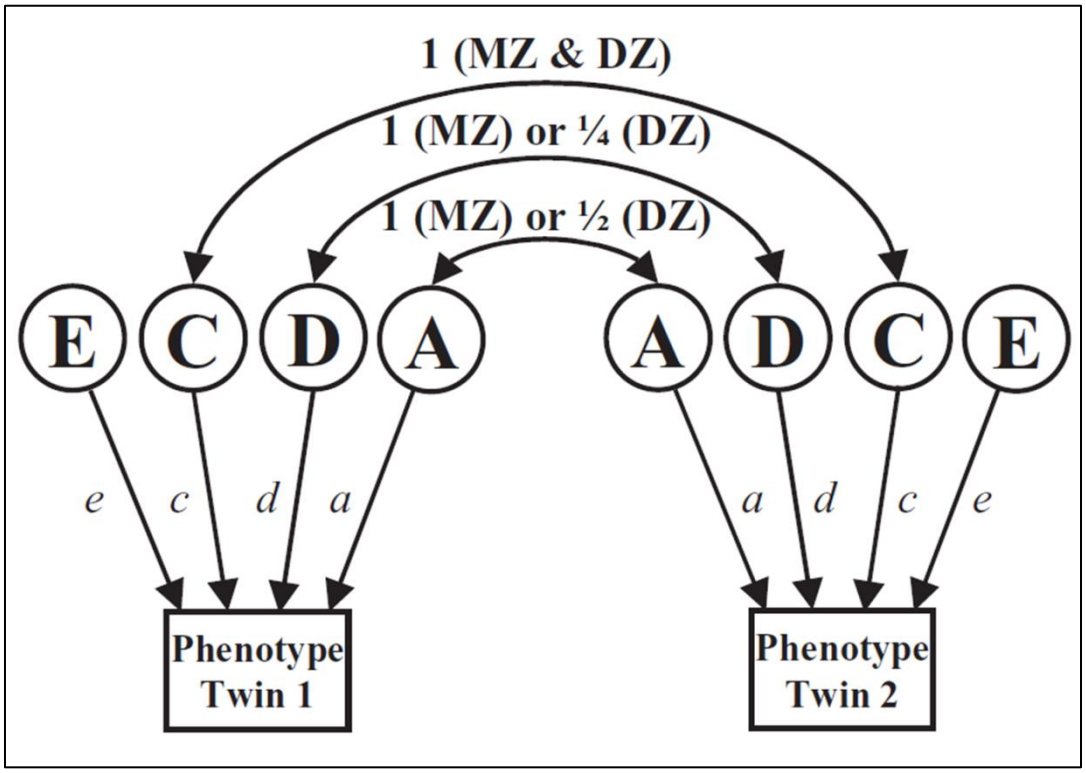


Figure 6. Path diagram for the basic univariate twin model. The additive "A" and dominance "D" factors have a correlation of 1 between MZ twins and 0.5 and 0.25 between DZ twins, respectively. Shared familial environment "C" has a correlation of 1 for both MZ and DZ twins who are reared together. "E" (unique environment) is the source of variance that will result in differences among members of one family; it is uncorrelated between members of MZ and DZ pairs. The path coefficients for "A", "D", "C" and "E" are represented by "a", "d", "c" and "e". Adapted from Rijsdijk and Sham (2002).

Though this way of decomposing the phenotypic variance to obtain estimates of different *latent* factors behind it has an enormous potential –as demonstrated by its popularity in the literature–, some points have to be addressed in order to use it in empirical studies of twin samples. For instance, this A + D + C + E model (or its A + C + E analogous) require relatively large sample sizes of both MZ and DZ twins (Posthuma and Boomsma, 2000; Draisma, 2011).

As an alternative, designs with only MZ twins have their own potential from the point of view of Quantitative Genetics, even when using moderate sample sizes (Hu *et al.*, 1998; Carlin *et al.*, 2005). Although these designs do not allow the dissection of phenotypic variance into the four different components described above, they provide a number of useful alternatives to parse out genes and environment:

i) specific non-shared environmental influences independent of genetic factors can be deduced from MZ twin data, since they are practically identical at the genetic level (Pike *et al.*, 1996);

ii) there is a relatively high degree of intrapair discordance in complex traits exhibited by MZ twins (Hall, 2003). This allows arranging genetically-informative designs with three groups of twin pairs: healthy (neither genetic nor environmental trait liability), discordant (unique environmental trait liability) and concordant (genetic liability) (Wolfensberger *et al.*, 2008; Borgwardt *et al.*, 2010; Ettinger *et al.*, 2012). By quantitatively comparing the traits across the three groups, some inferences on the genetic and environmental bases of some phenotypes can be elaborated;

iii) when using a MZ twin sample, associations between phenotypes can be decomposed into familial and unique environmental factors using statistical models for clustered data (Begg and Parides, 2003; Carlin *et al.*, 2005). This would be similar to separating a phenotypic association into P = F + E –where F = A + C cannot be further decomposed–, which can be done despite the absence of DZ twins in a given sample.

Besides, novel experimental designs on the epigenetic basis of behavior get an enormous profit of MZ twin samples (Wong *et al.*, 2005; Petronis, 2006; Ballestar, 2010); this will be discussed in detail in subsequent sections.

But how classical MZ twin studies, from Quantitative Genetics, could provide insights on the mechanisms leading to psychopathology through brain plasticity disruptions? An initial evident response could come from basic studies in Biology. For instance, recent research is highlighting the capacity of both cells and organisms with equal DNA sequences to produce different phenotypes in response to environmental and stochastic factors (Sultan, 2000; Raser and O'Shea, 2005). Though the very phenomenon of phenotypic flexibility may likewise be genetically fostered (Ayroles *et al.*, 2015), the current opinion in Epidemiological Epigenetics accepts the convention of phenotypic plasticity as environmentally driven (Wong *et al.*, 2005).

The same question can be answered from the very basis of human MZ twin research. Data from MZ twin studies supports the notion that an important extent of the human phenotypes arises from plasticity mechanisms (Visscher and Posthuma, 2010; Wells and Stock, 2011). A clear example of this point is the notion of “variability gene”, initially proposed by Berg (1988). Briefly, the concept of “variability gene” is built upon intrapair differences in MZ twins: it examines whether pairs of MZ twins carrying a certain genotype have larger phenotypic differences than other MZ twins with different genetic background. Studies developing this concept would thus allow determining which genetic load (if any) confers enhanced sensitivity in response to environmental factors. Though apparently outdated, the “variability gene” concept has recently been highlighted as a practical starting point to analyze novel hypotheses with modern research designs (Teare, 2011; van Dongen *et al.*, 2012).

1.2.3. Monozygotic twin designs: epigenetic perspectives

Epigenetics is commonly described as the study of gene expression changes that are produced by heritable, though potentially reversible, modifications of chromatin structure or DNA methylation (Henikoff and Matzke, 1997). Epigenetics has allowed challenging the dogma that complex phenotypes are the outcome of DNA sequence variations interacting with the environment (Petronis, 2006). With the advent of Epigenetics, the abovementioned equations to describe phenotypic variance (P = G + E) can be reformulated to include another component: P = G + E + EpiG (epigenetics) (Petronis, 2006). An overview of some epigenetic mechanisms can be found in Figure 7.

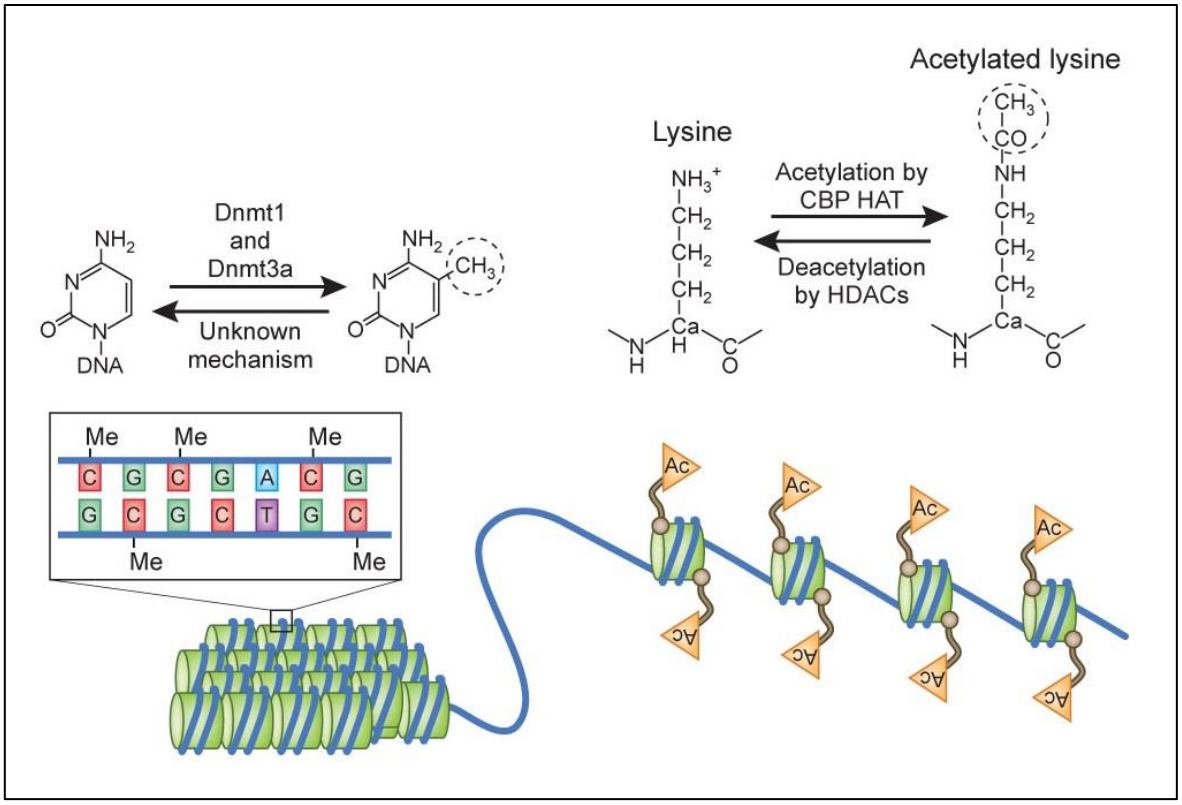


Figure 7. Schematic representation of DNA methylation and histone acetylation. Hypermethylated DNA recruits silencing transcription chromatin remodeling complexes with histone deacetylases and promotes chromatin condensation, whereas hypomethylated DNA unfolds into a 'beads-on-a-string' structure in which

histones are accessible for chromatin remodeling factors. Abbreviations: Ac, acetyl group; Me, methyl group. Adapted from Korzus (2010).

Amidst several different epigenetic marks, DNA methylation is particularly interesting in epidemiological studies, due to its accessibility, inter-individual variability and temporal stability (Foley *et al.*, 2009; Talens *et al.*, 2010). Remarkably, research has suggested that DNA methylation changes may be an important element underlying the etiopathology and clinical manifestation of a range of complex human disorders (Relton and Davey Smith, 2010). More specifically, DNA methylation is supposed to influence several psychopathological, neurocognitive and behavioral phenotypes (Abdolmaleky *et al.*, 2004; Sabunciyan *et al.*, 2012; Yu *et al.*, 2012; Baker-Andresen *et al.*, 2013; Grayson and Guidotti, 2013).

Among distinct functions, DNA methylation seemingly serves as a biological mechanism mediating the link between organismic adaptability/plasticity and disease states (Feinberg, 2007; Feinberg and Irizarry, 2010). More specifically, it has been proposed that some complex diseases may be partly due to losses of phenotypic plasticity could be epigenetically-mediated after the disruption of normal balance between gene-promoting and gene-silencing (Feinberg, 2007). This is schematically depicted in Figure 8.

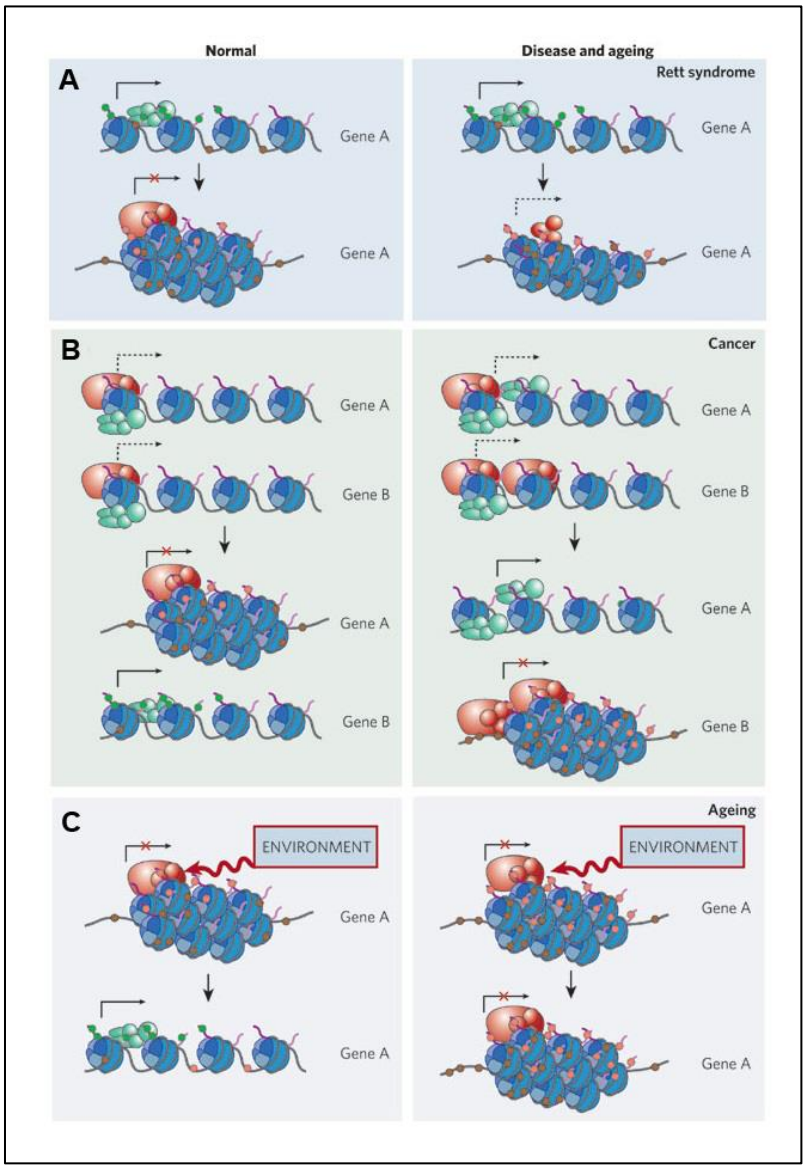


Figure 8. Phenotypic plasticity and the epigenetics of human disease and ageing. Commonly, epigenetic lesions in human disease affect a cell's ability to change its phenotype. A: in monogenic disorders, a defect in the epigenetic machinery hinders normal development. DNA methylation (brown circles) proceeds normally but is not recognized due to the absence of MeCP2 (large red oval). This leads to a failure to silence genes properly during development (dashed arrow). B: Cancer involves many epigenetic lesions that could affect a pluripotent program in tissue-specific stem cells (bivalent euchromatin and heterochromatin proteins) and normal tissue-specific silencing of gene A and activation of gene B after differentiation (lower left panel). C:

Ageing involves a loss of the normal plasticity of response to environmental signals. Adapted from Feinberg (2007).

There is also evidence suggesting that behavioral flexibility is sustained by similar DNA methylation changes (Baker-Andresen *et al.*, 2013; Barbier *et al.*, 2015). Overall, this background confers DNA methylation a key role in bridging the path between phenotypic flexibility in changing environments and in psychiatric disease.

However, though epigenetic modifications constitute a worthy landmark of the biological responses to the environment (Aguilera *et al.*, 2010), an important extent of the DNA methylation variability observed in a population is due to factors other than the environment (i.e., genetic variants altering epigenetic processes) (Kaminsky *et al.*, 2009). For this reason, Epigenetics research on complex diseases using DNA methylation measures has been notably fostered by MZ twin designs (Teare, 2011; van Dongen *et al.*, 2012). For instance, several pioneer studies in Psychiatry have suggested that DNA methylation differences may underlie the discordance for psychopathology observed within MZ twin pairs; this has been proposed for a range of phenotypes as diverse as schizophrenia (Dempster *et al.*, 2011), bipolar disorder (Kuratomi *et al.*, 2008), depression (Dempster *et al.*, 2011), and even personality traits (Kaminsky *et al.*, 2008).

Finally, it is worth noting that there are different temporal patterns of DNA methylation changes (Talens *et al.*, 2010). These timing patterns may somehow provide clues on the different types of plasticity described before (i.e., developmental and activational; see section 1.1.1). In the literature on the epigenetic epidemiology of psychiatry-related complex diseases, there are two popular timing patterns proposed for DNA methylation.

First, some reports suggest that transversally-measured DNA methylation levels may reflect previous environmental insults occurred even years before the epigenetic assessment. In this regard, the seminal work showing an association between prenatal famine and peripheral blood

DNA methylation status measured several decades later has been considered a strong proof of the fact that some epigenetic marks are established in specific lifetime windows and then remain fixed (Heijmans *et al.*, 2008; Tobi *et al.*, 2009). This would have large implications for psychiatric research since epidemiological studies show that specific adult psychiatric diseases may be the result of previous adverse environmental conditions occurring at different periods: i.e., while adverse obstetric histories have typically been linked to schizophrenia (Rapoport *et al.*, 2012), later factors during the infancy are commonly associated with depression (Dunn *et al.*, 2013). Remarkably, some studies also show that methylation of certain loci may be different depending on timing of event exposure (Klengel *et al.*, 2014). On this basis, one could hypothesize that exposure to risk factors, even when taking place during previous life stages, may leave a trace in the epigenome, thus justifying a retrospective research approach (i.e., the current methylation measurement may correlate with previous exposure to a given risk factor). This may, in a way, be related to the notion of *developmental plasticity*: perhaps a methylation mark established during a life stage may block later phenotypic plasticity.

In addition, a complementary approach consists in assessing the current epigenetic status in relation to the present psychopathology (i.e., does the current methylation represent an ongoing psychobiological imbalance?). The literature suggest this may also be possible (Liu *et al.*, 2013; Zhao *et al.*, 2013); rather than being in disagreement with the previous (retrospective) view, one could hypothesize that both views are valid and that different loci would be methylated according to one or the other mechanism. This process may perhaps be related to *activational plasticity*: some individuals could have a worse response to their experiences due to disruptions in their capacity to generate dynamic DNA methylation changes.

1.2.4. Imaging (epi)genetics

Findings from quantitative genetic studies using epidemiological methods –mainly family, twin and adoption studies– have significantly contributed to the development of Psychiatric Genetics during the last decades (Pardes *et al.*, 1989). These studies have justified the use of molecular genetics information to search for specific genetic markers of psychopathology, which is a project still under development (Plomin and Davis, 2009). Though molecular genetics research in Psychiatry has undoubtedly made significant progresses, the current advances in the field suggest that complementary approaches such as epigenetics (Bondy, 2011) and neuroimaging (Bigos and Weinberger, 2010) can provide new and highly relevant insights.

In this sense, modern research combining molecular genetics information with brain scans collected from the same individuals has raised the so-called field of *imaging genetics*. Imaging genetics constitutes a unique means to analyze the putative impact that functionally-relevant genetic variation can have on brain structure and function, which may ultimately lead to a better understanding of the biological foundations of brain alterations in behavioral and psychiatric phenotypes (Bigos and Weinberger, 2010; Tost *et al.*, 2014; Arslan, 2015).

Additionally, statistical studies have long emphasized that "association does not mean causation" (Miettinen, 1983), which consequently implies that the statistical link between a genetic variant and a clinical phenotype does not (*per se*) provide a straightforward biological mechanism of disease. This fact has also highlighted the potential role of imaging genetics as a valuable tool to uncover the biological mechanisms underlying statistical associations between genes and psychopathology (Bigos and Weinberger, 2010).

The advent of novel neuroimaging techniques has allowed the examination of the brain *in vivo* through several ways. Perhaps the most popular non-invasive techniques to examine the brain *in vivo* are those employing magnetic resonance imaging (MRI) scanners. Briefly, the MRI-based techniques are employed in medical radiology to analyze anatomical and physiological properties of

the brain in health and disease. By combining a powerful magnetic field, radio frequency pulses and computerized procedures, pictures of body organs such as the brain can be obtained. An MRI device first changes the aligning patterns of hydrogen atoms of the body, and then detects the energy they emit when going back to their normal alignment positions. As different tissues emit distinct energies when undergoing this process, MRI scanners reconstruct pictures of the tissues scanned (http://www.radiologyinfo.org/en/info.cfm?pg=bodymr). Some of the conventional MRI-based techniques used in Psychiatry include structural MRI (mainly used for volumetric analysis and brain morphology research), functional MRI (employed to assess brain function by analyzing blood-oxygen-level-dependent (BOLD) signals, a correlate of neural activity), diffusion weighted imaging (DWI, which analyzes the diffusion process of molecules such as water, and allows retrieving microscopic details on tissue architecture). A schematic summary of these techniques can be found in Figure 9.

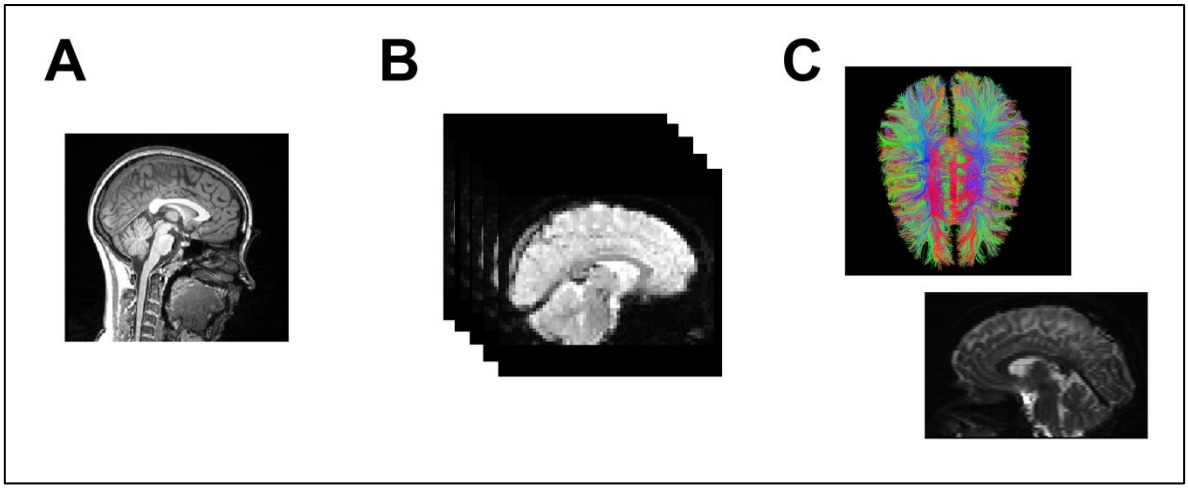


Figure 9. Examples of MRI-based techniques used in psychiatric research. A: 3D T1-weighted anatomical volume, for bran morphometry studies. B: Functional MRI (fMRI) time series acquired through a time interval. C: Diffusion-weighted-imaging (DWI) scan (bottom), which allows inferring a tractography image (top), to represent white matter fibers.

Several examples of imaging genetics studies can be found in the literature. For instance, genes such as disrupted in schizophrenia 1 (*DISC1*), interleukin-1β (*IL1B*) and zinc finger protein 804A (*ZNF804A*), which were previously associated with psychiatric conditions, have recently been shown to modify brain structure and function (Hashimoto *et al.*, 2006; Fatjo-Vilas *et al.*, 2012; Nenadic *et al.*, 2015). Likewise, a novel report has indicated that polymorphic variation in putative neuropsychiatric risk genes is associated with brain differences since the early brain developmental stages, emphasizing the role that genetic influences on fetal brain growth may have on psychiatric disease risk (Knickmeyer *et al.*, 2014). More specifically, genes such as catechol-O-methyltransferase (*COMT*), apolipoprotein E (*APOE*) and brain-derived neurotrophic factor (*BDNF*) were found associated with gray matter volume across a number of brain regions at birth (Figure 10).

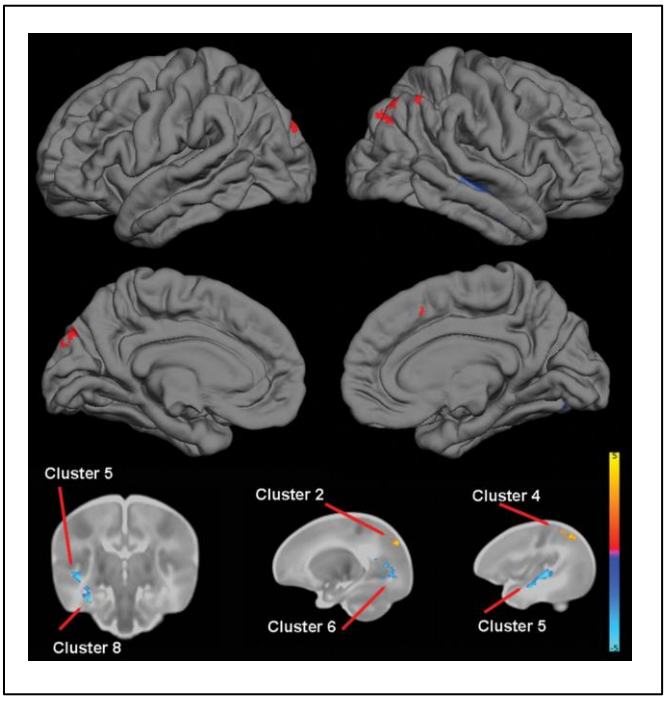


Figure 10. Effect of the rs4680 (*COMT*) genotype on brain structure. Top: locations of gray matter increases (red) and decreases (blue) in Val/Val homozygotes when compared with Met carriers. Bottom: selected 2D slices with significant clusters displayed on the atlas of the neonate brain. The color bar represents the *t*-value at each voxel. Red/yellow (blue/green) indicates Val/Val > (<) other. Adapted from Knickmeyer *et al.* (2014).

Overall, the aforesaid research proposes specific neurobiological mechanisms as mediators of associations between genetic variants and neuropsychiatric phenotypes. Of note, the applications of *imaging genetics* are not limited to the comprehension of mediator mechanisms. Perhaps one of the most innovative approaches outside that scope is the study of plasticity. Brain plasticity mechanisms in response to the experience may be largely influenced by genetic factors and are linked to both neurocognitive performance and behavioral phenotypes such as depression (Brans *et al.*, 2010; Chen *et al.*, 2013; Tost *et al.*, 2014). These findings are certainly relevant for contemporary research since they underline how genetically-driven dynamic pathways of neural activity might play an important role in health and disease.

Importantly, the study of neural plasticity in relation to psychopathology is not limited to combining information from *in vivo* brain structure and function with molecular biology data. The recent increase in popularity and availability of epigenetic techniques has given rise to the emergent field of *imaging epigenetics* (Wiers, 2012; Nikolova and Hariri, 2015), another promising tool to investigate plasticity changes in response to the experience. Since several brain changes observed through neuroimaging can be considered reflective of molecular mechanisms underlying neural plasticity, epigenetic processes have been proposed as mediators of behavioral flexibility disruptions in psychopathology (Johnstone *et al.*, 2013).

In this regard, a recent report showed associations between DNA methylation changes in *BDNF* and brain structure assessed with neuroimaging in depressed individuals (Choi *et al.*, 2015). This is outlined in Figure 11. The authors of that study hypothesize that the influence of *BDNF* methylation on depression risk may be through the epigenetic regulation of neural development and plasticity. This hypothesis is well supported by previous findings on epigenetic changes in *BDNF* that modify neural and cognitive profiles in depressed individuals (Kuhn *et al.*, 2014).

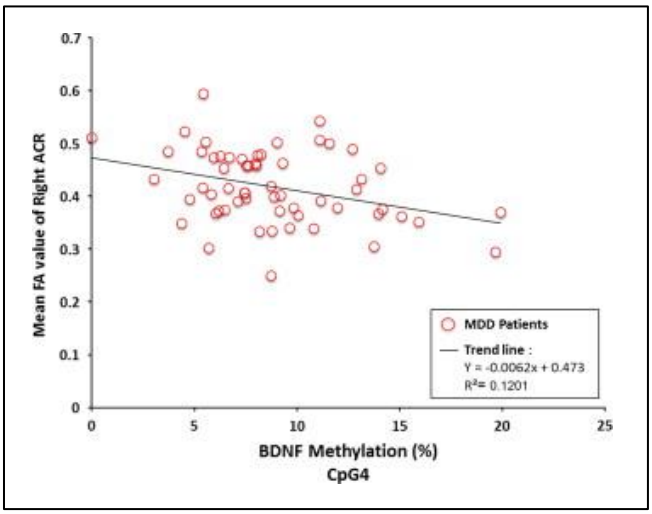


Figure 11. Correlation between methylation fraction of the *BDNF* and mean fractional anisotropy of the right anterior corona radiata. The CpG site analyzed here ("CpG4") was located at -688 bp from the transcriptional start site. The fractional anisotropy value was residualized to adjust for potential confounders. Adapted from Choi *et al.* (2015).

Similarly, based on empirical findings, Alvarado *et al.* (2015) have proposed that DNA methylation may serve as a mediator process in the relationship between stressful stimuli and long-term sensory, affective and cognitive disruptions. Considering the evidences of DNA methylation modifications in cognitive plasticity (Miller and Sweatt, 2007; Day and Sweatt, 2010), Alvarado *et al.* (2015) interpret their own research outcomes as evidence of epigenetically-mediated modifications of brain plasticity after stress exposure (Figure 12).

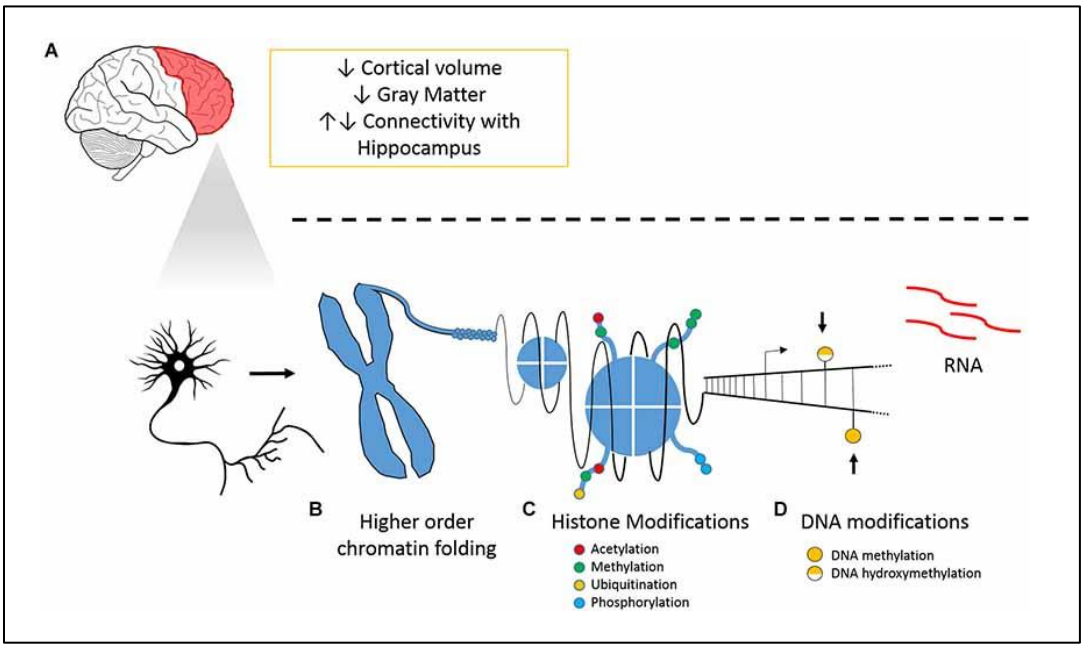


Figure 12. Tissue specific and cellular effects of chronic pain on the prefrontal cortex. A: Outline of neuroanatomical and molecular changes that accompany pain. B: Illustration of higher order chromatin modeling. C: Histone modification depiction. D: DNA modifications. Adapted from Alvarado *et al.* (2015).

1.3. Neural plasticity in depression-related phenotypes: evidences and perspectives

The preceding sections have underscored the importance of neurophysiological plasticity in response to the experience as a risk factor for behavioral disorders, and have shown how some genetically-informative designs may contribute to the understanding of such putative plasticity disruptions. For the sake of generality, they have not focused on any specific phenotype. It is worth noting that most research linking alterations of neural plasticity mechanisms and psychopathology has focused on individuals with psychotic disorders, and has been done mostly from the frame of developmental –but not activational– plasticity (Sanderson *et al.*, 2012; Burrows and Hannan, 2013; Waltereit *et al.*, 2014). However, despite some evidences supporting not only the

neurodevelopmental origins of depressive psychopathology (Ansorge *et al.*, 2007) but also some neuroplasticity disruptions in depressed individuals after stress (Pittenger, 2013), neither developmental nor activational plasticity alterations in depression have been studied in depth.

In view of this, the present section focuses on two main topics on developmental and activational plasticity in depression-related phenotypes: current evidences and future perspectives. First, a brief description of the research supporting the notion of developmental/activational neuroplasticity alterations in depression-related phenotypes is presented. Secondly, a subsection provides an overview of how novel technologies and research designs may converge to improve the understanding of several neurobiological mechanisms linking these two types of plasticity mechanisms and depression.

1.3.1. Developmental plasticity in depression-related phenotypes: early neurodevelopment and risk for adult depression

One of the most replicated risk factors for depressive psychopathology is stress exposure during early neurodevelopmental stages, in line with neurobiological theories of sensitive periods for the adverse effects of life stress in humans (Heim and Binder, 2012). It is widely accepted that the "developmental dimensions of genetic and environmental factors" (Ansorge *et al.*, 2007) play a foundational role in the etiology and pathophysiology of depressive phenotypes. In the psychiatric literature, perhaps the most popular neurobiological mechanism proposed as mediator of the link between early stress and depression is the hypothalamic-pituitary-adrenocortical (HPA) system model (Figure 13). This theory has become popular after research evidences showing that environmental developmental factors can have enduring effects in the HPA system physiology and –in parallel– in behavior; it proposes that early stress has a long-lasting effect on baseline measures of HPA metabolism measured years later in depressed individuals (Ansorge *et al.*, 2007). Notice that similar HPA system alterations also occur following adult stress (namely, in the *short-*

term) (Marco *et al.*, 2011), but this is discussed as part of activational plasticity mechanisms in a later subsection. Complementarily, it is worth noting that previous animal studies on the early life programing of the HPA axis in psychopathology have proposed neural plasticity alterations as a key mediator mechanism underlying non-adaptive emotional and social phenotypes (Marco *et al.*, 2011).

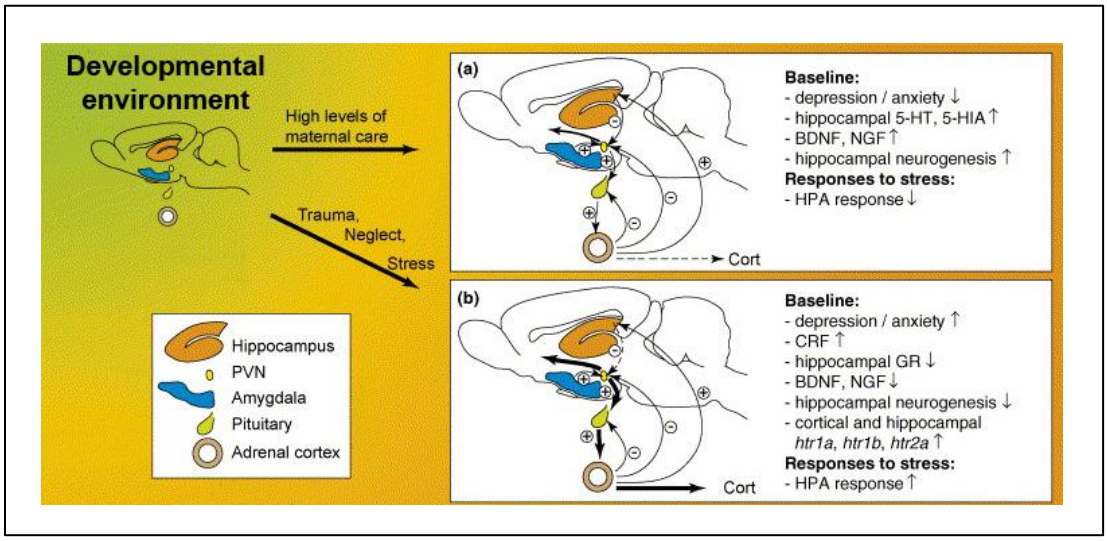


Figure 13. Consequences of the early life experience of stress on the HPA system and depression. High levels of maternal care lead to a suppressed HPA system and reduced depression- and anxiety-like behavior in the adult (box "a"). Conversely, stress during development leads to a hyperactive HPA system and increased depression- and anxiety-like behavior in the adult (box "b"). Adapted from Ansorge *et al.* (2007).

Overall, these and other related findings support the view of depression as a disorder of altered neuronal plasticity following stress exposure early in life (Calabrese *et al.*, 2009; Cattaneo *et al.*, 2015). Thus, the *neuroplasticity hypothesis* of depression could be postulated by systematically linking the concepts of developmental experiences, plasticity and psychopathology. This abstraction may allow new insights on the origins of depression, since the evolutionary roots of

psychopathology may be understood –at least partly– as neuroplasticity deficits following developmental adaptations in front of stressful experiences (Lee *et al.*, 2015).

1.3.2. Activational plasticity in depression-related phenotypes: emotional and cognitive flexibility disturbance in depression

Convergent evidence from transversal research designs in Psychiatric Genetics has led to authors such as Castren and Rantamaki (2010) to propose a *neuroplasticity hypothesis* of major depressive disorder. Though this may seem a wide-sense notion, these authors actually derive their hypothesis from the very punctual evidence that altered BDNF protein secretion may lead to dysfunctional emotional brain circuits in depression. This putative etiopathogenic mechanism has been supported by additional research in both animals and humans (McEwen, 2001; Calabrese *et al.*, 2009; Masi and Brovedani, 2011; Kuhn *et al.*, 2014; Cattaneo *et al.*, 2015). A proposed brain mechanism to explain how the BDNF and some epigenetic factors may prompt neuroplastic disruptions in depression after stress is schematized in Figure 14.

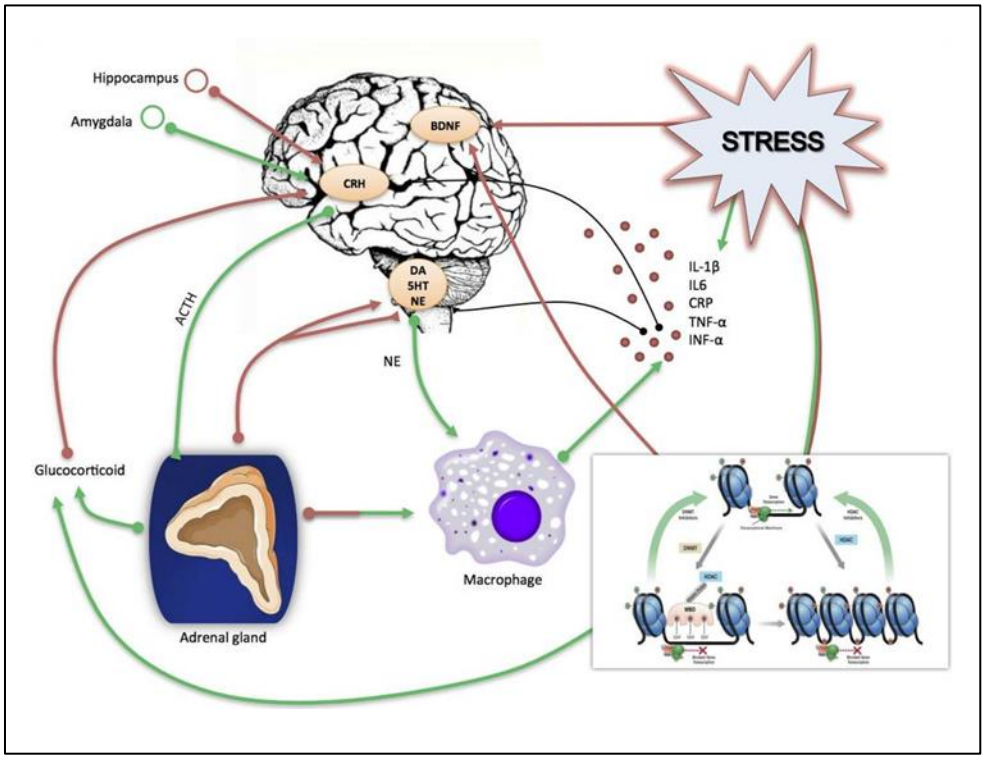


Figure 14. Schematic representation of the direct and indirect effect of stress on inflammation and neuroplasticity-related processes. Stress induces an immediate release of glucocorticoidas and pro-inflammatory cytokines; then, increased levels of glucocorticoids alter the CRH-ACTH brain signaling; afterwards, neurogenesis and neurotrophic factor production are altered. Likewise, proinflammatory cytokines can alter brain functioning and neurotrophins production and release. Additionally, stress can work indirectly by activating epigenetic mechanisms (inset box) which may act on the same target stress related genes. Adapted from Cattaneo *et al.* (2015).

Additionally, probably one of the most comprehensive literature reviews connecting short-term (activational?) plasticity and depression was recently conducted by Pittenger (2013). Remarkably, his work was not directly focused on the phenotype of depression; rather, he described how some neurocognitive endophenotypes of depression can be considered neuroplastic disruptions following stress exposure. He highlighted the evidences from animal research indicative

of plasticity impairments in the hippocampus following stressful events in experimental models of depression (Pittenger and Duman, 2008; Lupien *et al.*, 2009). While such hippocampal changes were mainly studied as triggers of cognitive damages, these and other studies in humans have also led to propose that pharmacological antidepressant treatments focused on the enhancement of plasticity have enormous therapeutic potential (Pittenger, 2013). Of note, a very recent clinical study in humans has shown that the electroconvulsive treatment of major depression can modify the structural plasticity of the hippocampus and the amygdala (Joshi *et al.*, 2015).

Taken together, the previous evidences indicate that the normal plasticity processes underlying emotional and cognitive mechanisms may be disrupted in depressive phenotypes. The short-term alterations of both the neurotrophic system (i.e., *BDNF* signaling) and the hippocampus outlined in several of these reports resemble activational plasticity deficits in depressed individuals, though these neurobiological principles still need to be confirmed (Cattaneo *et al.*, 2015).

1.3.3. Developmental plasticity and depression: additional research hypotheses

Despite the broad set of findings supporting developmental plasticity alterations in depression, further research can be conducted to determine the extent of this *neuroplasticity hypothesis*. Two main points could be addressed in this regard. First, what are the early developmental windows in which long-lasting alterations leading to depression may occur? And, secondly, what are the specific neurobiological mechanisms underlying developmental plasticity changes in depressed humans? Although this subsection does not pretend to answer these questions thoroughly, it will provide a number of points that remain unsolved and may be future research subjects.

First, despite the increasing interest in the early origins of adult diseases in both general pathology (Calkins and Devaskar, 2011; Bavamian *et al.*, 2015) and psychiatry (Cicchetti and Walker, 2003; Cicchetti and Cohen, 2006; Turecki *et al.*, 2014), still there is no clarification on the specific neurodevelopmental windows altering the risk for depression. A generally accepted view is

that stressful events during the first years of life may predispose an individual to depression during adulthood (Heim *et al.*, 2010; Heim and Binder, 2012). This would contrast with research in psychotic disorders such as schizophrenia and autism, where developmental insults occurring during prenatal stages are thought to predispose to psychopathology (Goldstein *et al.*, 2002). As discussed previously, some of the stressful events during the first years of life may lead to depression through developmental plasticity. But can fetal stress disrupt plasticity to predispose to depressive phenotypes? This is an interesting topic that could be examined further, since there are conflicting evidences in the literature (Wojcik *et al.*, 2013).

Thus, there would be an apparent paradox: both psychosis and depression would have developmental plasticity alterations, but while psychosis may be sensitive to fetal developmental damages, depression risk would be affected by post-natal life stress. This apparent inconsistency may be solved by concluding that damage to the nervous system *in utero* may imply different circuitry pathways than damage to the brain *early in life*. Nevertheless, the paradox gets even more intricate in view of other evidence from epidemiological psychiatry: some subclinical manifestations of psychopathology with a psychometric relationship with psychosis (i.e., psychotic experiences) are comorbid with the clinical manifestation of depression (Wigman *et al.*, 2012). This fact somehow supports the view of psychopathology as "a network of overlapping and reciprocally impacting dimension liabilities" (Wigman *et al.*, 2012). Nevertheless, the apparent neurobiological specificity mentioned above –psychosis resulting from prenatal stress and depression resulting from early life stress– gets blurred in presence of comorbidity evidences. What is more, Kelleher and Cannon (2011) have proposed that several risk factors for (clinically-defined) psychosis, including obstetric complications, also increase the risk for subclinical psychotic experiences. Hence, knowing that fetal damage increases the risk for both psychosis and psychotic experiences, and considering that psychotic experiences and depression are highly comorbid, why is there no conclusive association between obstetric complications and the risk for depression?

Several hypotheses can be feasibly postulated: first, psychotic experiences are connected with the clinical psychosis phenotype only in psychometric terms –but not so clearly at the population level– (Linscott and van Os, 2010). If this is correct, the high prevalence of subclinical psychosis in depressed individuals could not necessarily imply a large overlap in risk factors for both phenotypes. This has recently been highlighted by a report by Kounali *et al.* (2014), who concluded that psychotic experiences –a non-clinical psychosis phenotype– have only a week genetic and environmental risk overlap with schizophrenia. But by overemphasizing this population discontinuity between clinical and non-clinical psychosis, the data suggesting that individuals with non-clinical psychosis constitute a valid population in which to investigate the etiology of the clinical phenotype (Kelleher and Cannon, 2011) would perhaps be overly neglected.

Rather than staying with either a direct continuity view (Kelleher and Cannon, 2011) or a population discontinuity paradigm (Linscott and van Os, 2010; Kounali *et al.*, 2014), perhaps a third synthesizing model can be held. This would not only help uncover the relationship between clinical and non-clinical psychosis, but should also have larger implications for psychiatric research. In the present research setting, the aforesaid paradox of comorbidity between depression and non-clinical psychosis contrasting with an apparent specificity of neurodevelopmental windows leading to psychosis and depression may be elicited by adopting a third position. Namely, it may be reasonable emphasizing that there are some shared etiopathogenic mechanisms and perhaps intermediate phenotypes shared by all three psychopathological constructs.

Therefore, perhaps focusing on specific neurobiological endophenotypes (Gottesman and Gould, 2003) of relevance for depression and clinical and subclinical psychosis may shed light on the apparent paradoxes: it might be practical hypothesizing that the similarities and differences in neurophysiological pathways underlying the three phenotypes would provide new insights into the apparent phenomenological complexity of risk factors and psychopathological dimensions.

In this sense, novel technologies assessing neurobiological (endophenotypic) mechanisms *in vivo* in human individuals manifesting any of these three phenotypes can potentially be useful.

They may allow consolidating the developmental plasticity hypotheses of depression. For instance, with the advent of new epigenetic techniques, recent research has shown that the methylation of plasticity genes in children may be related to later risk for depression (Alisch *et al.*, 2014). This type of epigenetic mechanisms has likewise been proposed as mediator of the environmental impact of early neurodevelopmental insults in psychosis (Dong *et al.*, 2015). Additionally, it has been suggested that some epigenetic mechanisms may underlie distinct daily-life expressions of psychopathology –including subclinical psychosis– (Pishva *et al.*, 2014). Although research on the epigenetics of subclinical psychosis has just recently started and there are only a few studies on the subject, it somehow suggests that examining the neuroplastic bases of depression, in relation to both clinical and non-clinical psychosis may shed light on the specific and shared biological processes underneath psychopathology. Remarkably, this kind of translational neuroscience approaches may not only elicit etiological dynamics of each disorder, but might also be able to inform a clinically-oriented nosology (Hyman, 2007).

The former paragraphs thus make a case for the integration of epigenetic information into the research on developmental plasticity mechanisms in depression. Importantly, as noticed in subsection 1.2.4, the neurobiological landscapes below psychopathological phenotypes can be largely clarified by combining epigenetic information with human brain imaging data obtained *in vivo*. For instance, experimental research models of depression have indicated that early life stress predisposes to hippocampal plasticity changes, which may probably lead to affective disorders later in life (Herpfer *et al.*, 2012). The integration of these different sources of information, along with intra-phenotype contrasts using not only depression, but also related neurocognitive and psychopathological phenotypes, seems a good candidate strategy to unravel relevant etiopathogenic and dynamic factors in psychopathology. These new approaches may thus provide relevant clues on the specific neurobiological mechanisms underlying developmental plasticity changes in depressed humans.

1.3.4. Activational plasticity and depression: additional research hypotheses

So far, the *neuroplasticity hypothesis* of depression was formulated using the concept of developmental plasticity. Namely, the ideas proposed above tried to bridge the impact of early life stress on adult depression by using "developmental plasticity" as a connector. However, this hypothesis can be expanded further by incorporating the notion of "activational plasticity". As described in subsection 1.1.3, this concept refers to the capacity of showing a rich set of responses in front of diverse and changing experiential stimuli. Short-term rigidity when facing different experiences may thus be a disease trait. The differences between developmental and activational plasticity may probably be related to the complementary concepts of disease state (the final outcome after previous exposure to risk factors) and disease trait (an unadaptive mechanism of response).

A large number of new research findings could perhaps be used as support for the notion of activational plasticity disruptions in depression. Along with the previously mentioned neuroimaging findings (Joshi *et al.*, 2015), epigenetic evidences could also support activational plasticity deficits in depression (LaPlant *et al.*, 2010). These separate findings may foster new investigation proposals during the next years, probably integrating diverse sophisticated techniques.

2. HYPOTHESES AND OBJECTIVES

Based on the background mentioned in the Introduction, the main hypothesis guiding this thesis is:

Main hypothesis: Several etiopathogenic mechanisms of depression-related phenotypes can be clarified by expanding on processes of biobehavioral plasticity in response to the experience. This expansion can be elaborated on the basis of both neurodevelopmental phenomena (developmental plasticity) and novel biological mechanisms detectable through neuroimaging and epigenetics approaches (activational plasticity).

A few specific hypotheses can be drawn from above:

Specific hypothesis 1: ***[Depression and developmental plasticity.]*** Depression-related psychopathological phenotypes are induced by factors altering the early neurodevelopment, and these long-lasting changes can be assessed in adulthood.

Specific hypothesis 2: ***[Depression and activational plasticity.]*** The clinical manifestation of depression-related psychopathological phenotypes can be understood as activational plasticity deficits; these deficits can be assessed as neurobiological disease traits using novel epigenetic and neuroimaging techniques.

In relation to the *specific hypothesis 1*, the role of the obstetric risk factors for neurodevelopmental disorders was analyzed, with regard to depression-related phenotypes. This was done through the specific objectives 1 to 5. Each of these objectives is accompanied by a manuscript in the following sections.

Objective 1: The (still inconclusive) association between low birth weight and depression is examined using a genetically informative design. Namely, in recognition that both birth weight and depression are the result of genes and environment, a research protocol is developed to examine

whether the genetic (environmental) factors determining birth weight are shared with the genetic (environmental) factors influencing depression risk. This is investigated by means of an informative sample of adult twins from the University of Barcelona Twin Registry (*n* = 121 adult twin pairs). [Psychol Med 44, 1117-1119]

Objective 2: Since low birth weight has been associated with cortical surface area reductions, and considering that depressive individuals display volumetric brain reductions, the potential confounding role of these variables in morphological neuroimaging measures was analyzed in a sample of 48 twins (24 MZ pairs). [PLoS ONE 10(6), e0129616]

Objective 3: The role of birth weight and working memory (a cognitive endophenotype of psychopathology) in modulating DNA methylation levels of *IGF2* and related genes was evaluated in an informative sample of 34 twins (17 MZ pairs). [PLoS ONE 9(8), e103639]

Objective 4: The putative association between winter season of birth (a risk factor for neurodevelopmental psychotic disorders) and subclinical psychosis (a phenotype largely prevalent in depression, and assumedly linked to clinical psychosis) is examined by reviewing and meta-analyzing the existing literature and new data (*n* = 481 healthy adults). [Psychat Res 225(3), 227-235]

Objective 5: The putative mediating role of cortical thickness changes in the association between season of birth and (subclinical) psychotic experiences is analyzed using high-resolution 3D MRI scans in a sample of 48 twins (24 MZ pairs). [J Psychiat Res 56, 144-149]

In relation to the *specific hypothesis 2*, putative brain imaging and peripheral blood epigenetic signatures underlying the clinical manifestation of depression-related phenotypes were analyzed. This was done through the specific objectives 6 to 10. Each of these objectives is accompanied by a manuscript in the next sections.

Objective 6: The potential activational plasticity differences in cognition between individuals are assessed in a carefully selected sample of 54 MZ twin pairs (108 individuals). To this end, two polymorphisms in the epigenetic gene *DNMT3B* are analyzed using the “variability gene” approach. [Eur Psychiat 30(2), 303-308]

Objective 7: The relationship between *DEPDC7* epigenetic signatures in peripheral blood and depressive symptomatology during the last 30 days was analyzed in a sample of 17 MZ twin pairs (34 individuals) using an intrapair-differences model. [Eur Psychiat, *In press*]

Objective 8: The potential epigenetic signatures of depression were analyzed using a genome-wide design, with two distinct approaches: differential methylation and variable methylation assessment. This was analyzed in a sample of 17 MZ twin pairs (34 individuals). [Transl Psychiatry 5, e557]

Objective 9: The relationship between genotypic variation in *FKBP5* and hippocampal structural connectivity was analyzed in relation to depression risk, in a sample of 54 informative MZ twins (27 pairs), using a connectome-based approach. [*Submitted*]

Objective 10: The potential genetic or environmental roots of the association between amygdalar resting-state connectivity and depression was analyzed using two different time-series analysis approaches, in a sample of 48 informative MZ twins (24 pairs). [Human Brain Mapping, *In press*]

3. ADVISOR'S REPORT ON THE ARTICLES

The doctoral thesis "*Early neurodevelopment, adult human cognition and depressive psychopathology: analysis of neuroimaging brain correlates and epigenetic mediators*" is based on original results obtained by Aldo Córdova Palomera. These results have been published or submitted to international peer reviewed scientific journals. The impact factors of these journals demonstrate the quality of the research conducted, and are as follows:

1. **Low birth weight and adult depression: eliciting their association**, published in *Psychological Medicine* 44, 1117-1119. This is a leading international journal in the fields of psychiatry, related aspects of psychology and basic sciences. According to the Journal Citation Reports (JCR) Social Science Edition (2014), Psychological Medicine has an impact factor (IF) of 5.938, and is classified in the 1st decile of "Psychiatry" (ranking: 10/133) and "Psychology, Clinical" (ranking: 4/119).
2. **Birth weight and adult IQ, but not anxious-depressive psychopathology, are associated with cortical surface area: further evidences based on a twin study**, published in *PLoS ONE* 10(6), e0129616. This is one of the world's largest journals by number of papers published, covering primary research from any discipline within science and medicine. According to the JCR Science Edition (2014), PLoS ONE has an IF of 3.234, and is classified in the 1st quartile of "Multidisciplinary sciences" (ranking: 8/56).
3. **Birth weight, working memory and epigenetic signatures in *IGF2* and related genes: a MZ twin study**, published in *PLoS ONE* 9(8), e103639. This is one of the world's largest journals by number of papers published, covering primary research from any discipline within science and medicine. According to the JCR Science Edition (2014), PLoS ONE has an IF of 3.234, and is classified in the 1st quartile of "Multidisciplinary sciences" (ranking: 8/56).

4. **Season of birth and subclinical psychosis: systematic review and meta-analysis of new and existing data**, published in *Psychiatry Research* 225(3), 227-235. This journal provides very rapid publication of short but complete research reports in the field of psychiatry. According to the JCR Science Edition (2014), Psychiatry Research has an IF of 2.467, and is classified in the 2nd quartile in "Psychiatry" (ranking: 61/140).
5. **Cortical thickness correlates of psychotic experiences: examining the effect of season of birth using a genetically informative design**, published in *Journal of Psychiatric Research* 56, 144-149. This is an important journal that reports on the latest work in psychiatry and cognate disciplines. According to the JCR Science Edition (2014), Journal of Psychiatric Research has an IF of 3.957, and is classified in the 1st quartile of "Psychiatry" (ranking: 28/140).
6. **Polymorphic variation in the epigenetic gene *DNMT3B* modulates the environmental impact on cognitive ability: a twin study**, published in *European Psychiatry* 30(2), 303-308. This is the official journal of the European Psychiatric Association, the largest international association of psychiatrists in Europe, and publishes articles on topics relevant to all mental health clinicians, researchers and neuroscientists. According to the JCR Social Science Edition (2014), European Psychiatry has an IF of 3.439, and is classified in the 1st quartile in "Psychiatry" (ranking: 25/133).
7. **Further evidence of *DEPDC7* DNA hypomethylation in depression: a study in adult twins**, published in *European Psychiatry* (In press). This is the official journal of the European Psychiatric Association, the largest international association of psychiatrists in Europe, and publishes articles on topics relevant to all mental health clinicians, researchers and neuroscientists. According to the JCR Social Science Edition (2014), European Psychiatry has an IF of 3.439, and is classified in the 1st quartile in "Psychiatry" (ranking: 25/133).
8. **Genome-wide methylation study on depression: differential methylation and variable methylation in monozygotic twins**, published in *Translational Psychiatry* 5, e557. This

journal explores the translational pathway between research in neuroscience and conceptually novel treatments. According to the JCR Science Edition (2014), Translational Psychiatry has an IF of 5.62, and is classified in the 1st quartile in "Psychiatry" (ranking: 16/140).

9. **Polymorphic variation in *FKBP5* interacts with hippocampal connectivity to influence the risk for depression: a study in twins**, under review.
10. **Altered amygdalar resting-state connectivity in depression is explained by both genes and environment**, published in *Human Brain Mapping* (In press). This is an outstanding interdisciplinary journal covering interdisciplinary topics on basic, clinical, technical and theoretical research of the brain. According to the JCR Science Edition (2014), Human Brain Mapping has an IF of 5.969, and is classified in the 1st decile of "Radiology, Nuclear Medicine & Medical Imaging" (ranking 5/125), and in the 1st quartiles of "Neuroimaging" (ranking: 2/14) and "Neurosciences" (ranking 27/252).

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

Prof. Dr. Lourdes Fañanás Saura

Barcelona, June 30th 2015.

4. RESULTS - PUBLICATIONS

Low birth weight and adult depression: eliciting their association

Córdova-Palomera A, Goldberg X, Alemany S, Nenadic I, Gastó C, Fañanás L

Psychological Medicine (2014), 44, 1117-1119

DOI: http://dx.doi.org/10.1017/S0033291713002754

Psychological Medicine (2014), **44**, 1117–1119.

Correspondence

Psychological Medicine, **44** (2014).
doi:10.1017/S0033291713002754
First published online 11 November 2013

Letter to the Editor

Low birth weight and adult depression: eliciting their association

Theories supporting fetal origins of adult health and disease are nowadays widely accepted regarding some psychiatric conditions (Losh *et al.* 2012; Eide *et al.* 2013). However, whether genetic or environmental factors disrupting fetal growth might constitute a risk factor for depressive and/or anxious psychopathology remains still controversial.

A recent meta-analysis (Wojcik *et al.* 2013) evaluated the current evidence for an association between low birth weight (BW) and adult depression or psychological distress in the general population, and found no conclusive association between them. Remarkably, the systematic literature search performed by the authors allowed them to identify a couple of recent health register studies with positive results (Abel *et al.* 2010; Larsen *et al.* 2010). Although they were discarded from the statistical analyses of Wojcik *et al.* (2013) after considering that depression could be largely undiagnosed in the populations included therein, these important cohort designs – alongside the results from the meta-analysis itself – leave the door open to further scrutiny and debate.

Besides, despite the comprehensiveness of the above-mentioned meta-analysis, it is worth taking into account that fetal growth and psychopathology may share both genetic and environmental aetiological factors. In view of this, twin methodology can contribute to disentangle the putative origins of the controversial association discussed herein. Importantly, as heritability estimates of depression are relatively low (h^2 about 37%) and individual-specific environmental effects have a substantial influence on depressive phenotypes (Sullivan *et al.* 2000), it is likely that intra-uterine environmental factors affecting each of the co-twins' BW may play a role in engendering this psychopathology. In addition, previous epidemiological studies using twins have taken as their starting point inconclusive associations between low BW and later outcomes, to corroborate that non-genetic influences on BW may underlie the presence of disease (Villamor *et al.* 2009).

As monozygotic (MZ) twins are nearly identical at the DNA sequence level, their differences in BW provide a measure of non-genetic effects on fetal growth. Hence, a twin design constitutes an appropriate methodology to approach the current issue controlling for potential genetic confounders. Along these lines, if the BW–depression link were exclusively due to intra-uterine environmental impact on BW, this analysis would help to clarify the aetiology of this association and may possibly assist in the identification of at-risk individuals during early stages.

Here, the authors aimed to assess the presence of a link between BW and depression or anxiety and to determine whether the association can be explained by either familial factors (genetic plus shared environment) or within-pair differences in size at birth (i.e. unique environmental influences: does the twin with the lower BW have a higher risk for psychopathology than his co-twin?).

The variables of interest have been studied here using information from a representative sample of adult twins from the University of Barcelona Twin Registry (n=121 pairs). The presence of lifetime mental disorders was assessed in a face-to-face interview using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) by a trained clinical psychologist (X.G.). After excluding duos where at least one co-twin presented a neurological or psychiatric diagnosis other than depression or anxiety, 210 individuals (85 MZ and 20 dizygotic pairs; mean age=31 years, S.D.=13 years, 33% male) were selected for analysis. Taking into account the increasing evidence of shared aetiopathology and diagnostic criteria overlap for depressive and anxious disorders (Lowe *et al.* 2008), and given that previous reports have widely used instruments that measure symptoms of depression and anxiety together (Wojcik *et al.* 2013), patients with any of both lifetime diagnoses were grouped in a single set: D/A (affected by depression and/or anxiety). In all, 51 individuals (24% of the sample) had at least one of these lifetime diagnoses. Information on BW (and obstetric history) was collected by interviewing the twins' mothers (Walshe *et al.* 2011), using the Lewis–Murray Scale for Obstetric Complications (Lewis *et al.* 1989). BW distribution by gestational age of all subjects in the sample was in accordance with a previous report of twins (Glinianaia *et al.* 2000). In the overall sample, the mean BW was 2522 (S.D.=626, range 800–4150) g, and the observed mean intra-pair difference in BW was 279 (S.D.=254) g.

Corrections for sex, age and weeks of gestation of the twins were included in all the analyses. Participants gave their written informed consent, and

all procedures were carried out in accordance with the Declaration of Helsinki.

As a preliminary step, a logistic regression was performed in the above-mentioned subsample (n=210 individuals) to test for a potential direct relationship between BW and adult D/A. Huber–White estimators were applied to adjust for non-independence of the observations. No such association was found (β=0.31, S.E.=0.32, p=0.34).

It is worth noting that Pearson's correlation for BW of the MZ twin subset was r=0.83, which means that approximately 17% of the BW variance could be attributed to unique intra-uterine factors not shared by MZ twins. Thus, despite the previous (null) result, separating the variance of BW into familial and unique environmental factors was likely to provide additional information. This would clarify if the putative BW–D/A link was only due to one of these features and had been confounded by the other. In order to assess this hypothesis, a multivariate regression model solved by generalized estimating equations with an exchangeable correlation structure was applied using data from the group of 85 MZ twin pairs (15 D/A concordant, 14 D/A discordant and 56 healthy duos).

For the current aim, the logistic regression $\text{logit}(\pi_{ij}) = \beta_0 + \beta_B \mu_{i.} + \beta_W (X_{ij} - \mu_{i.})$ gives an estimate of both (i) genetic and shared environmental factors (β_B) and (ii) unique environmental events affecting each co-twin (β_W) (Begg & Parides, 2003) that confer risk for disease. Subindexes $i \in \{1, \ldots, n\}$ and $j \in \{1, 2\}$, respectively, stand for pair number (here, n=85 MZ pairs) and co-twin number (an arbitrarily assigned number within a pair: 1 or 2); π_{ij} represents the probability that co-twin j from the ith pair has of being affected by D/A; β_0 is the regression intercept; $\mu_{i.} = (X_{i1} + X_{i2})/2$ is the mean BW value of the ith pair; and $X_{ij} - \mu_{i.}$ represents the deviation of co-twin j from the pair's mean.

The so called unique environmental events ($X_{ij} - \mu_{i.}$) allow the quantification of the degree of (dis)advantage that each co-twin had during the pregnancy, as reflected in BW. In pairs where both twins had the same BW, $X_{ij} - \mu_{i.}$ equals 0, whereas positive or negative values of this term signify, respectively, that a co-twin had the higher or lower BW in his pair. Thus, β_B allows testing whether the twin with the lower BW has a higher risk for D/A than his heavier co-twin, which might indicate a role for a unique environment.

Results of this regression indicate no association between either genetic plus common environmental (β_B) or unique environmental events (β_W) and D/A (β_B=0.53, S.E.=0.37, p=0.15; β_W=–0.2, S.E.=0.79, p=0.79).

Although the sample size used for the current calculations was modest, all results were far from statistical significance, suggesting that they were not just related to lack of statistical power. They argued against a considerable effect size of the evaluated risk factors. Remarkably, demographic characteristics of this sample are representative of the general population for both obstetric and psychopathological profiles (both BW profile and sex distribution of D/A in the whole set of twins were in good agreement with the literature), which might render associations detectable.

The current results indicate that neither BW by itself nor environmental influences on BW are associated with adult depression. Thus, pregnancy factors associated with discordant BW in twins seem to not predispose to adult D/A. Remarkably, this latter finding is in agreement with a previous independent twin study indicating no differential risk for D/A diagnosis in MZ twins discordant for BW (Foley *et al.* 2000). Altogether, these research reports suggest that controversial results on the topic are probably not due to environmental influences on BW.

As stated by Wojcik *et al.* (2013), factors such as severity of symptoms may underlie the fact that both positive and negative results have been reported on the BW–depression association, particularly considering the fact that earlier studies have been based on heterogeneous research designs. In effect, the present analyses lacked the possibility of evaluating diverse disease severities, and advocate for further research on this issue as a putative means to clarify the controversial results. The relative contribution of genetic and/or environmental factors that may underlie the (potential) relationship between fetal growth and adult D/A should also be elucidated taking this fact into consideration, in order to gain more epidemiological insights.

Further data are available on request.

Acknowledgements

We acknowledge funding from the Ministry of Science and Innovation (SAF2008-05674), the European Twins Study Network on Schizophrenia Research Training Network (EUTwinsS, MRTN-CT-2006-035987) and the Comissionat per a Universitats i Recerca del DIUE of the Generalitat de Catalunya (2009SGR827).

Declaration of Interest

None.

References

Abel KM, Wicks S, Susser ES, Dalman C, Pedersen MG, Mortensen PB, Webb RT (2010). Birth weight, schizophrenia, and adult mental disorder: is risk confined

to the smallest babies? *Archives of General Psychiatry* **67**, 923–930.

Begg MD, Parides MK (2003). Separation of individual-level and cluster-level covariate effects in regression analysis of correlated data. *Statistics in Medicine* **22**, 2591–2602.

Eide MG, Moster D, Irgens LM, Reichborn-Kjennerud T, Stoltenberg C, Skjaerven R, Susser E, Abel K (2013). Degree of fetal growth restriction associated with schizophrenia risk in a national cohort. *Psychological Medicine* **43**, 2057–2066.

Foley DL, Neale MC, Kendler KS (2000). Does intra-uterine growth discordance predict differential risk for adult psychiatric disorder in a population-based sample of monozygotic twins? *Psychiatric Genetics* **10**, 1–8.

Glinianaia SV, Skjaerven R, Magnus P (2000). Birthweight percentiles by gestational age in multiple births. A population-based study of Norwegian twins and triplets. *Acta Obstetricia et Gynecologica Scandinavica* **79**, 450–458.

Larsen JK, Bendsen BB, Foldager L, Munk-Jørgensen P (2010). Prematurity and low birth weight as risk factors for the development of affective disorder, especially depression and schizophrenia: a register study. *Acta Neuropsychiatrica* **22**, 284–291.

Lewis SW, Owen MJ, Murray RM (1989). *Obstetric Complications and Schizophrenia: Methodology and Mechanisms*. Oxford University Press: New York.

Losh M, Esserman D, Anckarsater H, Sullivan PF, Lichtenstein P (2012). Lower birth weight indicates higher risk of autistic traits in discordant twin pairs. *Psychological Medicine* **42**, 1091–1102.

Lowe B, Spitzer RL, Williams JB, Mussell M, Schellberg D, Kroenke K (2008). Depression, anxiety and somatization in primary care: syndrome overlap and functional impairment. *General Hospital Psychiatry* **30**, 191–199.

Sullivan PF, Neale MC, Kendler KS (2000). Genetic epidemiology of major depression: review and meta-analysis. *American Journal of Psychiatry* **157**, 1552–1562.

Villamor E, Iliadou A, Cnattingius S (2009). Is the association between low birth weight and asthma independent of genetic and shared environmental factors? *American Journal of Epidemiology* **169**, 1337–1343.

Walshe M, McDonald C, Boydell J, Zhao JH, Kravariti E, Touloupoulou T, Fearon P, Bramon E, Murray RM, Allin M (2011). Long-term maternal recall of obstetric complications in schizophrenia research. *Psychiatry Research* **187**, 335–340.

Wojcik W, Lee W, Colman I, Hardy R, Hotopf M (2013). Foetal origins of depression? A systematic review and meta-analysis of low birth weight and later depression. *Psychological Medicine* **43**, 1–12.

A. CÓRDOVA-PALOMERA[1,2], X. GOLDBERG[1,2], S. ALEMANY[1,2], I. NENADIC[3], C. GASTÓ[2,4] AND L. FAÑANÁS[1,2]

[1] *Unitat d'Antropologia, Departament de Biologia Animal, Facultat de Biologia and Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain*

[2] *Centro de Investigaciones Biomédicas en Red de Salud Mental (CIBERSAM), Instituto de Salud Carlos III, Madrid, Spain*

[3] *Department of Psychiatry and Psychotherapy, Jena University Hospital, Jena, Germany*

[4] *Departamento de Psiquiatría, Instituto Clínico de Neurociencias, Hospital Clínico de Barcelona, and Instituto de Investigaciones Biomédiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain*

Address for correspondence: Dr L. Fañanás, Unitat d'Antropologia, Departament de Biologia Animal, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 643, 08028, Barcelona, Spain.
(Email: lfananas@ub.edu)

Advisor's report on the contribution of the Ph.D. candidate to the article

Prof. Dr. Lourdes Fañanás Saura, associate professor at the Faculty of Biology, University of Barcelona, and supervisor of the present doctoral thesis by Aldo Córdova Palomera, hereby certifies that the participation of the Ph.D. candidate in the article "Low birth weight and adult depression: eliciting their association" included the following tasks:

- Participation in study design.
- Statistical analyses.
- Writing of the first manuscript draft.
- Critical revision of the article.

Prof. Dr. Lourdes Fañanás Saura

Barcelona, June 30th 2015.

Birth weight and adult IQ, but not anxious-depressive psychopathology, are associated with cortical surface area: further evidences based on a twin study

Córdova-Palomera A, Fatjó-Vilas M, Falcón C, Bargalló N, Alemany S, Crespo-Facorro B, Nenadic I, Fañanás L

PLoS ONE (2015) 10(6), e0129616

DOI: http://dx.doi.org/10.1371/journal.pone.0129616

PLOS | ONE

RESEARCH ARTICLE

Birth Weight and Adult IQ, but Not Anxious-Depressive Psychopathology, Are Associated with Cortical Surface Area: A Study in Twins

Aldo Córdova-Palomera[1,2], Mar Fatjó-Vilas[1,2], Carles Falcón[3,4], Nuria Bargalló[2,3,5], Silvia Alemany[1,2], Benedicto Crespo-Facorro[2,6,7], Igor Nenadic[8], Lourdes Fañanás[1,2]*

1 Unidad de Antropología, Departamento de Biología Animal, Facultad de Biología and Instituto de Biomedicina (IBUB), Universitat de Barcelona, Av. Diagonal, 643, 08028, Barcelona, Spain, **2** Centro de Investigaciones Biomédicas en Red de Salud Mental (CIBERSAM), C/Doctor Esquerdo, 46, 28007, Madrid, Spain, **3** Medical Image Core Facility, the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS); C/Rosselló, 149–153, 08036, Barcelona, Spain, **4** Centro de Investigación Biomédica en Red en Bioingeniería, Biomedicina y Nanomedicina (CIBER-BBN), C/ Poeta Mariano Esquillor, s/n., 50018, Zaragoza, Spain, **5** Centro de Diagnóstico por Imagen, Hospital Clínico, C/Villarroel, 170, 08036, Barcelona, Spain, **6** University Hospital Marqués de Valdecilla, Department of Psychiatry, School of Medicine, University of Cantabria, Av. Valdecilla, s/n, 39008, Santander, Cantabria, Spain, **7** IFIMAV, Instituto de Formación e Investigación Marqués de Valdecilla, Av. Valdecilla, s/n, 39008, Santander, Cantabria, Spain, **8** Department of Psychiatry and Psychotherapy, Jena University Hospital, Friedrich Schiller University Jena, Philosophenweg 3, 07743, Jena, Germany

* lfananas@ub.edu

Citation: Córdova-Palomera A, Fatjó-Vilas M, Falcón C, Bargalló N, Alemany S, Crespo-Facorro B, et al. (2015) Birth Weight and Adult IQ, but Not Anxious-Depressive Psychopathology, Are Associated with Cortical Surface Area: A Study in Twins. PLoS ONE 10(6): e0129616. doi:10.1371/journal.pone.0129616

Academic Editor: Judith Homberg, Radboud University, NETHERLANDS

Received: November 5, 2014

Accepted: May 11, 2015

Published: June 18, 2015

Data Availability Statement: Some access restrictions apply to the data underlying the findings. All data underlying the findings in this study are available only upon request because of an ethical restriction. As mentioned in the Ethics statement section of the manuscript, human participants gave written informed consent to be included in the study (Comissió de Bioètica de la Universitat de Barcelona; Institutional Review Board registry IRB00003099; Assurance number: FWA00004225; http://www.ub.edu/recerca/comissiobioetica.htm). In their signed consent forms, all participants agree to collaborate by

Abstract

Background

Previous research suggests that low birth weight (BW) induces reduced brain cortical surface area (SA) which would persist until at least early adulthood. Moreover, low BW has been linked to psychiatric disorders such as depression and psychological distress, and to altered neurocognitive profiles.

Aims

We present novel findings obtained by analysing high-resolution structural MRI scans of 48 twins; specifically, we aimed: i) to test the BW-SA association in a middle-aged adult sample; and ii) to assess whether either depression/anxiety disorders or intellectual quotient (IQ) influence the BW-SA link, using a monozygotic (MZ) twin design to separate environmental and genetic effects.

Results

Both lower BW and decreased IQ were associated with smaller total and regional cortical SA in adulthood. Within a twin pair, lower BW was related to smaller total cortical and regional SA. In contrast, MZ twin differences in SA were not related to differences in either IQ or depression/anxiety disorders.

allowing the research group "Gens i ambient en la comprensió de la diversitat de la conducta humana i de la etiopatogenia de la malaltia mental", led by Prof. Dr. Lourdes Fañanás, member of the Institute of Biomedicine of the University of Barcelona (IBUB; Address: Departament de Bioquímica i Biologia Molecular, Facultat de Biologia, Universitat de Barcelona. Avda. Diagonal, 645. 08028. Barcelona, Spain. Phone: (+34) 934 021 525. www.ub.edu/ibub/). Data requests may be addressed to the IBUB using these contact details.

Funding: This work was supported by the Spanish SAF2008-05674, European Twins Study Network on Schizophrenia Research Training Network (grant number EUTwinsS; MRTN-CT-2006-035987), the Catalan 2014SGR1636 and the PIM2010-ERN-00642 in frame of ERA-NET NEURON. A. Córdova-Palomera was funded by The National Council for Science and Technology (CONACyT, Mexico). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Conclusion

The present study supports findings indicating that i) BW has a long-lasting effect on cortical SA, where some familial and environmental influences alter both foetal growth and brain morphology; ii) uniquely environmental factors affecting BW also alter SA; iii) higher IQ correlates with larger SA; and iv) these effects are not modified by internalizing psychopathology.

Introduction

Human neurodevelopment is a highly intricate, stage-dependent, dynamic, lifetime process. Early periods of growth are of particular importance due to their enduring impact on the remaining sequence of anatomical maturational processes. In fact, intrauterine and neonatal brain insults have been shown to have a long-term impact on behaviour and neurological outcomes [1,2].

Factors such as prematurity and very low birth weight (BW) have been related to altered cortical brain features later in life [3–5]. As genetic and environmental influences modify brain features differently across stages [6] Bystron et al., 2008), studying which early modifications of the human cortex have long-lasting effects–and their potential origins, both genetic and non-genetic–may shed light on human brain maturational processes and their consequences for cognitive functioning, and mental health and disease.

Accordingly, models of developmental vulnerability to adult psychopathology have recently been fostered by twin and magnetic resonance imaging (MRI) studies. They have demonstrated that both genetic and environmental influences play a role in abnormal neurocognition and related mental health issues [7]. Genetically informative neuroimaging approaches have contributed considerably to the discipline of developmental psychopathology, since they allow links between genes, brain structure/function, and neurocognitive profiles associated with both normal and pathological psychological traits to be ascertained [8–10].

More explicitly, three recent MRI studies have consistently shown that BW variation within a normal range has an effect on cortical features detectable in children, adolescents and young adults. This effect seems to be environmentally driven and independent of major psychiatric diagnoses. Specifically, the findings indicate that BW has long-term consequences on total and regional cortical surface area (SA), but not on cortical thickness [11–13]. This is consistent with previous research showing that cortical thickness and surface are two phenotypes highly independent at the genetic level [14], which suggests their alterations could be associated to relatively distinct neuropsychiatric outcomes.

The direction of the association is the same in these studies: low BW individuals show reduced cortical SA. Nevertheless, reductions of cortical SA have been found in different regions when directly analysing the relationship between BW and SA [11, 13], from those when the focus is exclusively on environmental influences on BW, as measured by monozygotic (MZ) twin difference designs [12]. Given that MZ twins have the same genetic background, their phenotypic dissimilarities are believed to be environmentally-induced. Accordingly, the slight contrast in previous reports may indicate that the variety of genetic and environmental factors influencing BW [15,16] can alter cortical anatomy in specific ways. Remarkably, cortical brain surface alterations found by these authors are located in areas with relevance for psychiatric research, such as the temporal, superior frontal and cingulate regions [17–19].

As concluded by Walhovd et al. [13], BW differences across diagnostic groups and conditions may influence differences observed in cortical parameters assessed later in life in neuroimaging research. The present study aims to address three considerations in this regard.

First, it has been reported that human intelligence, measured by IQ, could correlate with brain volume [20,21]. Moreover, altered cognitive capabilities have been related to low BW [22,23]. Skranes et al. [4] reported that cognitive impairments in very-low-BW young adults may be due to decreases in cortical SA caused by altered foetal growth trajectories. It has similarly been proposed that both depressive and anxious pathologies are associated with lower IQ [24], which means both cortical SA and IQ need to be included when analysing the association between BW and cortical variables. Also, a phenotypic correlation between cognitive abilities and cortical SA has been shown in healthy individuals, with genetic factors accounting for ~86% of the association [25]. Hence, genetically informative designs (i.e., studies of MZ twins) may allow us to determine whether the proposed alteration of BW and SA due to uniquely environmental factors [12] holds independently of (likewise) environmental influences on IQ or internalizing psychopathology.

Secondly, there is some–so far inconclusive–evidence of fetal growth alterations as risk factor for adult internalizing disorders (namely, depression and psychological distress) [26,27]. Several cortical morphological brain alterations have been related to these psychopathological states [28–31]. Current evidence suggests that anxious and depressive disorders exhibit a wide degree of comorbidity, a common etiopathology and diagnostic criterion overlap [32–35]. This is probably reflected as shared brain morphometry alterations [36,37] and may also induce SA changes.

Finally, as different patterns of change in cortical and subcortical structures emerge across successive stages of normal development [38], the age range across which the BW-SA link is valid remains unidentified.

Hence, while the previous associations have consistently been demonstrated in children, adolescents and young adults, further verification in older samples is still necessary. Besides, no previous study has evaluated the potential role of anxious-depressive psychopathology in altering these associations.

Our study aims: i) to test the previously identified associations (BW-SA; and MZ differences in both BW and SA) using a sample of middle-aged adults; and ii) to evaluate whether such associations persist regardless of internalizing (anxious-depressive) disorders and differences in IQ profiles.

Methods

Ethics statement

Written informed consent was obtained from all participants after a detailed description of the study aims and design, approved by the institutional ethics committee (Comissió de Bioètica de la Universitat de Barcelona (CBUB); Institutional Review Board registry IRB00003099; Assurance number: FWA00004225; http://www.ub.edu/recerca/comissiobioetica.htm). All procedures were in accordance with the Declaration of Helsinki.

Sample description

Participants of this study were part of a larger twin sample consisting of 242 European descent Spanish adult twins from the general population who gave permission to be contacted for research purposes. The current sample consisted of a 54-individual (27-twin-pair) subset of participants extracted from the initial group. For the current sample, the exclusion criteria

applied included age under 18 and over 65, a medical history of neurological disturbance, presence of sensory or motor alterations and current substance misuse or dependence.

Medical records and a battery of psychological and neurocognitive tests were obtained in face-to-face interviews by trained psychologists. Additionally, peripheral blood or saliva samples were obtained from all participants, and zygosity of the pairs was determined by genotyping 16 highly polymorphic microsatellite loci from DNA samples (SSRs; PowerPlex 16 System Promega Corporation). Identity on all the markers can be used to assign monozygosity (i.e., whether twins of a given pair were born from a single fertilized ovum, and are so identical at the DNA sequence level) with greater than 99% accuracy [39].

From the previous sample, a group of 54 middle-aged participants (27 MZ twin pairs; age range 22–56, median age 38; 47% female), who were informative for psychopathology, neurocognition and early stress factors, accepted to participate in an ongoing research project relating cognitive performance, brain function and epigenetic signatures.

The twins included in this subset of 54 participants met the following criteria: a) age at scan between 20 and 56 years, b) both twins right-handed and c) neither twin had a lifetime diagnosis other than depression and/or anxiety. Pairs where one or both twins met the criteria for a lifetime psychiatric diagnosis other than depression or anxiety, or with either neurological or major medical illnesses, were excluded (see *c. Clinical and Neurocognitive Assessment*).

After this point, due to image artifacts and a lack of data on some participants, the final sample (i.e., the subset included in all the statistical analysis) consisted of 48 individuals (24 twin pairs) (mean (SD) age = 36 (11) years; 42% male); there were 6 diagnosis-concordant (anxiety/depression) and 8 diagnosis-discordant pairs, and 10 healthy control twin pairs. All analyses described below refer to this 48-individual sample. Further demographic and descriptive details of this group of twins can be found elsewhere [40] and below.

Clinical and Neurocognitive Assessment

A trained clinical psychologist applied the Structural Clinical Interview for DSM-IV Axis I Disorders (SCID-I) [41] in a face-to-face interview to screen for the presence of any lifetime depression (major depressive disorder or depressive disorder not otherwise specified) or anxiety spectrum disorders (panic disorder with/without agoraphobia, specific/social phobia, generalized anxiety disorder, agoraphobia without history of panic disorder, anxiety disorder not otherwise specified or obsessive-compulsive disorder).

Individuals meeting the diagnostic criteria for at least one lifetime diagnosis of anxiety or depression were classified as affected by a stress-related disorder, and "concordant", "discordant" and "healthy" statuses of twin pairs were defined accordingly (Table 1). Most of the affected individuals in this sample experienced a first episode of any anxiety or depressive psychopathology during their adolescence, consistent with previous epidemiological data [42].

Intelligence quotient (IQ) was estimated from five subtests (block design, digit span, matrix reasoning, information and vocabulary) of the Wechsler Adult Intelligence Scale (WAIS-III) [43,44] by trained psychologists.

Participants were asked to report if they had received pharmacological 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 drug treatment for anxiety or depression.

Birth weight

Information on obstetric complications was collected by direct interviews with the participants' mothers [45] by means of the Lewis-Murray Obstetric Complications Scale [46]. BW

Table 1. Demographic, clinical, neurocognitive, obstetric and cortical variables for concordant, discordant and healthy MZ twin pairs.

	CONCORDANT (12 subjects)		DISCORDANT (16 subjects)		HEALTHY (20 subjects)		Group comparison
	Number of individuals	%	Number of individuals	%	Number of individuals	%	X-square[a]; *p*
Gender (m/f)	2/10	16.6/83.3	6/10	37.5/62.5	12/8	60/40	5.97; 0.052
Depression[+]	4	16.6	4	12.5	-	-	-
Anxiety[+]	6	25	1	3.1	-	-	-
Comorbid[+]	2	8.3	3	9.4	-	-	-
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	X-square[b]; *p*
Age	40.8 (13.3)	23–56	33.1 (12.2)	20–53	35.2 (7.9)	22–48	3.34; 0.188
IQ	103 (13.7)	83–127	106.3 (11.6)	87–131	107.4 (6.9)	96–118	0.9; 0.639
BW (grams)	2625 (508)	1900–3360	2421 (424)	1800–3000	2482 (536)	1400–3350	1.17; 0.557
ICV (cm^3)	1282 (257)	890–1592	1420 (212)	1106–1829	1533 (78)	1440–1719	9.42; 0.009*
Total SA (mm^2)	152600 (15287)	136200–174500	159900 (17158)	133500–185600	164400 (8658)	151900–182400	3.53; 0.172
Intrapair BW diff. (grams)	303 (361)	50–1000	334 (336)	100–1000	315 (277)	0–1000	0.41; 0.82
Intrapair IQ diff.	6.7 (7.2)	1–18	6.6 (4.6)	1–13	4.4 (3.9)	0–12	1.16; 0.56
Intrapair total SA diff. (mm^2)	2425 (1960)	144–5399	5874 (5765)	275–17330	3191 (3515)	109–9772	1.22; 0.542

Abbreviations: m = males; f = females;

[+] = lifetime diagnoses according to the Diagnostic and Statistical Manual of Mental Disorders; SD = standard deviation; IQ = intellectual quotient; BW = birth weight; ICV = total intracranial volume; SA = surface area

[a] = X-square and *p*-value estimates for gender data were obtained using Monte Carlo tests with 10^6 replicates

[b] = Kruskal-Wallis X-square, as these variables were continuous

* = statistically significant *p*-value.

doi:10.1371/journal.pone.0129616.t001

distribution by gestational age of all the subjects in the sample was in accordance with a previous report of Caucasian twins [47].

MRI acquisition and postprocessing

High-resolution 3D structural datasets, using a T1-weighted magnetization-prepared rapid gradient echo, were acquired at the MRI Unit of the Image Platform (IDIBAPS, Hospital Clínic de Barcelona) by means of a TIM TRIO 3 T scanner (Siemens, Erlangen, Germany), with the parameters: 3D T1-weighted MPRAGE sequence, TR = 2300 ms, TE = 3.03 ms, TI = 900 ms, flip angle = 9°, 192 slices in the sagittal plane, matrix size = 256×256, 1 mm^3 isometric voxel, 8-channel coil.

MRI scans were processed and analysed using the freely available software FreeSurfer (version 5.1.0; http://surfer.nmr.mgh.harvard.edu/), run on Ubuntu with the Linux 2.6.28-11-generic kernel. Further technical details of FreeSurfer can be found in the literature [48–52].

Cortical SA was measured over the interface between grey and white matter, at the so-called *white matter surface*, as this matches a morphological trait and has lower sensitivity to cortical thickness than the outermost surface [53]. The cerebral cortex was parcellated into 148 units (hereafter regions; 74 per hemisphere) based on gyral and sulcal structure [54]. Cortical SA measurements were obtained for all the subjects for each region and for the total cortical mantle. Regions of interest (ROIs) were defined from previous reports (see *Statistical analysis*), by combining some of the 148 available regions. Afterwards, total intracranial volume (ICV) was

estimated [55]. As volume estimates do not increase linearly with SA parameters, ICV was raised to the power of 0.754 (hereafter $ICV^{0.754}$) for later use as a covariate in statistical analysis. Both the cortical parcellation and intracranial volume calculation procedures have been validated against manual measurements (for details, see references above).

These procedures were fully automated; all scans were visually inspected, and slight manual corrections were applied when necessary, following standard procedures. General information on total SA and ICV measurements for the sample are given in Table 1.

Selection of MRI variables and statistical analysis

Cortical SA across the 148 regions of all 48 subjects were exported as a data matrix, and all statistical analysis was performed in the R Statistical Software [56]. Four different analyses based on multivariate linear regressions were performed. ICV was used as a covariate, given its potential to correlate with general brain features [14].

First, in order to test for associations between raw BW measures and SA phenotypes across the 48 individuals (i.e., considering each twin as an independent observation), linear mixed-effects (LME) models were implemented [57] using SA measurements (in square millimetres) from the dataset mentioned above. LME models allow corrections to be made for the correlated nature of data from twin pairs, thus providing appropriate regression estimates for specific outcomes of interest (here, SA of either total cerebral cortex or each of nine ROIs). Following previous reports on statistical analysis of twin data [58–60], pair id was included as a random effect, to apply a "random" shift in the intercept to both twins in every pair.

Consequently, the initial analysis implemented an LME regression to test for an association between BW, diagnostic status and a measure of total SA, controlling for gender, age, ICV and weeks of gestation [Total SA = $\beta_0 + \beta_1$(gender) + β_2(age) + $\beta_3(ICV^{0.754})$ + β_4(weeks of gestation) + β_5(BW) + β_6(IQ) + β_7(diagnosis)].

Next, nine similar analyses were carried out using ROIs over relevant Brodmann's areas [SA of ROI = $\beta_0 + \beta_1$(gender) + β_2(age) + $\beta_3(ICV^{0.754})$ + β_4(weeks of gestation) + β_5(BW) + β_6(IQ) + β_7(diagnosis)]. As shown in Fig 1, the ROIs covered, in the right hemisphere, *A*) middle, superior and transverse temporal, inferior insula, orbital medial olfactory and intermediate regions between them; *B*) middle posterior, posterior dorsal and marginalis cingulate regions, plus the paracentral area; *C*) subcallosal and frontal superior gyri, including the suborbital sulcus; and *D*) temporal pole. In the left hemisphere, ROIs were at *E*) temporal inferior gyrus, *F*) a cluster including the subcallosal, anterior cingulate and suborbital regions, *G*) middle and superior temporal cortex, *H*) orbital gyrus and H-shaped orbital sulcus, and *I*) frontal superior region. As discussed below, the Bonferroni correction to the statistical significance threshold was used for this set of regressions ($p_{Bonferroni} = 0.05 / 9 = 0.0056$). ROIs *A*, *B*, *C*, *E* and *F* were defined from a study of normal BW variation in the general population [13], whereas ROIs *D*, *G*, *H* and *I* were previously associated with (environmentally-driven) MZ twin pair differences in BW [12]. As discussed above (see *Introduction*), all these candidate areas have been shown relevance for neuropsychiatric outcomes [17–19].

Then, with the aim of evaluating whether lower BW within a twin pair was associated with smaller intrapair SA, regression models using twin pair differences [58,61] were applied. While structural equation modeling allows parsing out the specific genetic, shared and unique environmental factors underlying phenotypic relationships in relatively large samples of both MZ and dizygotic twins [62,63], it is not suited for the current sample with a moderate number of MZ twin pairs. In contrast, other statistical approaches based on regression modeling have been developed to be used with MZ twin data, and their usefulness and feasibility have been proven even for relatively small samples [58,61]. Since previous reports indicate that the

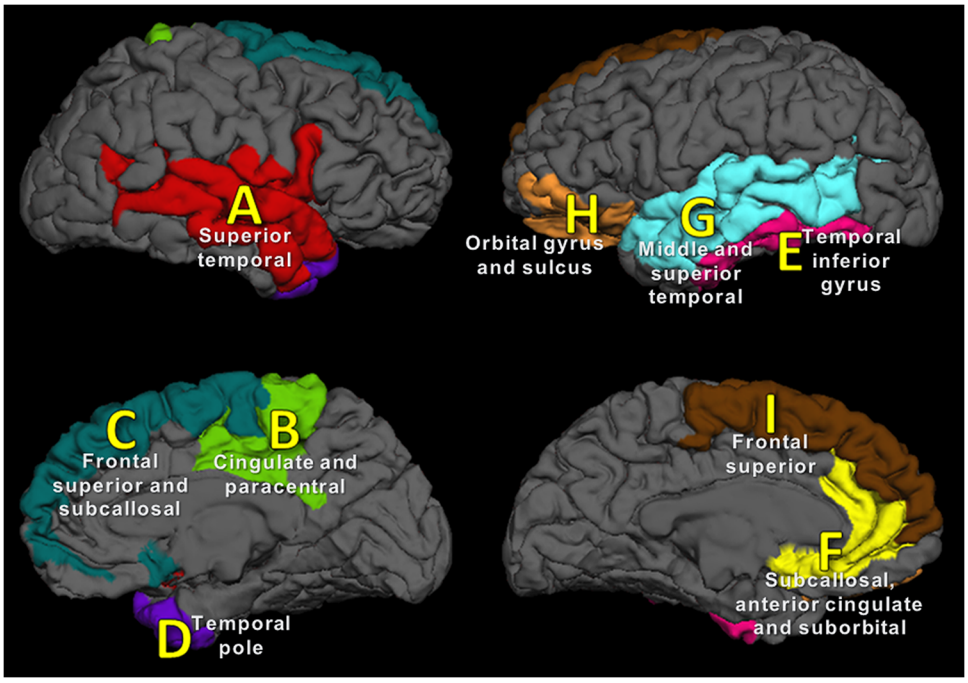


Fig 1. Nine anatomical ROIs for analysis of associations between SA and any of BW, IQ or depression/anxiety. Additional details on ROI selection and nomenclature can be found in *Methods*.

doi:10.1371/journal.pone.0129616.g001

association between BW and SA may largely be due to unique environmental factors [12,13], a common method to analyze (environmentally induced) differences within MZ twin pairs was adopted. Briefly, this approach consists in estimating the expected value of an outcome variable from: $E(D_i^Y) = \beta_{W1}D_i^X + \beta_{W2}D_i^{X'} + \beta_{W3}D_i^{X''} + \ldots$, where the outcome variable (here, intrapair differences in cortical SA) is $D_i^Y = Y_{i1} - Y_{i2}$; and the individual predictors are in the form $D_i^X = X_{i1} - X_{i2}$, $D_i^X = X'_{i1} - X'_{i2}$, and so on, with the set $\{X,X',X'',\ldots\}$ being the model predictors (here, ICV, IQ, BW and diagnostic, as mentioned below). The first subindex i stands for pair number, with $i \in \{1,\ldots,n\}$ (here, $n = 24$ MZ pairs), and the second subindex $j \in \{1,2\}$ is the randomly assigned co-twin number.

In the previous model, the β_W coefficients for covariates with the same value for both co-twins (i.e., age) cancel out by the subtraction $D_i^X = X_{i1} - X_{i2} = 0$, and only the variables that may show intrapair differences are thus included.

Initially, total (intrapair) differences in BW and total (intrapair) differences in SA were analysed. In contrast to the previous regressions, neither gender nor age was included as covariates from this point on, as they had the same value for both twins in each pair. A regressor variable corresponding to differences in diagnostic status was included. The models applied to assess intrapair differences included the variables of interest (BW, IQ and diagnostic status) and ICV as a covariate [Intrapair differences in total SA = β_0(intrapair differences in ICV$^{0.754}$) + β_1(intrapair differences in IQ) + β_2(intrapair differences in BW) + β_3(intrapair differences in diagnostic)].

Finally, to explore putative locations of origin for this last association, the nine ROIs were evaluated. Intrapair differences were tested following the technique mentioned in the preceding paragraph [Intrapair differences in SA of ROI = β_0(intrapair differences in ICV$^{0.754}$) + β_1(intrapair differences in IQ) + β_2(intrapair differences in BW) + β_3(intrapair differences in

diagnosis)]. Correspondingly, Bonferroni adjustments were applied by considering $p_{Bonferroni}$ = 0.05 / 9 = 0.0056.

As only 24 observations were included in the preceding tests of intrapair differences (one observation for each twin pair), *p*-values for this regression model were obtained from permutation tests, with the *lmPerm* R package [64]. Such permutation-based *p*-values are particularly suited to saturated experimental designs and datasets from non-normal populations or those with apparent outliers. Permutated *p*-values shown in the *Results* were in agreement with those obtained with ordinary least squares regressions.

Results

Table 1 shows descriptive sample data, arranged according to the psychopathological status of each twin pair (*concordant*, *discordant* or *healthy* pairs).

Among the three groups (*concordant*, *discordant* and *healthy*), no differences were found for either age, BW, IQ or total SA. ICV did show statistically significant inter-group differences ($p = 0.009$), which were seemingly driven by the *concordant* group (Kruskal-Wallis X-square for *discordant* vs. *healthy* $p = 0.286$), whose lower ICV mean value might have been due to the fact that there were 5 female and only 1 male pair. Consequently, adjustments for ICV were included in all subsequent tests (see *Methods*).

As a preliminary step, collinearity between BW and IQ, BW and diagnosis, and IQ and diagnosis were tested; none of them was found to be statistically significant.

BW and cortical SA: direct association

When evaluating all 48 observations independently (i.e., correcting for the clustered origin of observations from twin pairs), we found associations between total cortical SA and both BW ($\beta = 5.89$, $t = 2.79$, $p = 0.011$) and IQ ($\beta = 299.2$, $t = 2.67$, $p = 0.015$). Nonetheless, total SA was not related to diagnosis of internalizing psychopathology ($\beta = -2279.9$, $t = -1.52$, $p = 0.145$).

By examining the nine ROIs described above, it was found that the size of only one of them was associated with BW. Specifically, the dimension of ROI *B*, in the right cingulate and adjacent areas positively correlated with BW ($\beta = 0.34$, $t = 3.39$, $p_{Bonferroni} = 0.026$). Similarly, IQ score was directly proportional to size of the left subcallosal, anterior cingulate and suborbital cluster (ROI *F*, $\beta = 14.1$, $t = 3.27$, $p_{Bonferroni} = 0.034$) and also showed a trend towards association with the left temporal inferior gyrus (ROI *E*, $\beta = 13.5$, $t = 2.79$, $p_{Bonferroni} = 0.099$) (see Fig 1). No association was found between the size of any of these ROIs and diagnostic status.

Intrapair differences in BW and intrapair differences in cortical SA

Within a twin pair, smaller total cortical SA was associated with lower BW ($\beta = 7.6$, $p = 0.004$), but with no differences in either IQ ($\beta = 125.5$, $p = 0.2$) or diagnostic status ($\beta = -1677.3$, $p = 0.149$).

Finally, analysis of the nine ROIs detailed above showed that smaller intrapair area in the left middle and superior temporal cortical regions was related to lower BW within a pair (ROI *G*, $\beta = 0.8$, $p_{Bonferroni} = 0.029$) (see Fig 1). Intrapair differences in IQ and diagnosis had no effect on intrapair SA differences in these ROIs.

Discussion

These results, from a middle-aged adult sample, support an association between low BW and reduced cortical SA, in line with previous findings in younger samples. Such an association was found at the level of both individuals and MZ-differences, and by evaluating both total and

ROI SA. Neither the internalizing (i.e., anxious-depressive) psychopathological status nor the IQ scores of the participants altered this association.

BW and cortical SA

Initially, analysis was performed in order to search for influences of BW, IQ and diagnosis on cortical SA, across the 48 participants. BW was related to SA of the whole cortical mantle–each gram of BW accounted for approximately 5.94 mm^2 of adult SA–and of a region comprising the right cingulate and paracentral cortex. IQ was also associated with SA, in agreement with former publications showing larger brain volumes in people with higher IQ [20,21]. Specifically, higher IQ was correlated with larger total SA and larger SA of two regions in the left hemisphere: temporal inferior cortex and a cluster including cingulate, subcallosal and suborbital areas.

The analysis considered all subjects independently (i.e., as members of a general-population sample, correcting for the clustering of observations due to twin-pair relatedness). Remarkably, all associated ROIs (*B*, *E* and *F*, see Fig 1) corresponded to those derived from a study of a heterogeneous sample of healthy non-related individuals [13].

Intrapair differences in BW and intrapair differences in cortical SA

As mentioned above, it is known that BW and SA are the result of both genetic and environmental influences. Accordingly, by examining MZ twin pair differences, additional analysis explored the relationship of exclusively environmental effects on both phenotypes. We found that, within a twin pair, lower BW is associated with smaller total SA. Every gram of intrapair disadvantage in BW was associated with an average reduction of approximately 7.6 mm^2 in total cortical SA in adulthood. By comparing this result with those of previous sections, it is feasible to infer that SA is particularly sensitive to environmentally-driven BW variation. ROI analysis of intrapair differences indicated that a region covering the left middle and superior temporal cortex was specifically susceptible to environmental factors affecting BW. This area (ROI *G*, see Fig 1) was defined from a previous study of small intrapair BW differences of MZ twins [12].

While it was possible to show that non-genetic influences on foetal growth provoke changes in brain morphology, this was not the case for IQ. The detected IQ-SA link may be due to either familial factors (genes and shared environment) or gene-environment interactions, but solely environmental effects on both IQ and SA (as detected by MZ pair differences) were not associated with each other. Notably, this is also consistent with previous indications of the genetic origins of the association between intelligence and brain volume [21] and more recent evidence that most of the cognitive ability-SA relationship may be accounted for by genetic factors [25]. Our results are in agreement with these studies and also suggest the existence of environmental factors that commonly affect BW and SA, but not IQ and SA.

Lastly, all these relationships persisted independently of diagnosis of anxious-depressive disorders; this implies the results are robust, despite a putative confounding effect of clinical traits. Although inconclusive, there is some evidence linking foetal growth and risk for adult depression and/or psychological distress [26,27]. Hence, one could expect some differential brain morphological effect depending on diagnostic criteria. Nevertheless, this was not the case in the analysis carried out here: our results indicate that BW alters SA regardless of internalizing psychopathology traits. Further research using distinct severity of psychopathological status may clarify potential diagnosis-specific effects.

Limitations of the study

Finally, some limitations deserve consideration. First, the sample size is relatively small. Though replication using larger independent samples and with more severe phenotypic discordance is required, it is worth noting that the current findings are consistent with previous reports that show a long-lasting influence of early foetal growth alterations on adult brain morphology, which persist even despite the presence of psychotic psychopathology [11–13]. The present results partly replicate such studies in an independent sample, and suggest that the BW-SA relationship holds despite the presence of anxious/depressive disorders. This agreement probably suggests the presence of strong effect sizes for the above mentioned relationships.

Other putative limitation of this work is the phenotypical (i.e., clinical) heterogeneity across MZ twin groups, with an unbalanced distribution of concordant, discordant and healthy pairs. While cross-validating the present results with larger independent datasets from twin pairs with a narrower and more balanced phenotypic distribution is necessary, two features from the ongoing study should be noted. First, the clinical phenotype did not seem to modify any of the statistical associations described here. Namely, both BW-SA and IQ-SA associations were statistically significant across the diverse clinical-psychopathological composition of the MZ subgroups: the associations held across the set of all concordant, discordant and healthy pairs.

In addition, the only IQ measure employed here was derived from a full-scale assessment. While previous research indicates that both full-scale and performance IQ may be related to differences in BW of MZ co-twins [12], exploring the associations between performance IQ and BW may be difficult here mainly due to two reasons. First, using only a few intelligence subscales to build up a performance IQ measure may give rise to statistical distributions departing from normality. In this sense, the full-scale IQ measure was computed by averaging over a relatively large number of intelligence subscales, thus approaching a robust and normally-distributed variable. Secondly, in the larger UB twin registry dataset ($n > 200$ co-twins), not all IQ subscales seem associated with BW, seemingly due to the moderate (average) intra-pair difference in BW. Importantly, the associations described here between cortical SA and IQ are in agreement with former reports and show consistency with biological mechanisms proposed by recent literature.

Conclusion

The present study supports previous findings indicating that BW has a long-lasting effect on cortical SA, where a mixture of familial (genes and shared environment) and solely environmental interactions may influence both foetal growth and brain morphology; and environmental factors affecting BW have a specific effect on SA as well: a portion of SA which is entirely driven by the environment seems to be modified by the fraction of BW that is also determined by non-genetic influences. This distinction is particularly interesting given that ROI analysis indicate that the left temporal cortex is sensitive to environmental influences on BW, but it is not determined by the whole BW variation; which indicates that diverse determinants of BW (genes, environment and their interplay) may affect SA differently. Additionally, higher IQ scores correlate with larger SA; this relationship does not seem to be driven by unique environmental factors. None of these associations were modified by the presence of internalizing (anxious-depressive) disorders.

Acknowledgments

Supported by the Spanish SAF2008-05674, European Twins Study Network on Schizophrenia Research Training Network (grant number EUTwinsS; MRTN-CT-2006-035987), the Catalan

2014SGR1636 and the PIM2010-ERN-00642 in frame of ERA-NET NEURON. Special thanks are given to all twin pairs who participated in this study. We are indebted to the Medical Image core facility of the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) for the technical help. Ximena Goldberg, PhD, and MRI technicians César Garrido and Santi Sotés contributed to this work. A. Córdova-Palomera was funded by The National Council for Science and Technology (CONACyT, Mexico).

Author Contributions

Conceived and designed the experiments: ACP SA LF. Performed the experiments: ACP SA CF NB LF. Analyzed the data: ACP SA CF. Contributed reagents/materials/analysis tools: NB BCF IN LF. Wrote the paper: ACP MFV CF NB SA BCF IN LF. Interviewed the participants: SA. Pre-processed MRI data: ACP CF.

References

1. Bilbo SD, Schwarz JM (2009) Early-life programming of later-life brain and behavior: a critical role for the immune system. Front Behav Neurosci 3: 14. doi: 10.3389/neuro.08.014.2009 PMID: 19738918
2. Mwaniki MK, Atieno M, Lawn JE, Newton CR (2012) Long-term neurodevelopmental outcomes after intrauterine and neonatal insults: a systematic review. Lancet 379: 445–452. doi: 10.1016/S0140-6736(11)61577-8 PMID: 22244654
3. Bjuland KJ, Lohaugen GC, Martinussen M, Skranes J (2013) Cortical thickness and cognition in very-low-birth-weight late teenagers. Early Hum Dev 89: 371–380. doi: 10.1016/j.earlhumdev.2012.12.003 PMID: 23273486
4. Skranes J, Lohaugen GC, Martinussen M, Haberg A, Brubakk AM, Dale AM (2013) Cortical surface area and IQ in very-low-birth-weight (VLBW) young adults. Cortex 49: 2264–2271. doi: 10.1016/j.cortex.2013.06.001 PMID: 23845237
5. Zubiaurre-Elorza L, Soria-Pastor S, Junque C, Sala-Llonch R, Segarra D, Bargallo N, et al. (2012) Cortical thickness and behavior abnormalities in children born preterm. PLoS ONE 7: e42148. doi: 10.1371/journal.pone.0042148 PMID: 22860067
6. Toga AW, Thompson PM, Sowell ER (2006) Mapping brain maturation. Trends Neurosci 29: 148–159. PMID: 16472876
7. Viding E, McCrory EJ (2012) Genetic and neurocognitive contributions to the development of psychopathy. Dev Psychopathol 24: 969–983. doi: 10.1017/S095457941200048X PMID: 22781866
8. Bigos KL, Weinberger DR (2010) Imaging genetics—days of future past. Neuroimage 53: 804–809. doi: 10.1016/j.neuroimage.2010.01.035 PMID: 20080192
9. Fatjo-Vilas M, Pomarol-Clotet E, Salvador R, Monte GC, Gomar JJ, Sarro S, et al. (2012) Effect of the interleukin-1beta gene on dorsolateral prefrontal cortex function in schizophrenia: a genetic neuroimaging study. Biol Psychiatry 72: 758–765. doi: 10.1016/j.biopsych.2012.04.035 PMID: 22763186
10. Jaffee SR, Price TS, Reyes TM (2013) Behavior genetics: past, present, future. Dev Psychopathol 25: 1225–1242. doi: 10.1017/S0954579413000588 PMID: 24342837
11. Haukvik UK, Rimol LM, Roddey JC, Hartberg CB, Lange EH, Vaskinn A, et al. (2014) Normal birth weight variation is related to cortical morphology across the psychosis spectrum. Schizophr Bull 40: 410–419. doi: 10.1093/schbul/sbt005 PMID: 23419977
12. Raznahan A, Greenstein D, Lee NR, Clasen LS, Giedd JN (2012) Prenatal growth in humans and postnatal brain maturation into late adolescence. Proc Natl Acad Sci U S A 109: 11366–11371. doi: 10.1073/pnas.1203350109 PMID: 22689983
13. Walhovd KB, Fjell AM, Brown TT, Kuperman JM, Chung Y, Hagler DJ, et al. (2012) Long-term influence of normal variation in neonatal characteristics on human brain development. Proc Natl Acad Sci U S A 109: 20089–20094. doi: 10.1073/pnas.1208180109 PMID: 23169628
14. Panizzon MS, Fennema-Notestine C, Eyler LT, Jernigan TL, Prom-Wormley E, Neale M, et al. (2009) Distinct genetic influences on cortical surface area and cortical thickness. Cereb Cortex 19: 2728–2735. doi: 10.1093/cercor/bhp026 PMID: 19299253
15. Valero De Bernabe J, Soriano T, Albaladejo R, Juarranz M, Calle ME, Martinez D, et al. (2004) Risk factors for low birth weight: a review. Eur J Obstet Gynecol Reprod Biol 116: 3–15. PMID: 15294360
16. Yaghootkar H, Freathy RM (2012) Genetic origins of low birth weight. Curr Opin Clin Nutr Metab Care 15: 258–264. doi: 10.1097/MCO.0b013e328351f543 PMID: 22406741

17. Almeida LG, Ricardo-Garcell J, Prado H, Barajas L, Fernandez-Bouzas A, Avila D, et al. (2010) Reduced right frontal cortical thickness in children, adolescents and adults with ADHD and its correlation to clinical variables: a cross-sectional study. J Psychiatr Res 44: 1214–1223. doi: 10.1016/j.jpsychires.2010.04.026 PMID: 20510424

18. Haukvik UK, Lawyer G, Bjerkan PS, Hartberg CB, Jonsson EG, McNeil T, et al. (2009) Cerebral cortical thickness and a history of obstetric complications in schizophrenia. J Psychiatr Res 43: 1287–1293. doi: 10.1016/j.jpsychires.2009.05.001 PMID: 19473666

19. Takei Y, Suda M, Aoyama Y, Yamaguchi M, Sakurai N, Narita K, et al. (2013) Temporal lobe and inferior frontal gyrus dysfunction in patients with schizophrenia during face-to-face conversation: a near-infrared spectroscopy study. J Psychiatr Res 47: 1581–1589. doi: 10.1016/j.jpsychires.2013.07.029 PMID: 23978395

20. McDaniel MA (2005) Big-brained people are smarter: A meta-analysis of the relationship between in vivo brain volume and intelligence. Intelligence 33: 337–346.

21. Posthuma D, De Geus EJ, Baare WF, Hulshoff Pol HE, Kahn RS, Boomsma DI (2002) The association between brain volume and intelligence is of genetic origin. Nat Neurosci 5: 83–84. PMID: 11818967

22. Huang C, Martorell R, Ren A, Li Z (2013) Cognition and behavioural development in early childhood: the role of birth weight and postnatal growth. Int J Epidemiol 42: 160–171. doi: 10.1093/ije/dys207 PMID: 23243117

23. Raikkonen K, Forsen T, Henriksson M, Kajantie E, Heinonen K, Pesonen AK, et al. (2009) Growth trajectories and intellectual abilities in young adulthood: The Helsinki Birth Cohort study. Am J Epidemiol 170: 447–455. doi: 10.1093/aje/kwp132 PMID: 19528290

24. Rajput S, Hassiotis A, Richards M, Hatch SL, Stewart R (2011) Associations between IQ and common mental disorders: the 2000 British National Survey of Psychiatric Morbidity. Eur Psychiatry 26: 390–395. doi: 10.1016/j.eurpsy.2010.07.002 PMID: 20933370

25. Vuoksimaa E, Panizzon MS, Chen CH, Fiecas M, Eyler LT, Fennema-Notestine C, et al. (2014) The Genetic Association Between Neocortical Volume and General Cognitive Ability Is Driven by Global Surface Area Rather Than Thickness. Cereb Cortex.

26. Cordova-Palomera A, Goldberg X, Alemany S, Nenadic I, Gasto C, Fañanás L. (2014) Letter to the editor: low birth weight and adult depression: eliciting their association. Psychol Med 44: 1117–1119. doi: 10.1017/S0033291713002754 PMID: 24229470

27. Wojcik W, Lee W, Colman I, Hardy R, Hotopf M (2013) Foetal origins of depression? A systematic review and meta-analysis of low birth weight and later depression. Psychol Med 43: 1–12. doi: 10.1017/S0033291712000682 PMID: 22717127

28. Odlaug BL, Chamberlain SR, Derbyshire KL, Leppink EW, Grant JE (2014) Impaired response inhibition and excess cortical thickness as candidate endophenotypes for trichotillomania. J Psychiatr Res.

29. Venkatasubramanian G, Zutshi A, Jindal S, Srikanth SG, Kovoor JM, Kumar JK, et al. (2012) Comprehensive evaluation of cortical structure abnormalities in drug-naive, adult patients with obsessive-compulsive disorder: a surface-based morphometry study. J Psychiatr Res 46: 1161–1168. doi: 10.1016/j.jpsychires.2012.06.003 PMID: 22770508

30. Wagner G, Schultz CC, Koch K, Schachtzabel C, Sauer H, Schlosser RG (2012) Prefrontal cortical thickness in depressed patients with high-risk for suicidal behavior. J Psychiatr Res 46: 1449–1455. doi: 10.1016/j.jpsychires.2012.07.013 PMID: 22868048

31. Yoon S, Jun CS, Jeong HS, Lee S, Lim SM, Ma J, et al. (2013) Altered cortical gyrification patterns in panic disorder: deficits and potential compensation. J Psychiatr Res 47: 1446–1454. doi: 10.1016/j.jpsychires.2013.06.015 PMID: 23871448

32. Mosing MA, Gordon SD, Medland SE, Statham DJ, Nelson EC, Heath AC, et al. (2009) Genetic and environmental influences on the co-morbidity between depression, panic disorder, agoraphobia, and social phobia: a twin study. Depress Anxiety 26: 1004–1011. doi: 10.1002/da.20611 PMID: 19750555

33. Ressler KJ, Mayberg HS (2007) Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. Nat Neurosci 10: 1116–1124. PMID: 17726478

34. Wittchen HU, Nocon A, Beesdo K, Pine DS, Hofler M, Lieb R, et al. (2008) Agoraphobia and panic. Prospective-longitudinal relations suggest a rethinking of diagnostic concepts. Psychother Psychosom 77: 147–157. doi: 10.1159/000116608 PMID: 18277061

35. Zbozinek TD, Rose RD, Wolitzky-Taylor KB, Sherbourne C, Sullivan G, Stein MB, et al. (2012) Diagnostic overlap of generalized anxiety disorder and major depressive disorder in a primary care sample. Depress Anxiety 29: 1065–1071. doi: 10.1002/da.22026 PMID: 23184657

36. Kroes MC, Rugg MD, Whalley MG, Brewin CR (2011) Structural brain abnormalities common to posttraumatic stress disorder and depression. J Psychiatry Neurosci 36: 256–265. doi: 10.1503/jpn.100077 PMID: 21418787

37. van Tol MJ, van der Wee NJ, van den Heuvel OA, Nielen MM, Demenescu LR, Aleman A, et al. (2010) Regional brain volume in depression and anxiety disorders. Arch Gen Psychiatry 67: 1002–1011. doi: 10.1001/archgenpsychiatry.2010.121 PMID: 20921116

38. Tamnes CK, Walhovd KB, Dale AM, Ostby Y, Grydeland H, Richardson G, et al. (2013) Brain development and aging: overlapping and unique patterns of change. Neuroimage 68: 63–74. doi: 10.1016/j.neuroimage.2012.11.039 PMID: 23246860

39. Guilherme R, Drunat S, Delezoide AL, Oury JF, Luton D (2009) Zygosity and chorionicity in triplet pregnancies: new data. Hum Reprod 24: 100–105. doi: 10.1093/humrep/den364 PMID: 18945712

40. Alemany S, Mas A, Goldberg X, Falcon C, Fatjo-Vilas M, Arias B, et al. (2013) Regional gray matter reductions are associated with genetic liability for anxiety and depression: An MRI twin study. J Affect Disord 149: 175–181. doi: 10.1016/j.jad.2013.01.019 PMID: 23433857

41. First MB (1997) Structured clinical interview for DSM-IV axis I disorders: SCID—I: clinician version: administration booklet. Washington, D.C.: American Psychiatric Press.

42. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE (2005) Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 62: 593–602. PMID: 15939837

43. Sattler JM (2001) Assessment of children: cognitive applications. San Diego: J.M. Sattler.

44. Wechsler D, Cordero Pando A, Yela Granizo M, Zimmerman IL, Woo-Sam JM, Glasser AJ (1997) WAIS escala de inteligencia de Wechsler para adultos. Madrid; Barcelona: TEA Ediciones.

45. Walshe M, McDonald C, Boydell J, Zhao JH, Kravariti E, Touloupoulou T, et al. (2011) Long-term maternal recall of obstetric complications in schizophrenia research. Psychiatry Res 187: 335–340. doi: 10.1016/j.psychres.2011.01.013 PMID: 21324530

46. Lewis SW, Owen MJ, Murray RM (1989) Obstetric complications and schizophrenia: methodology and mechanisms; Schulz SC, Tamminga CA, editors. New York: Oxford University Press.

47. Glinianaia SV, Skjaerven R, Magnus P (2000) Birthweight percentiles by gestational age in multiple births. A population-based study of Norwegian twins and triplets. Acta Obstet Gynecol Scand 79: 450–458. PMID: 10857868

48. Dale AM, Fischl B, Sereno MI (1999) Cortical surface-based analysis. I. Segmentation and surface reconstruction. Neuroimage 9: 179–194. PMID: 9931268

49. Fischl B, Liu A, Dale AM (2001) Automated manifold surgery: constructing geometrically accurate and topologically correct models of the human cerebral cortex. IEEE Trans Med Imaging 20: 70–80. PMID: 11293693

50. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, et al. (2002) Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33: 341–355. PMID: 11832223

51. Fischl B, Salat DH, van der Kouwe AJ, Makris N, Segonne F, Quinn BT, et al. (2004) Sequence-independent segmentation of magnetic resonance images. Neuroimage 23 Suppl 1: S69–84. PMID: 15501102

52. Fischl B, Sereno MI, Tootell RB, Dale AM (1999) High-resolution intersubject averaging and a coordinate system for the cortical surface. Hum Brain Mapp 8: 272–284. PMID: 10619420

53. Winkler AM, Sabuncu MR, Yeo BT, Fischl B, Greve DN, Kochunov P, et al. (2012) Measuring and comparing brain cortical surface area and other areal quantities. Neuroimage 61: 1428–1443. doi: 10.1016/j.neuroimage.2012.03.026 PMID: 22446492

54. Fischl B, van der Kouwe A, Destrieux C, Halgren E, Segonne F, Salat DH, et al. (2004) Automatically parcellating the human cerebral cortex. Cereb Cortex 14: 11–22. PMID: 14654453

55. Buckner RL, Head D, Parker J, Fotenos AF, Marcus D, Morris JC, et al. (2004) A unified approach for morphometric and functional data analysis in young, old, and demented adults using automated atlas-based head size normalization: reliability and validation against manual measurement of total intracranial volume. Neuroimage 23: 724–738. PMID: 15488422

56. R Development Core Team (2012) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. doi: 10.1002/jcc.22917 PMID: 22278855

57. Pinheiro J, Bates D, DebRoy S, Sarkar D, Team RDC (2015) nlme: Linear and Nonlinear Mixed Effects Models.

58. Carlin JB, Gurrin LC, Sterne JA, Morley R, Dwyer T (2005) Regression models for twin studies: a critical review. Int J Epidemiol 34: 1089–1099. PMID: 16087687

59. Hu FB, Goldberg J, Hedeker D, Henderson WG (1998) Modelling ordinal responses from co-twin control studies. Stat Med 17: 957–970. PMID: 9612884

60. Visscher PM, Benyamin B, White I (2004) The use of linear mixed models to estimate variance components from data on twin pairs by maximum likelihood. Twin Res 7: 670–674. PMID: 15607018

61. Córdova-Palomera A (2015) mztwinreg: Regression Models for Monozygotic Twin Data.

62. Draisma HHM (2011) Analysis of metabolomics data from twin families: Division of Analytical Biosciences, Leiden/Amsterdam Center for Drug Research (LACDR), Faculty of Science, Leiden University.

63. Rijsdijk FV, Sham PC (2002) Analytic approaches to twin data using structural equation models. Brief Bioinform 3: 119–133. PMID: 12139432

64. Wheeler B (2010) lmPerm: Permutation tests for linear models.

Advisor's report on the contribution of the Ph.D. candidate to the article

Prof. Dr. Lourdes Fañanás Saura, associate professor at the Faculty of Biology, University of Barcelona, and supervisor of the present doctoral thesis by Aldo Córdova Palomera, hereby certifies that the participation of the Ph.D. candidate in the article "Birth weight and adult IQ, but not anxious-depressive psychopathology, are associated with cortical surface area: further evidences based on a twin study" included the following tasks:

- MRI data pre- and post-processing.
- Participation in study design.
- Statistical analyses.
- Writing of the first manuscript draft.
- Critical revision of the article.

Prof. Dr. Lourdes Fañanás Saura

Barcelona, June 30th 2015.

Birth weight, working memory and epigenetic signatures in* IGF2 *and related genes: a MZ twin study

Córdova-Palomera A, Alemany S, Fatjó-Vilas M, Goldberg X, Leza JC, González-Pinto A, Nenadic I, Fañanás L

PLoS ONE (2014), 9(8), e103639

DOI: http://dx.doi.org/10.1371/journal.pone.0103639

OPEN ACCESS Freely available online

PLOS ONE

Birth Weight, Working Memory and Epigenetic Signatures in *IGF2* and Related Genes: A MZ Twin Study

Aldo Córdova-Palomera[1,2], Silvia Alemany[1,2], Mar Fatjó-Vilas[1,2], Ximena Goldberg[1,2], Juan Carlos Leza[2,3], Ana González-Pinto[2,4], Igor Nenadic[5], Lourdes Fañanás[1,2]*

1 Unitat d'Antropologia, Departament de Biologia Animal, Facultat de Biologia and Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain, **2** Centro de Investigaciones Biomédicas en Red de Salud Mental (CIBERSAM), Instituto de Salud Carlos III, Madrid, Spain, **3** Department of Pharmacology, Faculty of Medicine, Universidad Complutense, Madrid, Spain, and Instituto de Investigación Hospital 12 de Octubre (I+12), Madrid, Spain, **4** Psychiatry Service, Santiago Apóstol Hospital, EMBREC, EHU/UPV University of the Basque Country, Vitoria, Spain, **5** Department of Psychiatry and Psychotherapy, Jena University Hospital, Jena, Germany

Abstract

Neurodevelopmental disruptions caused by obstetric complications play a role in the etiology of several phenotypes associated with neuropsychiatric diseases and cognitive dysfunctions. Importantly, it has been noticed that epigenetic processes occurring early in life may mediate these associations. Here, DNA methylation signatures at *IGF2* (insulin-like growth factor 2) and *IGF2BP1-3* (IGF2-binding proteins 1-3) were examined in a sample consisting of 34 adult monozygotic (MZ) twins informative for obstetric complications and cognitive performance. Multivariate linear regression analysis of twin data was implemented to test for associations between methylation levels and both birth weight (BW) and adult working memory (WM) performance. Familial and unique environmental factors underlying these potential relationships were evaluated. A link was detected between DNA methylation levels of two CpG sites in the *IGF2BP1* gene and both BW and adult WM performance. The BW-*IGF2BP1* methylation association seemed due to non-shared environmental factors influencing BW, whereas the WM-*IGF2BP1* methylation relationship seemed mediated by both genes and environment. Our data is in agreement with previous evidence indicating that DNA methylation status may be related to prenatal stress and later neurocognitive phenotypes. While former reports independently detected associations between DNA methylation and either BW or WM, current results suggest that these relationships are not confounded by each other.

Citation: Córdova-Palomera A, Alemany S, Fatjó-Vilas M, Goldberg X, Leza JC, et al. (2014) Birth Weight, Working Memory and Epigenetic Signatures in *IGF2* and Related Genes: A MZ Twin Study. PLoS ONE 9(8): e103639. doi:10.1371/journal.pone.0103639

Editor: Yan Zhang, Harbin Medical University, China

Received April 3, 2014; **Accepted** July 1, 2014; **Published** August 29, 2014

Data Availability: The authors confirm that, for approved reasons, some access restrictions apply to the data underlying the findings. All data underlying the findings in this study are available upon request because of an ethical restriction. As mentioned in the Ethics statement section of the manuscript, human participants gave written informed consent to be included in the study (Comissió de Bioètica de la Universitat de Barcelona; Institutional Review Board registry IRB00003099; Assurance number: FWA00004225; http://www.ub.edu/recerca/comissiobioetica.htm). In their signed consent forms, all participants agree to collaborate by allowing the research group "Gens i ambient en la comprensió de la diversitat de la conducta humana i de la etiopatogenia de la malaltia mental", led by Prof. Dr. Lourdes Fañanás, member of the Institute of Biomedicine of the University of Barcelona (IBUB; Address: Departament de Bioquímica i Biologia Molecular, Facultat de Biologia, Universitat de Barcelona. Avda. Diagonal, 645. 08028. Barcelona, Spain. Phone: (+34) 934 021 525. www.ub.edu/ibub/). Data requests may be addressed to the IBUB using these contact details.

Funding: This work is supported by the Spanish SAF2008-05674-C03-01, European Twins Study Network on Schizophrenia Research Training Network (grant number EUTwinsS; MRTN-CT-2006-035987; local PIs: F.L. and N.I.), the Catalan 2014SGR1636 and the Ministry of Science and Innovation (PIM2010ERN-00642) in frame of ERA-NET NEURON. A.C.P. was funded by CONACyT (Mexico). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: lfananas@ub.edu

Introduction

Prenatal growth in humans has been linked to several complex disorders in adulthood. In this regard, epidemiological studies demonstrate that poor intrauterine environment induces offspring phenotypes which are characterized by an increased risk of developing different chronic diseases [1]. In particular, indirect markers of prenatal suffering such as low birth weight (BW) have been shown to influence risk for neurodevelopmental disorders involving high cognitive dysfunction, such as schizophrenia and autism [2–4]. However, further studies indicate that low BW may not influence risk for other mental conditions such as anxiety or depression [5,6], probably suggesting some specificity between this risk factor and a number of neurodevelopment-related psychiatric and cognitive outcomes.

Remarkably, recent research has shown that epigenetic processes may mediate associations between environmental insults originating low BW, and several pathological conditions across the human lifespan [7]. Of note, among the different epigenetic marks, DNA methylation is particularly interesting in this context, since there is evidence that large inter-individual differences in methylation levels occur at regions covering mammalian developmental genes, and that this variability may correlate with phenotypic plasticity in changing environments [8]. Importantly, additional investigation on this topic has led to propose that some of these so-called variably methylated regions show temporal stability over periods of years and covary with individual traits [9], probably underlying the relationship between prenatal events and DNA methylation in adulthood [10].

In view of it, studies of the insulin-like growth factors and related genes are relevant as regards neurodevelopmental alterations, as it is widely recognized that the proteins they codify participate in complex signaling pathways affecting fetal and postnatal development [11]. Accordingly, epigenetics research indicates that DNA methylation levels of the insulin-like growth factor 2 (*IGF2*) and related developmental genes are linked to human prenatal insults such as maternal malnutrition and stress, fetal insults and low BW [12–16], and correlate with particular neuroanatomical features such as cerebral and cerebellar weight [17–19]. From these studies, it is feasible inferring that *IGF2* DNA methylation marks (established early in life and measured in adulthood) influencing fetal growth and development could also have some relationship with adult brain outcomes such as neurocognitive and neuropsychiatric traits.

Accordingly, expression levels, polymorphic variants and other biologically relevant features of the *IGF2* gene have frequently been associated with neurodevelopmentally induced behavioral traits, neurogenesis and cognitive phenotypes [20–24]. Of note, among several neurocognitive functions, *IGF2* has repeatedly been linked not only to modulation of memory consolidation and enhancement [25,26], but also to working memory (WM) performance [21,24]. WM designates a mechanism by which things are kept in mind when complex tasks are executed [27], and involves the activation of several brain regions implicated in other types of memory [28]. Its basic structure is thought to develop from around 6 years of age through adolescence [29,30], and it may probably be modified later in response to training [31].

Remarkably, these two previously proposed links are not definitely clear: on one side, locus-specific *IGF2* DNA methylation has been suggested to remain as a fingerprint reflecting fetal growth disturbances; in contrast, though WM performance evolves during later ontogenetic stages, it has also been related to *IGF2* signaling networks. Furthermore, while some studies suggest adverse prenatal events may modify adult WM performance [32,33] the evidence for a link between BW and WM in the adult general population is still not conclusive [34]. With this background, it is feasible hypothesizing that plasticity of cognitive functioning could somehow arise in response to biochemical alterations left printed early in life as DNA methylation marks.

Notably, published research reports showing relationships between adult *IGF2* methylation and previous fetal development do not typically control for the putative relationship between this adult epigenetic mark and neuropsychological performance, as reflected in psychometric measures. In addition, to the knowledge of authors, studies relating psychometric outcomes and *IGF2* DNA methylation (rather than expression levels or polymorphic variants) are scarce.

Thus, by exploring DNA methylation levels at *IGF2* and in three genes codifying for allied factors (IGF2-binding proteins 1–3, *IGF2BP1-3*), the current study was aimed at evaluating epigenetic correlates of BW and adult WM performance in a monozygotic (MZ) twin sample. Models implemented here assessed a putative link between DNA methylation and either BW or WM, controlling for each other. In addition to testing for direct associations, using MZ twins also allowed evaluating methylation changes and their putative phenotypic correlates controlling for confounding factors common to both twins (i.e. genes and shared environment).

Materials and Methods

a. Ethics statement

Written informed consent was obtained from all participants after a detailed description of the study aims and design, approved by the institutional ethics committee (Comissió de Bioètica de la Universitat de Barcelona (CBUB); Institutional Review Board registry IRB00003099; Assurance number: FWA00004225; http://www.ub.edu/recerca/comissiobioetica.htm). All procedures were in accordance with the Declaration of Helsinki.

b. Sample description

Participants of this study were part of a larger twin sample consisting of 242 European descent Spanish adult twins from the general population who gave permission to be contacted for research purposes. The current sample consisted of a 34-individual (17-twin-pair) subset of participants extracted from the initial group of participants. For the current sample, exclusion criteria applied included age under 21 and over 65, a medical history of neurological disturbance, presence of sensory or motor alterations and current substance misuse or dependence.

Medical records and a battery of psychological and neurocognitive tests were obtained in face-to-face interviews by trained psychologists (S.A and X.G.). Additionally, peripheral blood or saliva samples were obtained from all participants, and zygosity of the pairs was determined by genotyping 16 highly polymorphic microsatellite loci from DNA samples (SSRs; PowerPlex 16 System Promega Corporation). Identity on all the markers can be used to assign monozygosity (i.e., whether twins of a given pair were born from a single fertilized ovum, and are so identical at the DNA sequence level) with greater than 99% accuracy [35].

From the previous sample, a group of 34 middle-aged participants (17 MZ twin pairs; age range 22–56, median age 38; 47% female), who were informative for psychopathology, neurocognition and early stress factors, accepted to participate in an ongoing research project relating cognitive performance, brain function and genome-wide epigenetic signatures. Peripheral blood was available for all members of this group. All analyses described below refer to this 34-individual subset (Table 1).

c. Methylation data

The Illumina Infinium HumanMethylation450 (450K) BeadChip [36,37] was used. Briefly, by genotyping sodium bisulfite treated DNA, this platform assays DNA methylation at 482,421 CpG sites across the genome at single base resolution; afterwards, bisulfite-converted DNA undergoes whole-genome amplification, before being fragmented and hybridized to microarray probes. Indexes of DNA methylation fraction of each CpG site are estimated as $\beta = M/(M + U + \alpha)$; M and U stand for methylated and unmethylated fluorescence intensities, and α is an arbitrary offset applied to stabilize β values with low intensities.

d. CpG region selection

The microarray data contained methylation levels of 248 CpG sites mapped to locations at the four genes of interest (*IGF2* (11p15.5), *IGF2BP1* (17q21.32), *IGF2BP2* (3q27.2), and *IGF2BP3* (7p15.3)) in the human genome (hg19).

High intrapair correlation coefficients in methylation fractions were observed among MZ twin pairs when comparing their 248 CpG sites of interest (Spearman's rho for each of the 17 pairs ranging from 0.973 to 0.993).

Afterwards, variation across each of the 248 regions was evaluated, both at the whole-sample level and considering intrapair differences, in order to define regions with substantial inter-individual variation (i.e., informative variably methylated regions). Briefly, on the basis of a previously described procedure [9], the median absolute deviation (MAD) was estimated for each CpG site considering all 34 individuals. MAD provides a measure of variability in a distribution which is less biased by outliers than

Table 1. Descriptive data for variables included in the analyses.

Total sample		
***n*=34 (17 MZ twin pairs, 47% female)**		
	Mean (SD)	**Range**
Individual-level description		
Age (years)	37.8 (11.2)	22–56
Weeks of gestation	36.9 (2.4)	30–39
BW (kilograms)	2.4 (0.5)	1.4–3.4
WM score	110.4 (13.4)	89–142
Methylation fraction*	43.4 (11.9)%	20.7–60.8%
MZ twin intrapair differences		
BW differences (kilograms)	0.3 (0.3)	0–1
WM score differences	8.8 (6.1)	0–17
Methylation fraction* differences	4.4 (6.7)%	0.3–28.8%

MZ = monozygotic; SD = standard deviation; BW = birth weight; WM = working memory; IQ = intellectual quotient; *: average methylation fraction of cg07075026 and cg20966754 (*IGF2BP1*) (see Materials and methods: b. CpG region selection).
doi:10.1371/journal.pone.0103639.t001

standard deviation. In the same way, after calculating the absolute value of the intrapair difference across the 248 CpG sites for the 17 twin pairs, median values (of the differences) were computed. Large median values would indicate the presence of relatively large MZ twin differences at a given CpG, and allow evaluating whether or not inter-individual variation (i.e., in the whole sample) is accompanied by intrapair differences.

Further information about these CpG sites in relation to the UCSC Genome Browser (GRCh37/hg19) [38] coordinates and CpG islands can be found in Fig. 1 and Tables 1 and 2.

e. Obstetric data

Information about obstetric complications was collected by direct interviews with the participants' mothers by means of the Lewis-Murray Obstetric Complications Scale [39]. Long-term maternal recall of obstetric complications has been shown to be accurate enough for the current purposes [40]. From this questionnaire, a continuous measure of BW was obtained, and it was subsequently used along with adjustments for weeks of gestational age, as it may confound statistical associations between DNA methylation and other measures of fetal growth [12]. Also, previous systematic evidence review has pointed gestational age adjustment as an indicator of study quality when assessing relationships between BW and adult outcomes [6]. Use of weeks of gestation in linear regression analysis (see below g. Statistical analyses) is justified in this context since prior research has shown that, within the common gestational age range, fetal growth may be almost linearly related to gestational age [41]. Mean (SD) BW of the 34 individuals was 2.448 kilograms (SD = 0.492 kilograms). Mean intrapair difference of BW was 0.288 kilograms, ranging from 0 to 1 kilogram (Table 1). BW distribution by gestational age of all subjects in the sample was in accordance to a previous report of European descent twins [42].

f. Neurocognitive assessment

WM performance was estimated from two subtests (digit span and letter number sequencing) of the Wechsler Adult Intelligence Scale (WAIS-III) [43,44]. As it has been suggested that intelligence quotient (IQ) could be associated with WM [45,46], it was estimated from five WAIS-III subtests (block design, digit span, matrix reasoning, information and vocabulary), and included as covariate (Table 1).

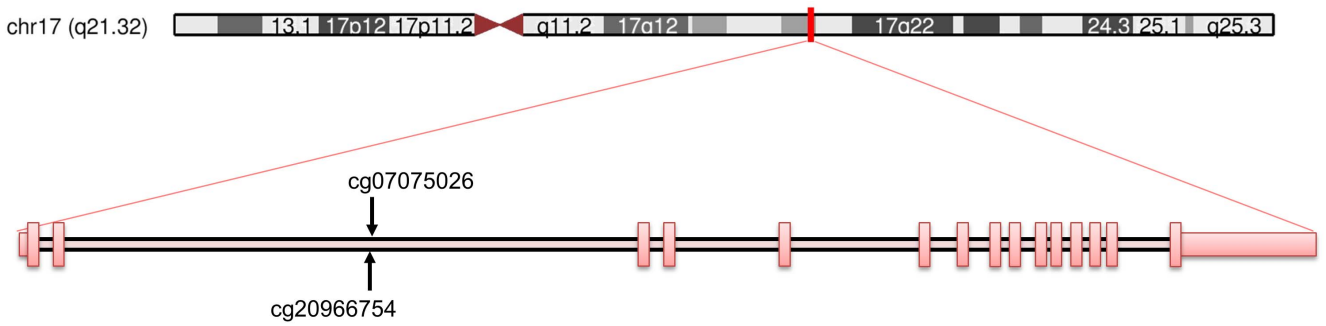


Figure 1. Schematic diagram of the studied CpG sites in the *IGF2BP1* gene. Top: depiction of chromosome 17 with a red mark indicating the locus of *IGF2BP1* (chr17:47,074,774–47,133,012). Bottom: location of cg07075026 and cg20966754 CpG sites, represented over an intron in a transcript of *IGF2BP1* at chr17:47,074,774–47,133,507. Red vertical squares crossing the transcript cover exonic regions. Adapted from the UCSC Genome Browser (GRCh37/hg19; http://genome.ucsc.edu).
doi:10.1371/journal.pone.0103639.g001

Table 2. Information of the studied CpG probes.

IlmnID	Chr	GRCh37 coordinates	Gene Name (UCSC)	Gene region feature category (UCSC)	CpG island \name (UCSC)	Relation to UCSC CpG Island
cg07075026	17	47091521	*IGF2BP1*	Body	chr17:47091037	Island
					-47091567	
cg20966754	17	47091339	*IGF2BP1*	Body	chr17:47091037	Island
					-47091567	

IlmnID = Unique CpG locus identifier from the Illumina CG database.
doi:10.1371/journal.pone.0103639.t002

g. Statistical analyses

Two different multivariate linear regression tests were performed, using only one methylation fraction outcome selected from the initial pool 248 CpG sites in four candidate genes (see b. CpG region selection and Results). First, considering each individual separately (i.e., correcting for clustered responses from twin families), the association between methylation fraction at a given CpG site (as defined in b. CpG region selection) and both BW and WM. Secondly, unique environmental influences (as derived from MZ twin pair differences) on both BW and WM were studied in relation to methylation fraction, using a regression procedure described elsewhere [47].

Briefly, the regression $Y_{ij}=\beta_0+\beta_B[(X_{i1}+X_{i2})/2]+\beta_W\left[X_{ij}-(X_{i1}+X_{i2})/2\right]$ allows estimating both a) familial factors (genes plus shared environment, β_B) and b) unique environmental influences (non-shared events within a pair, β_W) underlying statistical relationships. Subindex $i\in\{1,\ldots,n\}$ stands for pair number (here, $n=17$ MZ pairs) and $j\in\{1,2\}$ refers to co-twin number (randomly assigned). Y_{ij} represents the DNA methylation fraction at a given genomic region of co-twin j from the i-th pair. β_0 stands for intercept, $(X_{i1}+X_{i2})/2$ represents the mean BW or WM score of the i-th pair and $X_{ij}-(X_{i1}+X_{i2})/2$ denotes the deviation of co-twin j from the pair's mean score.

Gender, age, IQ and weeks of gestation were included as covariates in all analyses. All analyses were performed with R [48]. Linear mixed-effects regressions were executed with package lme4 [49,50], including family membership as a random effect. Additionally, to reduce the number of regressors, BW and WM were internally adjusted by weeks of gestation and IQ, and all tests were repeated. Since significance of results did not change when introducing this modification, only outcomes from the first set of regressions were considered. Also, as some of the participants showed liability to anxious-depressive psychopathology, analyses were repeated accounting for this fact, but significance of outcomes remained unchanged. Hence, only the former results are presented.

Results

Variability of methylation fraction across participants at all 248 CpG sites was assessed as both inter-individual (median absolute deviation, MAD) and within-pair (median value of absolute intrapair pair differences) dispersion levels. All 248 sites displayed low intrapair variability (maximum median intrapair difference at a given CpG $<|5.5|\%$). CpG site cg07075026 (in *IGF2BP1*) showed substantially higher inter-individual variability than the other 247 regions (MAD = 0.119); the CpG site with the second largest inter-individual dispersion score (cg20966754, MAD = 0.085) was located in a CpG island (the same as cg07075026), within an intronic region in the gene body of *IGF2BP1* (chr17:47,091,037-47,091,567, see Fig. 1). Consequently, as expected from the physical proximity of these CpG sites, they showed highly correlated values at the intra-individual level (Spearman's rho = 0.956). Thus, a mean methylation value of both sites was used as outcome of interest in all successive calculations. Across the 34 participants, mean methylation fraction for these combined score was 0.43 (SD: 0.12, range: 0.21–0.61).

Although median values of intrapair differences at either cg07075026 or cg20966754 were not particularly large, they were in the upper third of the distribution. Each of them showed moderate intrapair correlation rates (Spearman's rho = 0.809 and 0.762, respectively), indicating a role for unique environmental influences on their basis.

Associations were detected between *IGF2BP1* methylation fraction and both BW ($\beta=83.3\times10^{-3}$, $p=0.033$) and WM ($\beta=-4.4\times10^{-3}$, $p=0.009$) (see Table 3, Figure 2 and Figure 3). Thus, in this model, each BW kilogram increase correlated with approximately 8.33% rise in methylation fraction, whereas a 10-point upsurge in WM performance score would be associated to a 4.4% methylation level reduction.

Besides, analyses of familial and unique environmental influences indicated that the association between methylation and BW may be due to unique environmental influences on BW. Nonetheless, this result was statistically significant only at a trend level ($\beta_B=89.4\times10^{-3}$, $p=0.299$; $\beta_W=70.9\times10^{-3}$, $p=0.085$) (see Table 4 and Figure 2); hence, in this regression, every kilogram of intrapair advantage over the pair's mean BW value would be associated with a 7.09% increase in DNA methylation. The relationship between WM and *IGF2BP1* methylation was mainly due to shared genetic and environmental factors, although unique environmental influences also showed a trend towards significance ($\beta_B=-9.8\times10^{-3}$, $p=0.001$; $\beta_W=-2.9\times10^{-3}$, $p=0.086$) (see Table 4 and Figure 3): 10-point rises in the pair's mean WM score (i.e., WM's familial component) would account for an approximate reduction of 9.8% in methylation, whereas a 10-point advantage over a duo's average WM value would correlate with a 2.9% methylation level reduction.

Discussion

The current work suggests a putative link between both fetal growth and adult WM, and peripheral blood DNA methylation signatures at a region in the *IGF2BP1* gene, in agreement with previous literature [51,52]. Besides, while the former reports separately detected associations between DNA methylation and either early development or WM, current results expand on the subject to indicate that, in the ongoing independent sample, relationships between *IGF2BP1* DNA methylation and either BW or WM phenotypes are not confounded by each other. In view of the current working hypothesis, results aid to speculate that *IGF2BP1* methylation levels may be determined by early

Table 3. Results of the linear regression testing the association between *IGF2BP1* DNA methylation levels and both BW and WM.

	β	SE	*t*	Pr(>|*t*|)
Birth weight	83.3×10^{-3}	0.037	2.245	0.033
Working memory	-4.4×10^{-3}	0.002	−2.775	0.009

Mean methylation percentage of cg07075026 and cg20966754 was used as outcome. Analyses were adjusted for gender, age, weeks of gestation and IQ, and accounted for correlated responses from twin pairs using a mixed effects model. BW was introduced in kilograms and WM in standard units. SE: Standard error.
doi:10.1371/journal.pone.0103639.t003

environmental factors and that later compensatory brain mechanisms in healthy individuals could participate in raising cognitively normal profiles.

IGF2BP1 is a member of the highly conserved VICKZ (Vg1 RBP/Vera, IMP1-3, CRDBP, KOC, and ZBP1) family of RNA-binding proteins [53]. Remarkably, several functions within the central nervous system have previously been described for the IGF2BP1 and other members of VICKZ, suggesting their involvement in synaptic plasticity and hippocampal development. For instance, ZBP1 has been shown to interact with BDNF to regulate plasticity [54] and influence growth cone guidance

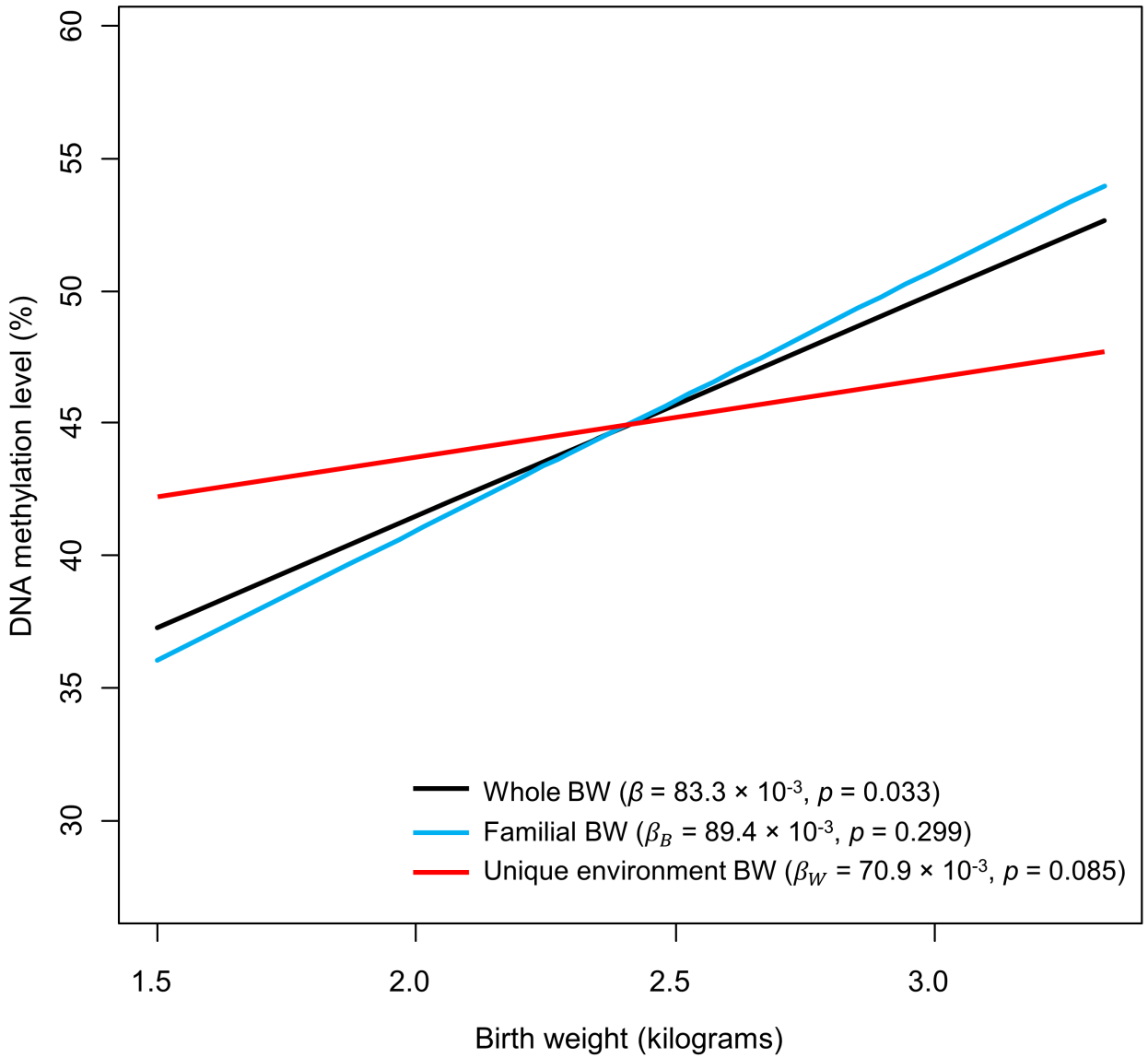


Figure 2. Representation of the association between birth weight and DNA methylation level of cg07075026 and cg20966754. The black line (''Whole BW'') was obtained from the first regression test (i.e., using raw BW from each of the 34 individuals), whereas blue and red lines (''Familial BW'' and ''Unique environment BW'') represent outcomes from the model evaluating familial and unique environmental factors.
doi:10.1371/journal.pone.0103639.g002

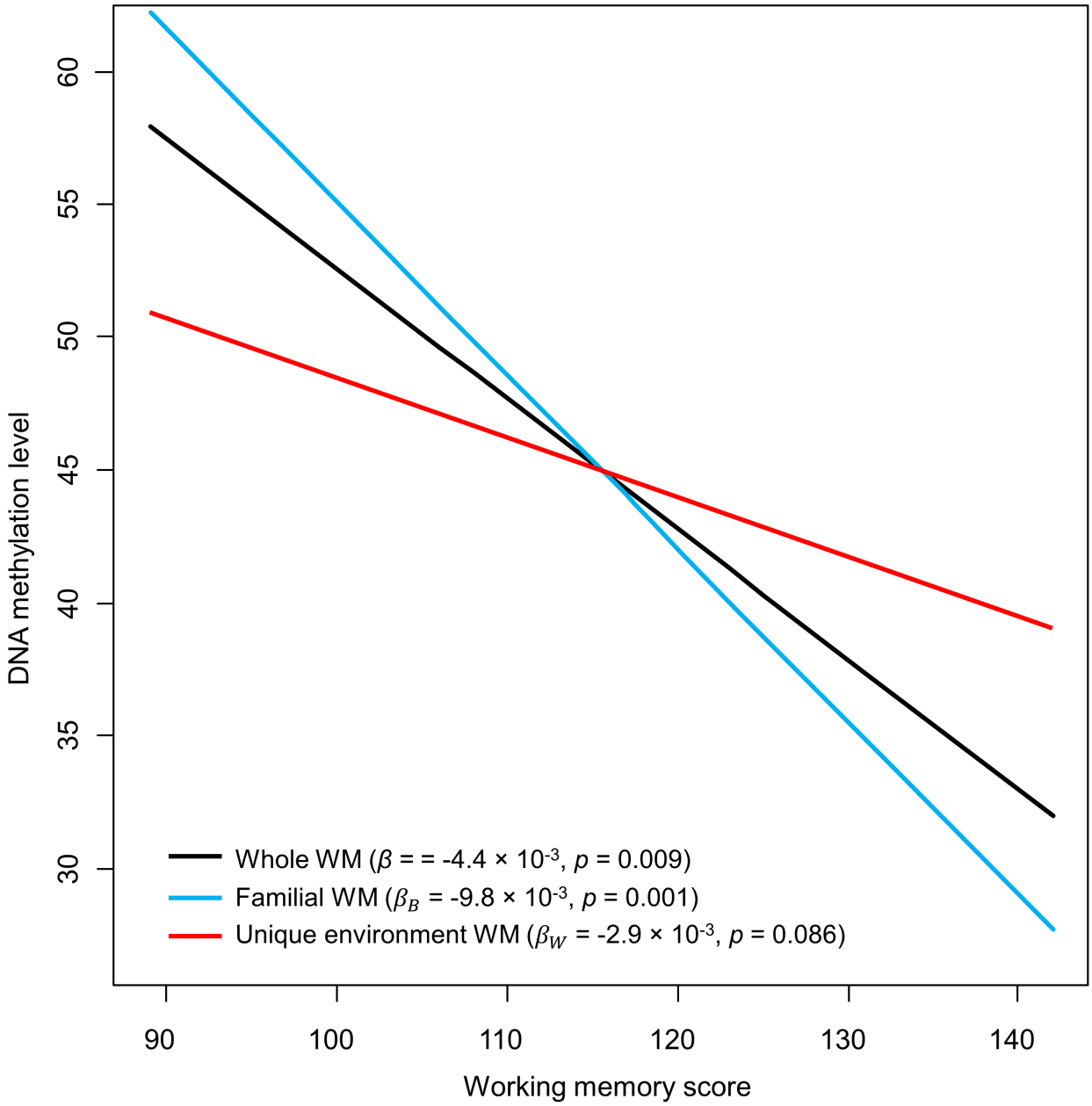


Figure 3. Representation of the association between working memory and DNA methylation level of cg07075026 and cg20966754. The black line ("Whole WM") was obtained from the first regression test (i.e., using raw WM from each of the 34 individuals), whereas blue and red lines ("Familial WM" and "Unique environment WM") represent outcomes from the model evaluating familial and unique environmental factors.
doi:10.1371/journal.pone.0103639.g003

[55,56]. Besides, ZBP1 participates in prenatal hippocampal cell signaling and development and signaling [57,58].

As regards potential functional consequences of the presently found epigenetic signature, it is worth mentioning that, while hypermethylation has typically been associated with gene silencing, recent evidence indicates this is not always true, and the inverse relationship has also been detected across several genomic regions [59]. Increased methylation of intragenic regions generally correlates with increased transcription [60,61]. Hence, one could posit a direct correlation between methylation and gene expression at the locus discussed here in *IGF2BP1*. Speculation on the directions of regression slopes obtained here should be accordingly derived. First, lower BW could correlate with gene silencing and reduced protein activity; secondly, since WM consolidation takes place during childhood and later developmental windows, healthy individuals (such as those in this sample) with reduced *IGF2BP1* transcription may have improved their WM performance to counteract potentially harmful effects of growth impairments. Further conjectures are elaborated below.

Concerning human fetal growth, it is worth noticing that a recent manuscript found an association between human fetal leukocyte DNA methylation of the *IGF2BP1* and gestational age [51], thus indicating that methylation of this gene could be a marker of developmental impairment. However, the 6 *IGF2BP1*'s CpG sites these authors found associated with gestational age

Table 4. Results of the linear regression testing the association between *IGF2BP1* DNA methylation level and the familial and unique environmental factors of both BW and WM.

	β	SE	t	Pr(>|t|)
Birth weight				
Familial factors (β_B)	89.4×10^{-3}	0.084	1.061	0.299
Unique environment (β_W)	70.9×10^{-3}	0.039	1.794	0.085
Working memory				
Familial factors (β_B)	9.8×10^{-3}	0.003	−3.669	0.001
Unique environment (β_W)	-2.9×10^{-3}	0.002	−1.785	0.086

Mean methylation percentage of cg07075026 and cg20966754 was used as outcome. Analyses were adjusted for gender, age, weeks of gestation and IQ, and accounted for correlated responses from twin pairs using a mixed effects model. BW was introduced in kilograms and WM in standard units. SE: Standard error.
doi:10.1371/journal.pone.0103639.t004

showed neither inter-individual nor intrapair variability in this sample (see Fig. 1).

Furthermore, it has been suggested that some DNA methylation marks in adults may correlate with prenatal trajectories [10]. In view of it, the fact that current multivariate analyses detected a negative correlation between WM and methylation may lead to hypothesize that individuals who suffered early insults –which could have established long-lasting epigenetic signatures–, might raise some cognitive skills in order to attenuate/counteract the impact of such developmental injuries. In fact, a recent compensatory scheme of the neurodevelopmental underpinnings of schizophrenia suggests that adaptation reactions may arise in individuals who suffer early impairments, and thus disease status would be a consequence of a failure of the compensatory response [62]. Hence, as this study considered adults from the general population, one may speculate that the early impact of low BW on *IGF2BP1* methylation status may later be lessened by WM performance improvements.

In a second set of analyses, decomposing BW and WM into both familial and unique environmental components allowed detecting that the BW-*IGF2BP1* methylation may be due to unique environmental influences. Other studies have described a number of maternal, fetal and placental sources of twin BW discordance (i.e., specific intrauterine conditions which could account for the aforesaid "unique environment") [63]. Notably, since both genes and environment shape the human neonatal epigenome [64], it is worth mentioning that previous reports have indicated intrapair DNA methylation differences in between heaviest and lightest newborn twins [65]. Nonetheless, other studies of DNA methylation in adult twins who were discordant for BW have failed to detect this association, probably due to the methodological limitations of using peripheral DNA samples [66], among other factors such as between-study sample heterogeneity.

Although less studied, there is some evidence indicating that adult WM performance could correlate with peripheral blood DNA methylation levels [52]. While in a different locus, the present twin study suggests the presence of a WM-methylation link, and also points that it may be driven by both familial and unique environmental factors. As adult WM is influenced by genes and environment [67], the same may be proposed for its relationship with DNA methylation, even though further research is needed to disentangle this potential relationship. Furthermore, since hippocampal synaptic plasticity influences WM [68], it is not surprising that Mukhopadhyay et al. [69] described how intrauterine insults may alter *IGF2BP1* gene expression in the developing hippocampus and cause long-term cognitive damage through functional compromise of hippocampal neurons.

Additionally, it is worth noting that both BW and WM were studied in relation to epigenetic changes in molecular pathways involving the IGF2 family. Thus, the direction of associations found here may be limited to the locus studied. Moreover, as intrapair differences in methylation percentage across all 248 CpG sites initially considered were small (median difference at each CpG <5.5%), overall methylation profiles must have been highly influenced by genetic factors. Besides, while genome-wide DNA methylation profiles may be influenced by single nucleotide polymorphisms (SNPs), data from dbSNP 138 [38,70] indicates there are no validated common SNPs in the genomic loci of these CpG sites for European descent populations.

A final limitation of this work should be noted apropos the relationship between DNA methylation in peripheral blood and brain regions. Although large epigenetic differences between some tissues have been documented in previous studies [71,72], some evidence from animal research suggests correlation between DNA methylation patterns across peripheral lymphocytes and a number of brain regions, presumably reflecting early environmental exposures [73–75]. Accordingly, the growing amount of publications in the literature showing significant DNA methylation alterations in peripheral cells of individuals with mental health conditions [76] suggests that this epigenetic mark, as measured in blood, could be suitable for research of complex brain-related phenotypes. Also, other authors have summarized published studies of psychiatric disorders, to suggest a high correlation between blood and brain methylation signatures [77]. Nevertheless, while a blood/brain DNA methylation correlation may exist for the genomic region studied here, the argument is still speculative and future research should correspondingly address the issue. As long as this study is exploratory, results must be taken with caution. Replication of the findings is needed in larger independent samples.

Acknowledgments

This work is supported by the Spanish SAF2008-05674-C03-01, European Twins Study Network on Schizophrenia Research Training Network (grant number EUTwinsS; MRTN-CT-2006-035987; local PIs: F.L. and N.I.), the Catalan 2014SGR1636 and the Ministry of Science and Innovation (PIM2010ERN-00642) in frame of ERA-NET NEURON. A. Córdova-Palomera was funded by CONACyT (Mexico). Authors are indebted to Xabier Agirre and Felipe Prósper for technical advice, and to J. Ignacio Martín-Subero for critical reading of the manuscript. Authors are indebted to the Genotype service at Centro Nacional de Investigaciones Oncologicas (CNIO Madrid, Spain) for performing array hybridization and providing technical support.

Author Contributions

Conceived and designed the experiments: ACP SA MFV XG JCL AGP IN LF. Performed the experiments: ACP SA MFV XG JCL AGP IN LF. Analyzed the data: ACP SA MFV LF. Contributed reagents/materials/analysis tools: IN LF. Contributed to the writing of the manuscript: ACP SA MFV XG JCL AGP IN LF.

References

1. Barker DJ (2004) The developmental origins of adult disease. J Am Coll Nutr 23: 588S–595S.
2. Eack SM, Bahorik AL, McKnight SA, Hogarty SS, Greenwald DP, et al. (2013) Commonalities in social and non-social cognitive impairments in adults with autism spectrum disorder and schizophrenia. Schizophr Res 148: 24–28.
3. Losh M, Esserman D, Anckarsater H, Sullivan PF, Lichtenstein P (2012) Lower birth weight indicates higher risk of autistic traits in discordant twin pairs. Psychol Med 42: 1091–1102.
4. Matheson SL, Shepherd AM, Laurens KR, Carr VJ (2011) A systematic meta-review grading the evidence for non-genetic risk factors and putative antecedents of schizophrenia. Schizophr Res 133: 133–142.
5. Cordova-Palomera A, Goldberg X, Alemany S, Nenadic I, Gasto C, et al. (2013) Letter to the Editor: Low birth weight and adult depression: eliciting their association. Psychol Med: 1–3.
6. Wojcik W, Lee W, Colman I, Hardy R, Hotopf M (2013) Foetal origins of depression? A systematic review and meta-analysis of low birth weight and later depression. Psychol Med 43: 1–12.
7. Burdge GC, Lillycrop KA (2010) Nutrition, epigenetics, and developmental plasticity: implications for understanding human disease. Annu Rev Nutr 30: 315–339.
8. Feinberg AP, Irizarry RA (2010) Evolution in health and medicine Sackler colloquium: Stochastic epigenetic variation as a driving force of development, evolutionary adaptation, and disease. Proc Natl Acad Sci U S A 107 Suppl 1: 1757–1764.
9. Feinberg AP, Irizarry RA, Fradin D, Aryee MJ, Murakami P, et al. (2010) Personalized epigenomic signatures that are stable over time and covary with body mass index. Sci Transl Med 2: 49ra67.
10. Reynolds RM, Jacobsen GH, Drake AJ (2013) What is the evidence in humans that DNA methylation changes link events in utero and later life disease? Clin Endocrinol (Oxf) 78: 814–822.
11. Vardatsikos G, Sahu A, Srivastava AK (2009) The insulin-like growth factor family: molecular mechanisms, redox regulation, and clinical implications. Antioxid Redox Signal 11: 1165–1190.
12. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, et al. (2008) Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A 105: 17046–17049.
13. Hoyo C, Fortner K, Murtha AP, Schildkraut JM, Soubry A, et al. (2012) Association of cord blood methylation fractions at imprinted insulin-like growth factor 2 (IGF2), plasma IGF2, and birth weight. Cancer Causes Control 23: 635–645.
14. Hoyo C, Murtha AP, Schildkraut JM, Jirtle RL, Demark-Wahnefried W, et al. (2011) Methylation variation at IGF2 differentially methylated regions and maternal folic acid use before and during pregnancy. Epigenetics 6: 928–936.
15. Steegers-Theunissen RP, Obermann-Borst SA, Kremer D, Lindemans J, Siebel C, et al. (2009) Periconceptional maternal folic acid use of 400 microg per day is related to increased methylation of the IGF2 gene in the very young child. PLoS One 4: e7845.
16. Wehkalampi K, Muurinen M, Wirta SB, Hannula-Jouppi K, Hovi P, et al. (2013) Altered Methylation of Locus 20 Years after Preterm Birth at Very Low Birth Weight. PLoS One 8: e67379.
17. Pidsley R, Dempster E, Troakes C, Al-Sarraj S, Mill J (2012) Epigenetic and genetic variation at the IGF2/H19 imprinting control region on 11p15.5 is associated with cerebellum weight. Epigenetics 7: 155–163.
18. Pidsley R, Dempster EL, Mill J (2010) Brain weight in males is correlated with DNA methylation at IGF2. Mol Psychiatry 15: 880–881.
19. Pidsley R, Fernandes C, Viana J, Paya-Cano JL, Liu L, et al. (2012) DNA methylation at the Igf2/H19 imprinting control region is associated with cerebellum mass in outbred mice. Mol Brain 5: 42.
20. Sigurdsson T, Stark KL, Karayiorgou M, Gogos JA, Gordon JA (2010) Impaired hippocampal-prefrontal synchrony in a genetic mouse model of schizophrenia. Nature 464: 763–767.
21. Alfimova MV, Lezheiko TV, Gritsenko IK, Golimbet VE (2012) [Association of the insulin-like growth factor II (IGF2) gene with human cognitive functions]. Genetika 48: 993–998.
22. Bracko O, Singer T, Aigner S, Knobloch M, Winner B, et al. (2012) Gene expression profiling of neural stem cells and their neuronal progeny reveals IGF2 as a regulator of adult hippocampal neurogenesis. J Neurosci 32: 3376–3387.
23. Nyberg F, Hallberg M (2013) Growth hormone and cognitive function. Nat Rev Endocrinol 9: 357–365.
24. Ouchi Y, Banno Y, Shimizu Y, Ando S, Hasegawa H, et al. (2013) Reduced Adult Hippocampal Neurogenesis and Working Memory Deficits in the Dgcr8-Deficient Mouse Model of 22q11.2 Deletion-Associated Schizophrenia Can Be Rescued by IGF2. J Neurosci 33: 9408–9419.
25. Alberini CM, Chen DY (2012) Memory enhancement: consolidation, reconsolidation and insulin-like growth factor 2. Trends Neurosci 35: 274–283.
26. Chen DY, Stern SA, Garcia-Osta A, Saunier-Rebori B, Pollonini G, et al. (2011) A critical role for IGF-II in memory consolidation and enhancement. Nature 469: 491–497.
27. Baddeley A (2010) Working memory. Curr Biol 20: R136–140.
28. Baddeley A (2012) Working memory: theories, models, and controversies. Annu Rev Psychol 63: 1–29.
29. Gathercole SE, Pickering SJ, Ambridge B, Wearing H (2004) The structure of working memory from 4 to 15 years of age. Dev Psychol 40: 177–190.
30. Tamnes CK, Walhovd KB, Grydeland H, Holland D, Ostby Y, et al. (2013) Longitudinal working memory development is related to structural maturation of frontal and parietal cortices. J Cogn Neurosci 25: 1611–1623.
31. Shipstead Z, Redick TS, Engle RW (2012) Is working memory training effective? Psychol Bull 138: 628–654.
32. Bennett DS, Mohamed FB, Carmody DP, Malik M, Faro SH, et al. (2013) Prenatal tobacco exposure predicts differential brain function during working memory in early adolescence: a preliminary investigation. Brain Imaging Behav 7: 49–59.
33. Kodituwakku PW, Kalberg W, May PA (2001) The effects of prenatal alcohol exposure on executive functioning. Alcohol Res Health 25: 192–198.
34. Freedman D, Bao Y, Kremen WS, Vinogradov S, McKeague IW, et al. (2013) Birth weight and neurocognition in schizophrenia spectrum disorders. Schizophr Bull 39: 592–600.
35. Guilherme R, Drunat S, Delezoide AL, Oury JF, Luton D (2009) Zygosity and chorionicity in triplet pregnancies: new data. Hum Reprod 24: 100–105.
36. Bibikova M, Barnes B, Tsan C, Ho V, Klotzle B, et al. (2011) High density DNA methylation array with single CpG site resolution. Genomics 98: 288–295.
37. Sandoval J, Heyn H, Moran S, Serra-Musach J, Pujana MA, et al. (2011) Validation of a DNA methylation microarray for 450,000 CpG sites in the human genome. Epigenetics 6: 692–702.
38. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, et al. (2002) The human genome browser at UCSC. Genome Res 12: 996–1006.
39. Lewis S, Owen M, Murray R (1989) Obstetric complications and schizophrenia: methodology and mechanisms. New York: Oxford University Press.
40. Catov JM, Newman AB, Kelsey SF, Roberts JM, Sutton-Tyrrell KC, et al. (2006) Accuracy and reliability of maternal recall of infant birth weight among older women. Ann Epidemiol 16: 429–431.
41. Luecke RH, Wosilait WD, Young JF (1995) Mathematical representation of organ growth in the human embryo/fetus. Int J Biomed Comput 39: 337–347.
42. Glinianaia SV, Skjaerven R, Magnus P (2000) Birthweight percentiles by gestational age in multiple births. A population-based study of Norwegian twins and triplets. Acta Obstet Gynecol Scand 79: 450–458.
43. Sattler JM (2001) Assessment of children: cognitive applications. San Diego: J.M. Sattler.
44. Wechsler D, Cordero Pando A, Yela Granizo M, Zimmerman IL, Woo-Sam JM, et al. (1997) WAIS escala de inteligencia de Wechsler para adultos. Madrid; Barcelona: TEA Ediciones.
45. Goldberg X, Alemany S, Rosa A, Picchioni M, Nenadic I, et al. (2013) Substantial genetic link between iq and working memory: Implications for molecular genetic studies on schizophrenia. the european twin study of schizophrenia (EUTwinsS). Am J Med Genet B Neuropsychiatr Genet 162: 413–418.
46. Oberauer K, Schulze R, Wilhelm O, Suss HM (2005) Working memory and intelligence—their correlation and their relation: comment on Ackerman, Beier, and Boyle (2005). Psychol Bull 131: 61–65; author reply 72–65.
47. Begg MD, Parides MK (2003) Separation of individual-level and cluster-level covariate effects in regression analysis of correlated data. Stat Med 22: 2591–2602.
48. R Development Core Team (2011) R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.
49. Bates D, Maechler M, Bolker B (2013) lme4: Linear mixed-effects models using S4 classes.
50. Baayen RH (2011) languageR: Data sets and functions with "Analyzing Linguistic Data: A practical introduction to statistics".
51. Parets SE, Conneely KN, Kilaru V, Fortunato SJ, Syed TA, et al. (2013) Fetal DNA Methylation Associates with Early Spontaneous Preterm Birth and Gestational Age. PLoS One 8: e67489.
52. Ursini G, Bollati V, Fazio L, Porcelli A, Iacovelli L, et al. (2011) Stress-related methylation of the catechol-O-methyltransferase Val 158 allele predicts human prefrontal cognition and activity. J Neurosci 31: 6692–6698.
53. Yisraeli JK (2005) VICKZ proteins: a multi-talented family of regulatory RNA-binding proteins. Biol Cell 97: 87–96.
54. Waterhouse EG, Xu B (2009) New insights into the role of brain-derived neurotrophic factor in synaptic plasticity. Mol Cell Neurosci 42: 81–89.
55. Sasaki Y, Welshhans K, Wen Z, Yao J, Xu M, et al. (2010) Phosphorylation of zipcode binding protein 1 is required for brain-derived neurotrophic factor

signaling of local beta-actin synthesis and growth cone turning. J Neurosci 30: 9349–9358.
56. Welshhans K, Bassell GJ (2011) Netrin-1-induced local beta-actin synthesis and growth cone guidance requires zipcode binding protein 1. J Neurosci 31: 9800–9813.
57. Perycz M, Urbanska AS, Krawczyk PS, Parobczak K, Jaworski J (2011) Zipcode binding protein 1 regulates the development of dendritic arbors in hippocampal neurons. J Neurosci 31: 5271–5285.
58. Tiruchinapalli DM, Oleynikov Y, Kelic S, Shenoy SM, Hartley A, et al. (2003) Activity-dependent trafficking and dynamic localization of zipcode binding protein 1 and beta-actin mRNA in dendrites and spines of hippocampal neurons. J Neurosci 23: 3251–3261.
59. van Eijk KR, de Jong S, Boks MP, Langeveld T, Colas F, et al. (2012) Genetic analysis of DNA methylation and gene expression levels in whole blood of healthy human subjects. BMC Genomics 13: 636.
60. LaSalle JM, Powell WT, Yasui DH (2013) Epigenetic layers and players underlying neurodevelopment. Trends Neurosci 36: 460–470.
61. Rauch TA, Wu X, Zhong X, Riggs AD, Pfeifer GP (2009) A human B cell methylome at 100-base pair resolution. Proc Natl Acad Sci U S A 106: 671–678.
62. Maziade M, Paccalet T (2013) A protective-compensatory model may reconcile the genetic and the developmental findings in schizophrenia. Schizophr Res 144: 9–15.
63. Miller J, Chauhan SP, Abuhamad AZ (2012) Discordant twins: diagnosis, evaluation and management. Am J Obstet Gynecol 206: 10–20.
64. Ollikainen M, Smith KR, Joo EJ, Ng HK, Andronikos R, et al. (2010) DNA methylation analysis of multiple tissues from newborn twins reveals both genetic and intrauterine components to variation in the human neonatal epigenome. Hum Mol Genet 19: 4176–4188.
65. Gordon L, Joo JE, Powell JE, Ollikainen M, Novakovic B, et al. (2012) Neonatal DNA methylation profile in human twins is specified by a complex interplay between intrauterine environmental and genetic factors, subject to tissue-specific influence. Genome Res 22: 1395–1406.
66. Souren NY, Lutsik P, Gasparoni G, Tierling S, Gries J, et al. (2013) Adult monozygotic twins discordant for intra-uterine growth have indistinguishable genome-wide DNA methylation profiles. Genome Biol 14: R44.
67. Karlsgodt KH, Kochunov P, Winkler AM, Laird AR, Almasy L, et al. (2010) A multimodal assessment of the genetic control over working memory. J Neurosci 30: 8197–8202.
68. Malleret G, Alarcon JM, Martel G, Takizawa S, Vronskaya S, et al. (2010) Bidirectional regulation of hippocampal long-term synaptic plasticity and its influence on opposing forms of memory. J Neurosci 30: 3813–3825.
69. Mukhopadhyay P, Horn KH, Greene RM, Michele Pisano M (2010) Prenatal exposure to environmental tobacco smoke alters gene expression in the developing murine hippocampus. Reprod Toxicol 29: 164–175.
70. Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, et al. (2001) dbSNP: the NCBI database of genetic variation. Nucleic Acids Res 29: 308–311.
71. Davies MN, Volta M, Pidsley R, Lunnon K, Dixit A, et al. (2012) Functional annotation of the human brain methylome identifies tissue-specific epigenetic variation across brain and blood. Genome Biol 13: R43.
72. Varley KE, Gertz J, Bowling KM, Parker SL, Reddy TE, et al. (2013) Dynamic DNA methylation across diverse human cell lines and tissues. Genome Res 23: 555–567.
73. Provencal N, Suderman MJ, Guillemin C, Massart R, Ruggiero A, et al. (2012) The signature of maternal rearing in the methylome in rhesus macaque prefrontal cortex and T cells. J Neurosci 32: 15626–15642.
74. Szyf M (2013) DNA methylation, behavior and early life adversity. J Genet Genomics 40: 331–338.
75. Szyf M, Bick J (2013) DNA methylation: a mechanism for embedding early life experiences in the genome. Child Dev 84: 49–57.
76. Hayashi-Takagi A, Vawter MP, Iwamoto K (2013) Peripheral Biomarkers Revisited: Integrative Profiling of Peripheral Samples for Psychiatric Research. Biol Psychiatry.
77. Tylee DS, Kawaguchi DM, Glatt SJ (2013) On the outside, looking in: a review and evaluation of the comparability of blood and brain "-omes". Am J Med Genet B Neuropsychiatr Genet 162B: 595–603.

Advisor's report on the contribution of the Ph.D. candidate to the article

Prof. Dr. Lourdes Fañanás Saura, associate professor at the Faculty of Biology, University of Barcelona, and supervisor of the present doctoral thesis by Aldo Córdova Palomera, hereby certifies that the participation of the Ph.D. candidate in the article "Birth weight, working memory and epigenetic signatures in *IGF2* and related genes: a MZ twin study" included the following tasks:

- Participation in study design.
- Statistical analyses.
- Writing of the first manuscript draft.
- Critical revision of the article.

Prof. Dr. Lourdes Fañanás Saura

Barcelona, June 30th 2015.

Season of birth and subclinical psychosis: systematic review and meta-analysis of new and existing data

Córdova-Palomera A, Calati R, Arias B, Ibáñez MI, Moya J, Ortet G, Crespo-Facorro B, Fañanás L

Psychiatry Research (2015), 225(3), 227-235

DOI: http://dx.doi.org/10.1016/j.psychres.2014.11.072

Psychiatry Research 225 (2015) 227–235

Contents lists available at ScienceDirect

Psychiatry Research

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

Review article

Season of birth and subclinical psychosis: Systematic review and meta-analysis of new and existing data

Aldo Córdova-Palomera [a,b], Raffaella Calati [c], Bárbara Arias [a,b], Manuel-Ignacio Ibáñez [b,d], Jorge Moya [b,e], Generós Ortet [b,d], Benedicto Crespo-Facorro [b,f,g], Lourdes Fañanás [a,b,*]

[a] Unidad de Antropología, Departamento de Biología Animal, Facultad de Biología and Instituto de Biomedicina (IBUB), Universitat de Barcelona, Av. Diagonal, 643, 08028 Barcelona, Spain
[b] Centro de Investigaciones Biomédicas en Red de Salud Mental (CIBERSAM), C/Doctor Esquerdo, 46, 28007 Madrid, Spain
[c] INSERM U1061, FondaMental Foundation, University of Montpellier, Av. du doyen Gaston-Giraud, 191, 34295, Montpellier, France
[d] Department of Basic and Clinical Psychology and Psychobiology, Faculty of Health Sciences, Universitat Jaume I, Av. de Vicent Sos Baynat, s/n, 12071 Castelló, Spain
[e] Department of Pedagogy and Psychology, Faculty of Education, Psychology and Social Work, University of Lleida, Av. de l'Estudi General, 4, 25001 Lleida, Spain
[f] University Hospital Marqués de Valdecilla, Department of Psychiatry, School of Medicine, University of Cantabria,Av. Valdecilla, s/n, 39008 Santander Cantabria, Spain
[g] IDIVAL, Valdecilla Biomedical Research Institute, Av. Valdecilla, s/n, 39008, Santander, Spain

ARTICLE INFO

Article history:
Received 2 July 2014
Received in revised form
10 October 2014
Accepted 30 November 2014
Available online 11 December 2014

Keywords:
Season of birth
Schizotypy
Subclinical psychosis
Winter birth
General population
Seasonality

ABSTRACT

Season of birth (SOB) has been shown to modify the risk of several health outcomes, including a number of neuropsychiatric disorders. Empirical evidence indicates that subclinical forms of psychosis in the general population share some risk factors with categorical diagnoses of psychosis. Hence, by systematically reviewing and meta-analyzing new and existing data, the current work aimed to determine whether there is evidence of an association between winter SOB and subclinical psychosis in the general population. Our meta-analytic results do not indicate an association between winter SOB and schizotypy in adult populations, although they indicate winter SOB may be a risk factor for psychotic experiences or symptoms in children around 12–15 years (OR=1.12, 95%CI:1.03–1.21). In the whole new dataset for adults (n=481, mean age=22.8 years) no association was detected in either an unadjusted model or adjusting for gender and age. Overall, our results indicate that the association between winter SOB and increased subclinical psychosis may hold in children, but does not in the broad general adult population. Nevertheless, the epidemiological and clinicopathological significance of winter SOB as a risk factor for subclinical psychosis would probably be slight due to the small effect sizes indicated by the reports available to date.

Contents

* Corresponding author at: Unidad de Antropologia, Departamento de Biologia Animal, Facultad de Biología and Instituto de Biomedicina (IBUB), Universitat de Barcelona, Av. Diagonal, 645. Barcelona, Spain, 08028. Tel.: +34 093 402 1461; fax: +34 093 403 5740.
E-mail address: lfananas@ub.edu (L. Fañanás).

http://dx.doi.org/10.1016/j.psychres.2014.11.072
0165-1781/

1. Introduction

Season of birth (SOB) has been shown to modify the risk of several health outcomes, including a number of neuropsychiatric disorders (Brewerton et al., 2012; Cheng et al., in press; Davies et al., 2003; Disanto et al., 2012; Dome et al., 2010). There is evidence indicating that seasonality influences fetal growth and development (Currie and Schwandt, 2013; Flouris et al., 2009; Strand et al., 2011; Watson and McDonald, 2007), which bears significance for psychiatric research.

Some mechanisms have been proposed to explain how SOB affects early neurodevelopmental trajectories, including factors such as pollution, eating patterns, vitamin D deficits, maternal infections and temperature changes (Currie et al., 2009; Eyles et al., 2013; Schwartz, 2011; Siega-Riz et al., 2004). Recent epidemiological research has indicated that seasonality exerts a strong influence on fetal features such as gestation length and birth weight, and that these may be markedly be compelled by maternal influenza and pregnancy weight gain (Currie and Schwandt, 2013). In addition, research has suggested that SOB exerts a long-lasting effect on the embryonic brain; this may persist until adulthood (Giezendanner et al., 2013; Moore et al., 2001; Pantazatos, 2013), and is probably behind the enduring effect of the factors mentioned on mental health and disease.

While the psychiatric research mentioned above focuses on clinically-defined psychotic phenotypes, there is empirical evidence that attenuated (i.e., subclinical) forms of psychosis in the general population share many but not all risk factors with categorical diagnoses of psychosis (Breetvelt et al., 2010; Kelleher and Cannon, 2011; Linscott and van Os, 2010). Remarkably, despite the psychometric, phenomenological and temporal continuity between subclinical psychotic features and psychotic disorders, population structures ranging from normality to disease are probably discontinuous, and models that support a *continuum of psychosis* need further evaluation (David, 2010; Lawrie et al., 2010; Linscott and van Os, 2010, 2013). Hence, more research is needed to determine the precise extent of the overlap in risk and its putative epidemiological consequences.

Even though there is broad agreement between studies that winter SOB increases the risk for some psychotic conditions, studies that evaluate this effect for subclinical psychosis in the general population provide mixed results. Therefore, by reviewing and meta-analyzing previously published reports, the current work aims to determine whether there is evidence of an association between winter SOB and subclinical psychosis. New data from an independent community sample of adults is included to increase the statistical power and to replicate previous studies.

2. Materials and methods

2.1. Meta-analysis

2.1.1. Search strategy and inclusion criteria

A literature search was conducted using PubMed, The ISI Web of Science and PsycINFO to screen for studies that evaluate the association between SOB and subclinical psychosis in the general population. The string [(*"season of birth" OR "seasonality" OR "birth season"*) *AND* (*"psychotic experiences" OR "psychotic like" OR "psychosis like" OR "subclinical psychosis" OR schizotyp* OR schizoi**)], with proper syntax adjustments depending on the search engine, was applied to retrieve potentially relevant articles published before October 22nd 2013. There was no language restriction. In addition, the lists of references from the reports identified and other relevant publications were scrutinized to find further pertinent publications.

Papers were included if they: i) reported results from primary research, ii) examined the association between SOB and subclinical psychosis, iii) presented data using non-ill general population samples (or both patients and controls, but showed information for healthy subjects separately), iv) performed psychometric evaluations of individuals from the northern hemisphere, and v) considered psychotic experiences, schizotypal traits, or non-clinical psychotic symptoms as outcomes, and measures were obtained via self-rating scales. This apparently broad category of outcomes was considered in recognition of the fact that questionnaires evaluating schizotypal traits show an overlap with assessments of other psychosis-proneness traits and psychosis-spectrum symptoms in the general population (Barrantes-Vidal et al., 2013; Wang et al., 2012).

2.1.2. Data extraction

The search results were independently screened by two reviewers (ACP and RC) to identify relevant studies. A data extraction sheet was used to record important information such as the main outcome measure, psychometric scale used and number of items, definition of the seasons of the year, sample size, gender and ethnicity of participants, summary result and other comments. Also, the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement checklist (von Elm et al., 2007) was used to assess the accuracy and completeness of the observational studies reviewed. Briefly, this checklist consists of 22 items that consider six different sections of a report: 1) title and abstract, 2) introduction, 3) methods, 4) results, 5) discussion and 6) other information.

2.1.3. Data analysis

All statistical analyses were performed in R (R Development Core Team, 2011). Since not all studies provide the same effect size

measure (for example, when using continuous psychometric scales authors may report mean differences or *t*-statistics), odds ratios were estimated where necessary using R's compute.es package (Del Re, 2013). The package allows statistics from one study to be converted to many other common effect size estimates; it is based on previous literature on meta-analysis methodology (Cooper et al., 2009). Along with existing findings, results from an ongoing study were included as another independent study (see below: Section 2.2.).

Meta-analytic procedures were implemented with R's metafor package (Viechtbauer, 2010), and residual heterogeneity (random effects model) was accounted for through the DerSimonian–Laird (DL) approach. For comparison, sensitivity analyses included fixed effects models for meta-analytic procedures. As there were no large differences across models, and since random effects models are especially suitable for sets of studies with non-identical methods and samples (Viechtbauer, 2010), only results obtained with random effects are shown.

Between-study differences were similarly assessed. The following indicators of heterogeneity and variability are reported: τ^2 (estimated amount of total heterogeneity), I^2 (total heterogeneity/total variability), H^2 (total variability/sample variability) and results from Cochran's Q-test for residual heterogeneity (Cochran, 1954), which evaluates whether the variability in effect sizes or outcomes is greater than expected based on sampling variability. Statistically significant results from the last test indicate that effects or outcomes in a meta-analysis are heterogeneous.

2.2. New data

2.2.1. Sample description and measures

Data from a sample consisting of 561 individuals were gathered from both a university campus (Jaume I University; Castelló, Spain) and other university offices and technical schools in Barcelona, Spain, between 2005 and 2006. Recruiting was mainly conducted through advertisements in those institutions. The exclusion criteria applied were the presence of neurological conditions, medical illnesses affecting brain function, a history of head injury and a history of psychiatric treatment. These were screened via an interview based on selected items from other questionnaires (First, 1997; Maxwell, 1992). After applying the exclusion criteria and due to a lack of data about either date of birth or psychopathology for some participants, the final sample (i.e., the subset included in all analysis; hereafter "new data") consisted of 481 subjects (46.4% male; mean age: 22.8 years, S.D.: 5.3 years). Of these individuals, 80.7% were students.

Schizotypal personality traits were assessed using the Schizotypal Personality Questionnaire-Brief (SPQ-B) (Raine and Benishay, 1995), a brief, 32-item self-report screening instrument derived from the Schizotypal Personality Questionnaire (Raine, 1991). Items in the SPQ-B are scored "yes" or "no", which is later translated into either the presence or the absence of a schizotypal trait. Total schizotypal scores were calculated for each subject by adding all the SPQ-B items for which he/she answered "yes". Date of birth data was structured into winter (December 22nd–March 21st) and the rest of the year. This definition of the winter period was adopted following the conventional seasonal periodicity of annual cycles of meteorological and ecological patterns in several northern hemisphere countries, and in view of the facts that 1) all the studies included in the meta-analysis presented information suitable for comparison with the same yearly structure and 2) they and other psychiatric literature reports usually define similar periods as risk factors.

All participants were of Caucasian (Spanish) ancestry. They provided written informed consent after a detailed description of the study aims and design, approved by the local Ethics Committee.

Table 1
Demographic and psychopathological features of the new sample of 481-individuals introduced in this manuscript.

	Winter birth (n=119, 43% male)		Non-winter birth (n=362, 48% male)		Group comparison[a]	
	Mean	S.D.	*Mean*	S.D.	*X-squared*	*p*
Age	22.4	3.5	22.9	5.8	1.18	0.278
Schizotypal personality features[b]						
Cognitive-perceptual	1.3	1.5	1.5	1.5	1.27	0.26
Interpersonal	2.5	1.9	2.7	2.1	0.7	0.402
Disorganized	3.9	3.1	3.9	3.3	0.12	0.727
Total schizotypy score	7.7	5.1	8	5.5	0.19	0.662

[a] Kruskal–Wallis X-squared, as these variables were continuous.
[b] Schizotypal personality features were estimated using subscales of the SPQ.

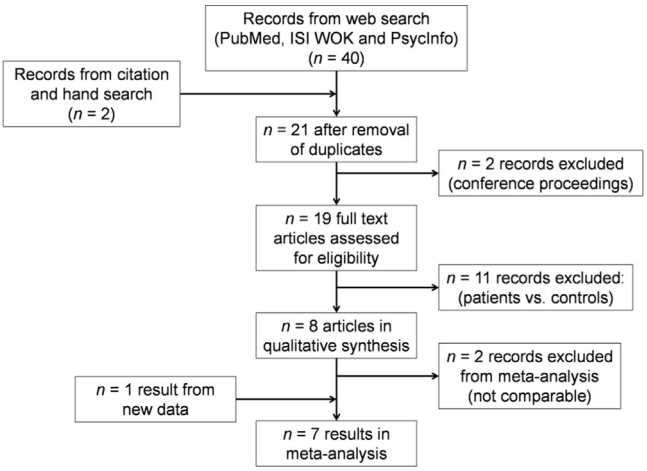


Fig. 1. Flowchart of study selection and inclusion of results. Seven papers were incorporated in the qualitative analysis (see Table 2), and results from six of them and the new data (i.e., a total of seven independent results) were included in the meta-analysis.

All procedures were in accordance with the Helsinki Declaration. Additional descriptive details of the sample can be found in Table 1 and elsewhere (Aguilera et al., 2009; Arias et al., 2012).

2.2.2. Statistical analysis of the new data

To include the new data in the meta-analysis, raw mean differences in total SPQ scores between individuals from the winter and the rest of the year births were obtained, and unadjusted odds ratios were estimated as described above (see Section 2.1.3.).

Afterwards, multivariate linear regression analysis was performed to evaluate the relationship between total schizotypal scores and SOB. Since some reports indicate that subclinical psychosis may be influenced by both gender and age (Ito et al., 2010; Miettunen and Jaaskelainen, 2010; Wigman et al., 2012), and as these variables may have accounted for the between-study heterogeneity in the previous meta-analytic section, additional analysis was performed to include them as covariates (i.e., schizotypy ~ gender + age + SOB). This was conducted using ordinary least squares in the regression tests. For comparison, permutation-based *p*-Values were also obtained for these linear tests. These *p*-Values are useful for saturated designs, non-normal data or with apparent outliers (Wheeler, 2010), and thus allowed us to lessen the probability of false positives due to some statistical artefacts. Since both ordinary least squares and permutation tests for linear regression produced similar results, only those from the former method are reported.

3. Results

3.1. Meta-analysis

3.1.1. Eligibility of studies

Fig. 1 depicts the search process. After applying the search strategy defined above and excluding duplicates and hits that were not scientific papers, 19 full-text papers were retrieved and assessed for eligibility. Eight studies met all inclusion criteria; description of these reports and the new data (from the independent sample characterized here) can be found in Table 2. From the nine data sources included in Table 2, an association between winter birth and subclinical psychosis is supported by three studies (Bolinskey et al., 2013; Hori et al., 2012; Tochigi et al., 2013); one study found increased risk in subjects born during summer (Kirkpatrick et al., 2008), and both the raw new data obtained here (see Section 2.2) and three other publications indicated no statistically significant association (Breetvelt et al., 2010; Cohen and Najolia, 2011; Reid and Zborowski, 2006). From the set of null studies, Reid and Zborowski (2006) report statistically significant results for the spring group (compared with all other births). Nevertheless, when combining data in their paper to arrange a winter/spring birth group, the significance of the effects is lost. It is worth noting that Kirkpatrick et al. (2008) conclude that summer births have increased risk of schizoid-like features, consistent with their previous findings in favor of a June/July excess of "deficit schizophrenia" births (Messias et al., 2004). However, this result could not be incorporated into the meta-analytic procedure due to the definition of exposure (June/July birth) and since the authors provide results from a subset of 171 high schizotypy scorers (i.e., there was no comparison with the low schizotypy scorers), wherein they evaluate the continuous psychopathological score with respect to birth season and gender.

The only adjusted OR came from the study of Breetvelt et al. (2010), who account for demographical risk factors and other psychopathological traits. While adjusted and unadjusted effect sizes could be combined in meta-analysis provided they address the same relationship (Voils et al., 2011), it was not included in most of the analyses since the psychometric assessment of schizotypy implemented therein is not comparable to others.

3.1.2. Features of the studies included in the review and meta-analysis

As shown in Table 2, two studies reported empirical data from children (Polanczyk et al., 2010; Tochigi et al., 2013). Hence, they were examined separately. The other five studies reported on adult populations. Whereas the new data and two other studies (Cohen and Najolia, 2011; Hori et al., 2012) analyze relationships between schizotypal personality traits and the SPQ, the reports by Bolinskey et al. (2013) and Reid and Zborowski (2006) were based on the Chapmann Psychosis Proneness Scales (CPPS) (Chapman et al., 1978; Eckblad and Chapman, 1983; Eckblad et al., 1982). Hence, these five studies were first divided into two subsets (schizotypal personality or psychosis proneness) and later combined into a larger 5-study block for comparison. Data from all seven studies included in the meta-analysis were introduced as unadjusted effect size estimates (raw ORs).

Fig. 2 depicts the results of the accuracy and completeness assessment of the studies using the STROBE checklist. Overall, all the studies include informative abstracts and accurate explanations of their scientific background, rationale, objectives and hypotheses. Nonetheless, they exhibit some weaknesses in their discussion sections, either by not offering a cautions interpretation of results or by not discussing the external validity (generalizability) of the outcomes. Meta-analytic tests were performed afterwards to attempt to overcome such limitations of the available literature.

Notably, a cluster of 4 high-quality comprehensive studies (Bolinskey et al., 2013; Breetvelt et al., 2010; Polanczyk et al., 2010; Tochigi et al., 2013) was observed, whose minor drawbacks were mainly in the above-mentioned discussion of results. In contrast, the manuscripts by Kirkpatrick et al. (2008) and Reid and Zborowski (2006) lacked precision in a number of items that evaluate their methods (setting, description of participants, variables, data sources, bias, and study size or statistics), results and discussion. It is worth noting that neither of these two studies seemed to bias subsequent results of the meta-analysis. First, using a very particular methodological design, Kirkpatrick et al. (2008) conclude that summer SOB is a risk factor for a (non-clinical) proxy for the schizophrenia deficit syndrome (Table 2). This conclusion is derived from a new psychometric measure in which scores from the Beck Depression Inventory are subtracted from those of the Social Anhedonia Scale (i.e., anhedonia in the absence of depression). While this new measure may be problematic given the statistical correlation among psychometric scales (Lewandowski et al., 2006), the finding served as a confirmation of the authors' previous results indicating a summer birth excess in clinically defined schizophrenia deficit syndrome (Kirkpatrick et al., 2002). That report was not included in the meta-analysis not only in view of the particular psychometric measure employed but also since its statistical approach compared SOB within a high-schizotypy group. Further research is needed to confirm this finding. Secondly, despite some methodological weaknesses, data from Reid and Zborowski (2006) indicate a very similar effect size to that found in other studies, including the new independent sample (see below). This probably suggests that raw CPPS questionnaire scores behave similarly in relation to winter SOB across studies. In fact, our meta-analytic results shown in subsequent sections do not seem to be biased by the presence or removal of this study.

In summary, there was no evident relationship between the STROBE quality assessment and the effect size derived from each report.

3.1.3. Association between winter birth and subclinical psychosis: meta-analysis results

Fig. 3 shows forest plots of two meta-analyses performed. Data from children suggest there is an association between winter/spring SOB and psychotic symptoms or experiences in the general population, though the effect size is relatively small (OR=1.12, 95% CI: 1.03–1.21, p_{OR}=0.009; τ^2: 0, I^2: 0%, H^2: 1, Q=0.53, p_Q=0.469). Publication bias did not seem to be an issue in this case since there was both a positive and a null result. It is worth noting that, despite providing a null result, inclusion of the study by Polanczyk et al. (2010) in the child meta-analysis did increase the overall effect size and narrow the confidence intervals, and Cochran's Q-test indicated no statistically significant between-study sampling heterogeneity. Furthermore, since the report is based on a population with a mean age of 12 years, and Tochigi et al. (2013) also report estimates for the youngest half of their sample (whose mean age should also be around 12 years), additional meta-analysis was performed to compare these two 12-year-old samples (Supplementary Fig. 1). Remarkably, an increase in effect size was observed, and indexes of heterogeneity were smaller (i.e., samples were more homogeneous) in the former case (OR=1.15, 95% CI: 1.03–1.29, p_{OR}=0.014; τ^2: 0, I^2: 0%, H^2: 1, Q=0.33, p_Q=0.563).

Data for adults did not support statistically significant associations (OR=1.22, 95% CI: 0.87–1.7, p_{OR}=0.256; τ^2: 0.09, I^2: 66.44%, H^2: 2.98, Q=11.92, p_Q=0.018) (Fig. 3), with no evidence of publication bias (test for funnel plot asymmetry: z=1.82, p=0.069).

Table 2
Summary of data considered for review and meta-analysis. All odds ratios (OR) shown were included in posterior procedures; statistics and descriptives from Kirkpatrick et al. (2008) are informative here but not suitable for direct comparison and inclusion in the meta-analysis.

Authors	Main outcome[a]	*n*	Country of origin	Gender (% male)	Mean age (S.D.) years	Ethnicity	Scale; # of items	Winter definition	Result OR (95% CI, *p*)[b] Winter: psychosis	Comments
New data	Schizotypal traits	481	Spain	46.4	22.78 (5.31)	Caucasian	Schizotypal Personality Questionnaire-Brief (SPQ-B); 32 items	Dec. 22–Mar. 21	OR = 0.89 (95% CI = 0.61–1.29, *p* = 0.526) Winter: no differential risk	-
Tochigi et al. (2013)	Psychotic-Like Experiences (PLEs)	17702	Japan	49.4	15.2[c] (1.7)	Japanese	Items from the Diagnostic Interview Schedule for Children (DISC-C); 4 items	Nov.–Mar.	OR = 1.11 (95% CI = 1.02–1.21, *p* = 0.016) Winter: increased risk	When stratifying by gender, the effect was only seen in females (OR = 1.13, 95% CI: 1.01–1.27, *p* = 0.03). When stratifying by age, the effect was only seen in the youngest set (mean age ~12 years, OR = 1.14, 95% CI: 1.02–1.29, *p* = 0.026)[c].
Bolinskey et al. (2013)	Psychometric schizotypy	84	USA	16.7	18.77 (1.02)	Mixed (Caucasian, African-American, Hispanic)	Chapman psychosis proneness scales (CPPS); 105 items	Dec. through mid-Mar. ("winter/early spring")	OR[d] = 3.69 (95% CI = 1.24–11.01, *p* = 0.009) Winter: increased risk	No statistically significant mean score differences were detected on any of the CPPS subscales by winter birth, either for the complete, combined sample, or within the "high-schizotypy" or "low-schizotypy" groups, separately.
Hori et al. (2012)	Schizotypal traits	451	Japan	24.8	45.2 (15.2)	Japanese	Schizotypal Personality Questionnaire (SPQ); 74 items	Dec., Jan. and Feb. ("Japanese winter")	OR = 1.72 (95% CI = 1.19–2.48, *p* = 0.004) Winter: increased risk	Initially, *n* = 451 individuals. Not clear if all of them were included in the analysis. Authors also reported statistically significant associations when controlling for gender and age. Afterwards, stratifying by gender and adjusting for age, the effect was detected only in females (*p* = 0.009).
Cohen and Najolia (2011)	Schizotypal traits	3485	USA	36.2	19.28 (2.26)	Mixed (Caucasian, African-American, Hispanic and "other")	Different versions of the Schizotypal Personality Questionnaire (SPQ); on average, 42 items answered by each individual	Dec. 22–Mar. 21	OR = 1.06 (95% CI = 0.76–1.5, *p* = 0.735) Winter: no differential risk	Authors found no statistically significant differences across either seasons or months.
Polanczyk et al. (2010)	Psychotic symptoms	2127	UK	49	12 (0)	Caucasian	Items from dunedin study and avon longitudinal study of parents and children interview protocols; 7 items	Not mentioned in article	OR = 1.28 (95% CI = 0.88–1.87, *p* = 0.196) Winter: no differential risk	Twin sample (statistical adjustment of responses to study each co-twin individually).
Breetvelt et al. (2010)	Non-clinical psychotic symptoms	4894	The Nether-lands	44.9	39 (12.6)	Mixed (Dutch, Western European, and a few from Surinam, Morocco and Turkey)	Dutch version of the symptom check list (SCL-90-R); 4 items	Jan., Feb. and Mar.	Adjusted OR = 0.98 (95% CI = 0.73–1.31, *p* = not significant) Winter: no differential risk	OR adjusted for demographical risk factors.
Kirkpatrick et al. (2008)	Schizoid-like features: "proxy for the deficit syndrome"	426	USA	28	20.1 (3.5)	Mixed (Caucasian, African-American, Asian/Pacific, Hispanic and "other")	Combined measure: chapman psychosis proneness scales (CPPS) and Beck Depression Inventory (BDI); 105 (CPPS) plus 21 (BDI) items	"Summer" was defined as Jun–July, and tests were about "Summer" versus other months	[Not comparable OR] (Summer: increased risk, *p* = 0.037)	[Not included in meta-analysis.] The outcome was a proxy for the deficit schizophrenia syndrome. Individuals born in summer were at increased risk in a model controlling for gender and age. These

Table 2 (*continued*)

Authors	Main outcome[a]	*n*	Country of origin	Gender (% male)	Mean age (S.D.) years	Ethnicity	Scale; # of items	Winter definition	Result OR (95% CI, *p*)[b] Winter: psychosis	Comments
										seasonality results were obtained by analyzing a 171-individual high-schizotypy group.
Reid and Zbor-owski (2006)	Schizotypy (PER-MAG)	452	USA	24.6	21.31 (5.05)	Mixed (White, Black, Hispanic, Asian, American-Indian and "other")	Perceptual Aberration-Magical Ideation (PER-MAG) scale; 65 items	Dec., Jan. and Feb.	OR = 0.94 (95% CI = 0.62–1.41, $p = 0.766$) Winter: no differential risk	Winter/Spring births associated with higher risk ($p = 0.01$); May births at higher risk than August births; spring births at higher risk than summer births ($p = 0.007$).

[a] Main outcome as described by the authors.
[b] Effect size for "winter: psychosis" was estimated when not directly provided in the paper, or recalculated when the data were available. *n*: Number of participants. S.D.: Standard seviation. CI: Confidence interval.
[c] Data for the youngest half of this sample were also used to recalculate meta-analytic measures (see Section 3.1.2).
[d] OR shown was calculated from data in the original report.

Complementary analyses were performed to explore these data, assorted by psychometric scale. Nevertheless, no associations were detected either when evaluating schizotypal personality traits (OR = 1.17, 95% CI: 0.8–1.71, $p_{OR} = 0.408$; τ^2: 0.08, I^2: 69.63%, H^2: 3.29, $Q = 6.59$, $p_Q = 0.037$) or when assessing psychosis proneness (OR = 1.69, 95% CI: 0.45–6.36, $p_{OR} = 0.439$; τ^2: 0.76, I^2: 81.04%, H^2: 5.28, $Q = 5.28$, $p_Q = 0.022$) (Supplementary Fig. 2).

3.2. Further results using new data

In the previous meta-analysis, mean differences in raw SPQ scores were used to compute ORs from the new data. This allowed comparison with other effect size estimates which were mostly also unadjusted. Hence, additional tests using linear regression models were performed to evaluate whether adjusting for gender and age (two important sources of heterogeneity in the former results, which indeed influence measures of subclinical psychosis) could provide additional insight.

As expected from the literature, higher schizotypy scores were found to be associated with both male gender and younger age ($\beta_{gender} = 1.91$, $t_{gender} = -3.95$, $p_{gender} < 10^{-4}$; $\beta_{age} = -0.19$, $t_{age} = -4.15$, $p_{age} < 10^{-4}$). Nevertheless, there was no association with winter SOB in the same regression test ($\beta_{SOB} = -0.36$, $t_{SOB} = -0.64$, $p_{SOB} = 0.521$; adjusted R^2 for the whole test = 0.055). The significance of these results did not change when including individuals with a previous history of psychiatric treatment.

4. Discussion

The present study aims to determine whether there is enough evidence to support the association between psychometrically-assessed subclinical psychosis and winter SOB, by evaluating previous results and new data. A total of nine independent results were included in a qualitative and systematic review, and seven of them were statistically assessed by means of meta-analytic procedures. New data was explored to control for potentially confounding demographic variables.

4.1. Interpretation of meta-analysis results and literature review

The meta-analysis results indicate that an association between winter SOB and childhood (~12–15 years old) psychotic symptoms/experiences is sustained by the current empirical evidence, though the effect size is relatively small (OR = 1.12, 95% CI: 1.03–1.21, $p = 0.009$). In the broad adult population, there was no association between SOB and subclinical psychosis, either when using an extensive definition of psychosis or when carefully separating reports according to their psychometrical approach to the assessment of psychopathology (i.e., independently examining schizotypal personality and psychosis proneness). It is noteworthy that the reports included in the meta-analysis of child psychotic symptoms/experiences display large sampling homogeneity, suggesting reliability of the winter SOB-psychosis relationship in child samples. However, currently available reports for adults may lack homogeneity. It is likewise worth noting that all these outcomes are based on unadjusted effect size estimates.

An important topic raised by these meta-analysis results is the contrast in the relationship between SOB and psychopathological profiles across ages: while winter SOB seems to increase the risk of psychotic symptoms in children, this may not be the case in adults. It is worth noting that lower schizotypal scores are typically found with increasing age in adults, as shown in the literature (Badcock and Dragović, 2006) and confirmed by the new community sample used here. One could speculate that, since the effect size of winter SOB on child subclinical psychosis is small, the continuous and

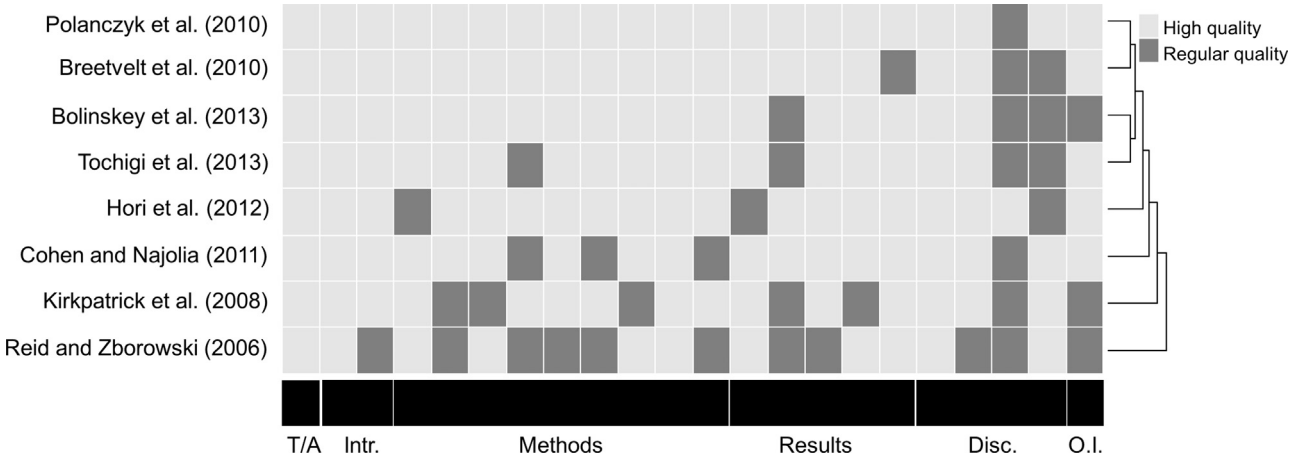


Fig. 2. Assessment of items that should be included in reports of observational studies, according to the STROBE Statement. After evaluating each of the 22 items in the STROBE checklist, hierarchical clustering analysis revealed similarities and differences in included items across the seven reports considered for review. Rows represent studies (references shown at the leftmost extreme), and columns indicate each of the 22 items in the checklist. Dark (light) gray rectangles represent low (high)-quality items according to the checklist. The discontinuous black bar at the bottom indicates the report section evaluated by sets of items. T/A: title and abstract. Intr.: introduction. Disc.: discussion. O.I.: other information.

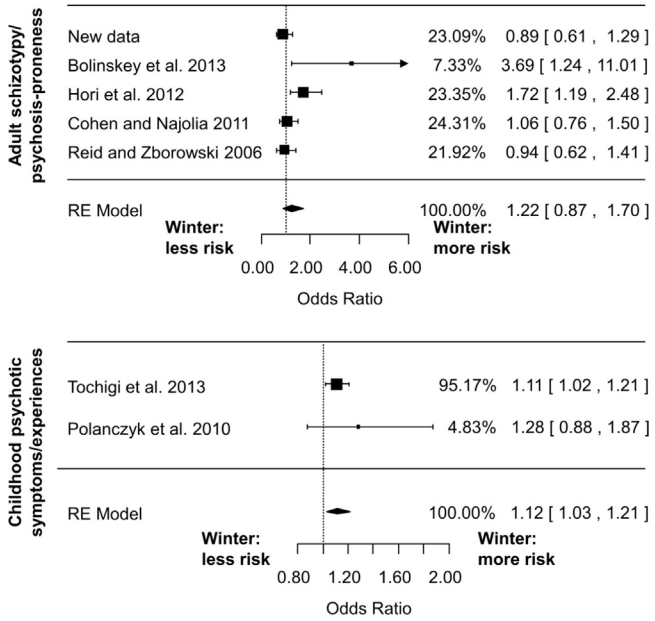


Fig. 3. Results of the random-effects meta-analysis of winter birth influence on subclinical psychosis. Top: adult data. Bottom: studies in children.

perhaps stronger influence of age may render SOB effects practically undetectable in adults.

As in all meta-analysis, the feasibility of results largely depends on the quality of the incorporated data. Although publication bias does not seem to be present in the studies included here (all null results were derived from reports emphasizing further positive findings), there was large study heterogeneity, ostensibly derived from differences in gender and age distributions, number of ethnic groups included and length of psychometric instruments used. It is worth noting that all previous reports openly supporting a winter SOB-subclinical psychosis association (Bolinskey et al., 2013; Hori et al., 2012; Tochigi et al., 2013) are derived from populations with large heterogeneity for such study attributes.

Remarkably, from these features, gender and age have widely been shown to modulate schizotypal traits; nevertheless, reports found in the literature irregularly discuss the putative effect these variables could have on the final outcomes. In addition, some authors have indeed described diverse effects when stratifying a population by gender or age. Their inclusion as covariates is recommended for future studies. It is worth noting that, when stratifying their sample by gender, Tochigi et al. (2013) found a significant effect in girls but not in boys.

4.2. Analysis of new data

Further analysis was performed with data from an independent adult sample, to evaluate the effect of the two aforementioned potentially confounding variables in the relationship between SOB and subclinical psychosis. Inclusion of this sample helped increase the statistical power in the meta-analysis and also allowed us to replicate prior studies. This new data came from individuals with no previous history of psychiatric drug consumption (another infrequently controlled variable in prior reports), though the results did not change when treated individuals were included in the analysis. Winter SOB was not associated with subclinical psychosis, either in a univariate model or adjusting for gender and age. Results from this independent sample were in agreement with a number of previously published reports for adult populations, and sensitivity analysis suggested its inclusion improved the meta-analysis.

4.3. Further issues and future directions

Some limitations of the current study and supplementary recommendations for subsequent research warrant mention. The limitations include the definition of seasonal exposure (winter SOB in the northern hemisphere), which was conventionally adopted due to its high rate of recurrence in research reports. Nevertheless, since SOB may be a proxy of prenatal insults occurring during developmental windows prior to birth, further contrast between seasons may lead to distinct outcomes. For instance, Reid and Zborowski (2006) report an association between winter/spring when compared to summer/fall births. However, such an association was driven by spring births, and comparison of winter versus other seasons led to the inclusion of data from their report as a non-significant odds ratio.

Recent epidemiological evidence provided by Currie and Schwandt (2013) is relevant in this context. They conclude that May conception (i.e., birth around mid-February) increases the risk of a short gestation and low birth weight, which is probably mediated by influenza exposure. Therefore, assessment of populations conceived during this

narrow window may help identify at-risk individuals. Also, those authors indicate that conception during summer may lead to high pregnancy weight gain, which is often reflected as high birth weight. Inclusion of individuals conceived in this season may possibly bias some results in epidemiological research.

The small number of reports may also affect meta-analysis results. Two points must be discussed in this regard. First, in the meta-analysis of children, combining studies gave optimal homogeneity parameters, indicating the association may have held in two independent samples. Secondly, while two adult samples considered in meta-analysis suggested statistically significant winter SOB-psychopathology associations (Bolinskey et al., 2013; Hori et al., 2012), one of them provided relatively large confidence intervals. Hence, evidence of a compelling association has only seldom been reported. Thus, meta-analysis results with (broad-sense) adult samples (indicating no statistical association) may be somehow realistic.

Overall, further research using appropriate epidemiological designs is needed to determine if the association is valid for specific demographical subgroups for which particular psychopathological profiles have previously been described. Certainly, the associations described here require validation through replication. From the two sets of analysis performed (meta-analysis and complementary scrutiny of independent data), it is reasonable to infer that an association cannot be detected when focusing on demographically diverse populations. Although more research is enthusiastically invited to address this topic, only mild effects could be expected on the basis of the current results. Hence, the clinicopathological significance of winter SOB on later subclinical psychotic outcomes may not be severe and the epidemiological relevance would probably be small.

Contributors

Study design: Aldo Córdova-Palomera, Raffaella Calati, Bárbara Arias, and Lourdes Fañanás.

Collection of new data (including sample recruitment and psychometric evaluations): Bárbara Arias, Manuel-Ignacio Ibáñez, Jorge Moya, Generós Ortet, and Lourdes Fañanás.

Data management and statistical analyses: Aldo Córdova-Palomera and Raffaella Calati.

Writing of the manuscript: Aldo Córdova-Palomera, Raffaella Calati, Bárbara Arias, Manuel-Ignacio Ibáñez, Jorge Moya, Generós Ortet, Benedicto Crespo-Facorro, and Lourdes Fañanás.

Role of funding source

Funding sources had no further role in study design, analysis and interpretation of the data, writing, or decision to submit the manuscript for publication. Authors are responsible for statements and assertions, which do not constitute the views of the funding sources.

Conflict of interest

Authors have no conflict of interest to declare.

Acknowledgment

This work received support from projects funded by the Spanish Ministry of Science and Innovation (Grant numbers SAF2008-05674-C03-00 and 03, PNSD2008-I090, PNSD2009-I019 and IT2009-0016), the Institute of Health Carlos III (CIBERSAM, research group 08), the Comissionat per a Universitats i Recerca, DIUE, Generalitat de Catalunya, Spain (Grant number 2014SGR1636), Fundació Caixa Castelló-Bancaixa (Grant numbers P1-1B2010-40 and P1-1B2011-47) and the Ministero dell'Istruzione, dell'Università e della Ricerca, Italy (IT107CB8DC). ACP was funded by CONACyT, Mexico (Grant number 310762). Authors are indebted to Prof. Dr. Alessandro Serretti for critical reading of the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.psychres.2014.11.072.

References

Aguilera, M., Arias, B., Wichers, M., Barrantes-Vidal, N., Moya, J., Villa, H., van Os, J., Ibanez, M.I., Ruiperez, M.A., Ortet, G., Fananas, L., 2009. Early adversity and 5-HTT/BDNF genes: new evidence of gene-environment interactions on depressive symptoms in a general population. Psychological Medicine 39, 1425–1432.

Arias, B., Aguilera, M., Moya, J., Saiz, P.A., Villa, H., Ibanez, M.I., Garcia-Portillo, M.P., Bobes, J., Ortet, G., Fananas, L., 2012. The role of genetic variability in the SLC6A4, BDNF and GABRA6 genes in anxiety-related traits. Acta Psychiatrica Scandinavica 125, 194–202.

Badcock, J.C., Dragović, M., 2006. Schizotypal personality in mature adults. Personality and Individual Differences 40, 77–85.

Barrantes-Vidal, N., Gross, G.M., Sheinbaum, T., Mitjavila, M., Ballespi, S., Kwapil, T.R., 2013. Positive and negative schizotypy are associated with prodromal and schizophrenia-spectrum symptoms. Schizophrenia Research 145, 50–55.

Bolinskey, P.K., Iati, C.A., Hunter, H.K., Novi, J.H., 2013. Season of birth, mixed-handedness, and psychometric schizotypy: preliminary results from a prospective study. Psychiatry Research 208, 210–214.

Breetvelt, E.J., Boks, M.P., Numans, M.E., Selten, J.P., Sommer, I.E., Grobbee, D.E., Kahn, R.S., Geerlings, M.I., 2010. Schizophrenia risk factors constitute general risk factors for psychiatric symptoms in the population. Schizophrenia Research 120, 184–190.

Brewerton, T.D., Dansky, B.S., O'Neil, P.M., Kilpatrick, D.G., 2012. Seasonal patterns of birth for subjects with bulimia nervosa, binge eating, and purging: results from the national women's study. The International Journal of Eating Disorders 45, 131–134.

Cochran, W.G., 1954. The combination of estimates from different experiments. Biometrics 10, 101–129.

Cohen, A.S., Najolia, G.M., 2011. Birth characteristics and schizotypy: evidence of a potential "second hit". Journal of Psychiatric Research 45, 955–961.

Cooper, H.M., Hedges, L.V., Valentine, J.C., 2009. The Handbook of Research Synthesis and Meta-Analysis, 2nd ed. Russell Sage Foundation, New York.

Currie, J., Neidell, M., Schmieder, J.F., 2009. Air pollution and infant health: lessons from New Jersey. Journal of Health Economics 28, 688–703.

Currie, J., Schwandt, H., 2013. Within-mother analysis of seasonal patterns in health at birth. Proceedings of the National Academy of Sciences of the United States of America 110, 12265–12270.

Chapman, L.J., Chapman, J.P., Raulin, M.L., 1978. Body-image aberration in schizophrenia. Journal of Abnormal Psychology 87, 399–407.

Cheng, C., Lin, C.-H., Chou, P.-H., Tsai, C.-J., Lan, T.-H., Nestadt, G. Season of birth in obsessice-compulsive disorder. Depression and Anxiety. http://dx.doi.org/10.1002/da.22200, in press.

David, A.S., 2010. Why we need more debate on whether psychotic symptoms lie on a continuum with normality. Psychological Medicine 40, 1935–1942.

Davies, G., Welham, J., Chant, D., Torrey, E.F., McGrath, J., 2003. A systematic review and meta-analysis of Northern Hemisphere season of birth studies in schizophrenia. Schizophrenia Bulletin 29, 587–593.

Del Re, A., 2013. compute. es: Compute effect sizes.

Disanto, G., Morahan, J.M., Lacey, M.V., DeLuca, G.C., Giovannoni, G., Ebers, G.C., Ramagopalan, S.V., 2012. Seasonal distribution of psychiatric births in England. PLoS One 7, e34866.

Dome, P., Kapitany, B., Ignits, G., Rihmer, Z., 2010. Season of birth is significantly associated with the risk of completed suicide. Biological Psychiatry 68, 148–155.

Eckblad, M., Chapman, L.J., 1983. Magical ideation as an indicator of schizotypy. Journal of Consulting and Clinical Psychology 51, 215–225.

Eckblad, M., Chapman, L.J., Chapman, J.P., Mishlove, M., 1982. The revised social anhedonia scale. Unpublished test.

Eyles, D.W., Burne, T.H., McGrath, J.J., 2013. Vitamin D, effects on brain development, adult brain function and the links between low levels of vitamin D and neuropsychiatric disease. Frontiers in Neuroendocrinology 34, 47–64.

First, M.B., 1997. Structured Clinical Interview for DSM-IV Axis I Disorders: SCID - I: Clinician Version: Administration Booklet. American Psychiatric Press, Washington, D.C.

Flouris, A.D., Spiropoulos, Y., Sakellariou, G.J., Koutedakis, Y., 2009. Effect of seasonal programming on fetal development and longevity: links with environmental

temperature. American Journal of Human Biology: The Official Journal of the Human Biology Council 21, 214–216.
Giezendanner, S., Walther, S., Razavi, N., Van Swam, C., Fisler, M.S., Soravia, L.M., Andreotti, J., Schwab, S., Jann, K., Wiest, R., Horn, H., Muller, T.J., Dierks, T., Federspiel, A., 2013. Alterations of white matter integrity related to the season of birth in schizophrenia: a DTI study. PLoS One 8, e75508.
Hori, H., Teraishi, T., Sasayama, D., Matsuo, J., Kawamoto, Y., Kinoshita, Y., Kunugi, H., 2012. Relationships between season of birth, schizotypy, temperament, character and neurocognition in a non-clinical population. Psychiatry Research 195, 69–75.
Ito, S., Okumura, Y., Sakamoto, S., 2010. Sex differences in the schizotypal personality questionnaire brief among japanese employees and undergraduates: a cross-sectional study. Personality and Individual Differences 48, 40–43.
Kelleher, I., Cannon, M., 2011. Psychotic-like experiences in the general population: characterizing a high-risk group for psychosis. Psychological Medicine 41, 1–6.
Kirkpatrick, B., Messias, E., LaPorte, D., 2008. Schizoid-like features and season of birth in a nonpatient sample. Schizophrenia Research 103, 151–155.
Kirkpatrick, B., Tek, C., Allardyce, J., Morrison, G., McCreadie, R.G., 2002. Summer birth and deficit schizophrenia in Dumfries and Galloway, southwestern Scotland. The American Journal of Psychiatry 159, 1382–1387.
Lawrie, S.M., Hall, J., McIntosh, A.M., Owens, D.G., Johnstone, E.C., 2010. The 'continuum of psychosis': scientifically unproven and clinically impractical. The British Journal of Psychiatry: The Journal of Mental Science 197, 423–425.
Lewandowski, K.E., Barrantes-Vidal, N., Nelson-Gray, R.O., Clancy, C., Kepley, H.O., Kwapil, T.R., 2006. Anxiety and depression symptoms in psychometrically identified schizotypy. Schizophrenia Research 83, 225–235.
Linscott, R.J., van Os, J., 2010. Systematic reviews of categorical versus continuum models in psychosis: evidence for discontinuous subpopulations underlying a psychometric continuum. Implications for DSM-V, DSM-VI, and DSM-VII. Annual Review of Clinical Psychology 6, 391–419.
Linscott, R.J., van Os, J., 2013. An updated and conservative systematic review and meta-analysis of epidemiological evidence on psychotic experiences in children and adults: on the pathway from proneness to persistence to dimensional expression across mental disorders. Psychological Medicine 43, 1133–1149.
Maxwell, M., 1992. Family Interview for Genetic Studies (FIGS): A Manual for FIGS.
Messias, E., Kirkpatrick, B., Bromet, E., Ross, D., Buchanan, R.W., Carpenter Jr., W.T., Tek, C., Kendler, K.S., Walsh, D., Dollfus, S., 2004. Summer birth and deficit schizophrenia: a pooled analysis from 6 countries. Archives of General Psychiatry 61, 985–989.
Miettunen, J., Jaaskelainen, E., 2010. Sex differences in wisconsin schizotypy scales – a meta-analysis. Schizophrenia Bulletin 36, 347–358.
Moore, P.B., El-Badri, S.M., Cousins, D., Shepherd, D.J., Young, A.H., McAllister, V.L., Ferrier, I.N., 2001. White matter lesions and season of birth of patients with bipolar affective disorder. The American Journal of Psychiatry 158, 1521–1524.
Pantazatos, S.P., 2013. Prediction of individual season of birth using MRI. NeuroImage 88C, 61–68.
Polanczyk, G., Moffitt, T.E., Arseneault, L., Cannon, M., Ambler, A., Keefe, R.S., Houts, R., Odgers, C.L., Caspi, A., 2010. Etiological and clinical features of childhood psychotic symptoms: results from a birth cohort. Archives of General Psychiatry 67, 328–338.
R Development Core Team, 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Raine, A., 1991. The SPQ: a scale for the assessment of schizotypal personality based on DSM-III-R criteria. Schizophrenia Bulletin 17, 555–564.
Raine, A., Benishay, D., 1995. The SPQ-B: a brief screening instrument for schizotypal personality disorder. Journal of Personality Disorders 9, 346–355.
Reid, H.M., Zborowski, M.J., 2006. Schizophrenia-proneness, season of birth and sleep: elevated schizotypy scores are associated with spring births and extremes of sleep. Personality and Individual Differences 41, 1185–1193.
Schwartz, P.J., 2011. Season of birth in schizophrenia: a maternal-fetal chronobiological hypothesis. Medical Hypotheses 76, 785–793.
Siega-Riz, A.M., Savitz, D.A., Zeisel, S.H., Thorp, J.M., Herring, A., 2004. Second trimester folate status and preterm birth. American Journal of Obstetrics and Gynecology 191, 1851–1857.
Strand, L.B., Barnett, A.G., Tong, S., 2011. The influence of season and ambient temperature on birth outcomes: a review of the epidemiological literature. Environmental Research 111, 451–462.
Tochigi, M., Nishida, A., Shimodera, S., Okazaki, Y., Sasaki, T., 2013. Season of birth effect on psychotic-like experiences in Japanese adolescents. European Child & Adolescent Psychiatry 22, 89–93.
Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor package. Journal of Statistical Software 36, 1–48.
Voils, C.I., Crandell, J.L., Chang, Y., Leeman, J., Sandelowski, M., 2011. Combining adjusted and unadjusted findings in mixed research synthesis. Journal of Evaluation in Clinical Practice 17, 429–434.
von Elm, E., Altman, D.G., Egger, M., Pocock, S.J., Gotzsche, P.C., Vandenbroucke, J.P., Initiative, S., 2007. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. PLoS Medicine 4, e296.
Wang, Y., Neumann, D., Shum, D.H., Chan, R.C., 2012. A cross-validation study of clustering of schizotypy using a non-clinical Chinese sample. Psychiatry Research 200, 55–58.
Watson, P.E., McDonald, B.W., 2007. Seasonal variation of nutrient intake in pregnancy: effects on infant measures and possible influence on diseases related to season of birth. European Journal of Clinical Nutrition 61, 1271–1280.
Wheeler, B., 2010. lmPerm: Permutation tests for linear models.
Wigman, J.T., van Nierop, M., Vollebergh, W.A., Lieb, R., Beesdo-Baum, K., Wittchen, H.U., van Os, J., 2012. Evidence that psychotic symptoms are prevalent in disorders of anxiety and depression, impacting on illness onset, risk, and severity – implications for diagnosis and ultra-high risk research. Schizophrenia Bulletin 38, 247–257.

Advisor's report on the contribution of the Ph.D. candidate to the article

Prof. Dr. Lourdes Fañanás Saura, associate professor at the Faculty of Biology, University of Barcelona, and supervisor of the present doctoral thesis by Aldo Córdova Palomera, hereby certifies that the participation of the Ph.D. candidate in the article "Season of birth and subclinical psychosis: systematic review and meta-analysis of new and existing data" included the following tasks:

- Participation in study design.
- Statistical analyses.
- Writing of the first manuscript draft.
- Critical revision of the article.

Prof. Dr. Lourdes Fañanás Saura

Barcelona, June 30th 2015.

Cortical thickness correlates of psychotic experiences: examining the effect of season of birth using a genetically informative design

Córdova-Palomera A, Alemany S, Falcón C, Bargalló N, Goldberg X, Crespo-Facorro B, Nenadic I, Fañanás L

Journal of Psychiatric Research (2014), 56, 144-149

DOI: http://dx.doi.org/10.1016/j.jpsychires.2014.05.014

Journal of Psychiatric Research 56 (2014) 144–149

Contents lists available at ScienceDirect

Journal of Psychiatric Research

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

Cortical thickness correlates of psychotic experiences: Examining the effect of season of birth using a genetically informative design

A. Córdova-Palomera [a, b], S. Alemany [a, b], C. Falcón [c, d], N. Bargalló [b, c, e], X. Goldberg [a, b], B. Crespo-Facorro [b, f, g], I. Nenadic [h], L. Fañanás [a, b, *]

[a] Unidad de Antropología, Departamento de Biología Animal, Facultad de Biología and Instituto de Biomedicina (IBUB), Universitat de Barcelona, Av. Diagonal, 643, 08028 Barcelona, Spain
[b] Centro de Investigaciones Biomédicas en Red de Salud Mental (CIBERSAM), C/Doctor Esquerdo, 46, 28007 Madrid, Spain
[c] Medical Image Core Facility, the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), C/Rosselló, 149-153, 08036 Barcelona, Spain
[d] Centro de Investigación Biomédica en Red en Bioingeniería, Biomedicina y Nanomedicina (CIBER-BBN), C/Poeta Mariano Esquillor, s/n, 50018 Zaragoza, Spain
[e] Centro de Diagnóstico por Imagen, Hospital Clínico, C/Villarroel, 170, 08036 Barcelona, Spain
[f] University Hospital Marqués de Valdecilla, Department of Psychiatry, School of Medicine, University of Cantabria, Av. Valdecilla, s/n, 39008 Santander, Cantabria, Spain
[g] IFIMAV, Instituto de Formación e Investigación Marqués de Valdecilla, Av. Valdecilla, s/n, 39008 Santander, Cantabria, Spain
[h] Department of Psychiatry and Psychotherapy, Jena University Hospital, Friedrich Schiller University Jena, Philosophenweg 3, 07743 Jena, Germany

ARTICLE INFO

Article history:
Received 4 December 2013
Received in revised form
13 May 2014
Accepted 14 May 2014

Keywords:
Cortical thickness
Psychotic experiences
Season of birth
Winter/Spring
MRI
Monozygotic twins

ABSTRACT

Season of birth has been shown to influence risk for several neuropsychiatric diseases. Furthermore, it has been suggested that season of birth modifies a number of brain morphological traits. Since cortical thickness alterations have been reported across some levels of the psychosis-spectrum, this study was aimed at i) assessing the scarcely explored relationship between cortical thickness and severity of subclinical psychotic experiences (PEs) in healthy subjects, and ii) evaluating the potential impact of season of birth in the preceding thickness–PEs relationship. As both PEs and brain cortical features are heritable, the current work used monozygotic twins to separately evaluate familial and unique environmental factors.

High-resolution structural MRI scans of 48 twins (24 monozygotic pairs) were analyzed to estimate cortical thickness using FreeSurfer. They were then examined in relation to PEs, accounting for the effects of birth season; putative differential relationships between PEs and cortical thickness depending on season of birth were also tested.

Current results support previous findings indicative of cortical thickening in healthy individuals with high psychometrically assessed psychosis scores, probably in line with theories of compensatory aspects of brain features in non-clinical populations. Additionally, they suggest distinct patterns of cortical thickness–PEs relationships depending on birth seasonality. Familial factors underlying the presence of PEs may drive these effects.

1. Introduction

Consistent epidemiological evidence demonstrates that genetic background and neurodevelopmental disruptions play a role in the etiology of schizophrenia (SZ) (Gejman et al., 2011; Matheson et al., 2011). Likewise, subclinical phenotypes such as psychotic experiences (PEs) share some genetic and early risk factors with SZ, and may also have similar neuroanatomical correlates with this psychotic disorder (Kelleher and Cannon, 2011). Consequently, clinicopathological significance of obstetric complications on PEs could be studied in relation to SZ.

Among the most broadly studied obstetric risk factors for SZ is being born during Winter or Spring in the Northern Hemisphere (Davies et al., 2003). Nevertheless, currently available studies evaluating its association with subclinical psychotic phenotypes offer inconclusive results (Cohen and Najolia, 2011; Tochigi et al., 2013; Zammit et al., 2009). A number of mediating processes

* Corresponding author. Unitat d'Antropologia, Dep. Biologia Animal, Facultat Biologia, Universitat de Barcelona, Av. Diagonal, 645, Barcelona 08028, Spain. Tel.: +34 93 402 1461; fax: +34 93 403 5740.
E-mail address: lfananas@ub.edu (L. Fañanás).

http://dx.doi.org/10.1016/j.jpsychires.2014.05.014
0022-3956/

have been suggested for the association between birth seasonality and SZ risk, such as maternal infections, vitamin D deficits and maternal chronobiology alterations due to temperature changes (Schwartz, 2011).

Accordingly, these three risk factors have independently been linked to fetal brain development disturbances (Boksa, 2010; Eyles et al., 2013; Garbett et al., 2012; Schwartz, 2011). Similarly, maternal prenatal infections and vitamin D deficits have formerly been examined in relation to brain cortical thickness, especially in animal studies. Cortical thickness is a measure of the average distance between the pial and white matter cortical surfaces. It is linked to the number of neurons within a cortical column (Rakic, 2008), and it is relevant in this context due to its high sensitivity to brain development across stages (Sowell et al., 2003). While some have proposed that the aforesaid exposures correlate with cortical thinning (Carpentier et al., 2013; Eyles et al., 2003; Fatemi et al., 1999; Hatfield et al., 2011), others have found that they may lead to increases in cortical thickness (Smith et al., 2012; Willette et al., 2011). Though conflicting results may be caused by design heterogeneity, all these reports agreed in suggesting that cortical thickness is influenced by immune responses in-utero.

Neuroimaging evidence indicates that several brain features may be different depending on season of birth, in both healthy adults (Pantazatos, 2013) and within groups of individuals suffering neuropsychiatric disorders (d'Amato et al., 1994; Giezendanner et al., 2013; Kaasinen et al., 2012; Moore et al., 2001; Sacchetti et al., 1992). Nevertheless, research on seasonal effects on cortical thickness of subjects manifesting SZ and related phenotypes is still scarce. This is an important issue since several studies using novel neuroimaging techniques have consistently shown widespread decreases of cortical thickness in SZ patients (Goldman et al., 2009; Nesvag et al., 2008; Rimol et al., 2012; Schultz et al., 2010). Besides, although the relationship between cortical thickness and subclinical psychosis in the general population has been less studied, a recent report found increased cortical thickness in subjects from a healthy population exhibiting high scores on a schizotypy assessment (Kuhn et al., 2012), consistent with the theory that some brain volumetric and functional features of individuals with psychotic traits may act as protective/compensating factors (Hazlett et al., 2008; Suzuki et al., 2005) thus avoiding transitions to more severe psychotic conditions.

Considering these elements, the current study was aimed at: *i*) testing whether factors involved in the expression of PEs are associated with brain cortical thickness, and *ii*) assessing the impact of birth seasonality on this potential relationship.

As the role of gene-environment interactions in early neurodevelopment has been previously underscored due to its potential to shed light on psychiatric research (Demjaha et al., 2012; Rapoport et al., 2012), and since both genes and environment influence cortical thickness (Panizzon et al., 2009) and PEs (Lataster et al., 2009), a genetically informative design was implemented here to test for associations. Insofar as members of a monozygotic (MZ) twin pair have identical DNA sequences, this work studied their phenotypic similarities and differences in order to obtain insights on familial and environmental influences.

2. Methods

2.1. Sample description

The present sample was gathered from a set of 115 Spanish Caucasian adult twin pairs (230 individuals) from the general population, who gave permission to be contacted for research purposes. All twins were contacted by telephone and invited to participate in a general study of early risk factors and adult cognitive and psychopathological traits. A battery of psychological and neurocognitive tests was administered to the twins by trained psychologists (S.A. and X.G.). Similarly, they were interviewed for medical records (S.A. and X.G.). Exclusion criteria applied were age under 17 and over 65 years, current substance misuse or dependence, a medical history of neurological disturbance and presence of sensory or motor alterations. 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 in accordance with the Declaration of Helsinki.

Zygosity of all pairs was assessed by genotyping 16 highly polymorphic microsatellite loci from DNA samples (SSRs; PowerPlex® 16 System Promega Corporation). Identity on all the markers can be used to assign monozygosity with greater than 99% accuracy (Guilherme et al., 2009). In the whole sample (115 twin pairs), 86 duos were MZ.

From that group of participants, using the previously collected data, a subset of 54 individuals (27 MZ twin pairs) was selected, as they were informative for obstetric and psychopathological traits and gave consent to participate in the MRI part of the present study.

Twins included in this subset of 54 participants met the following criteria: a) age at scan between 20 and 56 years, b) both twins right-handed, and c) none of the twins manifested liability for DSM-IV-R psychiatric diagnoses other than depression and/or anxiety. Pairs where one or both twins manifested either neurological or major medical illnesses were excluded as well (see Measures).

After this point, due to image artifacts or lack of data about six participants, the final sample (i.e., the subset included in all statistical analyses) consisted of 48 individuals (20 males).

2.2. Measures

To evaluate liability for psychopathology in this general population sample, a clinical psychologist (X.G.) applied the Structural Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (First, 1997) in a face-to-face interview to screen for presence of any lifetime psychiatric disorder.

Then, dimension-specific (positive, negative and depressive) PEs were assessed by means of the Community Assessment of Psychic Experiences (CAPE) (Stefanis et al., 2002), a 42-item self-report questionnaire measuring subclinical manifestations of psychosis in the general population. This dimensional representation of PEs somehow parallels the fact that psychotic patients manifest symptom clusters, and supports the view of psychosis as a quantitative trait in which symptoms may co-occur together. The CAPE evaluates lifetime prevalence of PEs using a frequency scale ranging from "never" to "nearly always", and provides a distress score for each item ranging from "not distressful" to "very distressful". Examples of items assessing positive, negative and depressive PEs in this questionnaire are, respectively, "do you ever feel as if there is a conspiracy against you?", "do you ever feel that you are spending all your days doing nothing?", and "do you ever feel pessimistic about everything?".

Participants were asked to report if they had received pharmacological 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 drug treatment for anxiety or depression.

Information about obstetric complications was collected by direct interviews with the participants' mothers (Walshe et al., 2011) by means of the Lewis–Murray Obstetric Complications Scale (Lewis et al., 1989). Using birth data included herein, birth season of each twin pair was classified as either Winter/Spring (risk factor) or Summer/Autumn.

Since epidemiological findings indicate that Winter/Spring could be considered a psychosis risk factor when compared to Summer/Autumn births (Davies et al., 2003), current data was coded into this two-category structure. In the current data, this structure was also advantageous due to the limited number of degrees of freedom (see 2.4. Statistical analyses). Following standard conventions, seasons of the year were defined as Winter/Spring (December 22nd-June 21st) and Summer/Autumn (June 22nd-December 21st).

2.3. MRI acquisition and post-processing

High resolution 3D structural datasets, with a T1-weighted magnetization prepared rapid gradient echo, were acquired at the MRI Unit of the Image Platform (IDIBAPS, Hospital Clínic de Barcelona) by means of a TIM TRIO 3T 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°, 192 slices in the sagittal plane, matrix size = 256 × 256, 1 mm^3 isometric voxel, 8-channel coil.

MRI scans were processed and analyzed using the freely available software FreeSurfer (version 5.1.0; http://surfer.nmr.mgh.harvard.edu/), ran on Ubuntu with the Linux 2.6.28-11-generic kernel. The processing stream consists in removal of non-brain tissue, mapping to Talairach-like space and segmentation of the gray–white matter and pial boundaries. To define cortical features, a two-dimensional tessellated mesh of more than 160,000 vertices per hemisphere was demarcated over the white surface to distinguish the gray–white matter boundary, and it was subsequently expanded to the gray–pial surface edge. At each vertex, cortical thickness was measured by estimating the shortest distance from white to pial surfaces. Further technical details on FreeSurfer can be found in prior publications (Dale et al., 1999; Fischl et al., 2001; Fischl et al., 2002, 2004; Fischl et al., 1999). These procedures were fully automated; all scans were visually inspected and slight manual corrections were done when necessary following standard procedures.

2.4. Statistical analyses

Vertex-wise *p*-maps of cortical thickness were generated across all >160,000 vertices in each hemisphere, to test for *i*) an association between cortical features and either familial factors or unique environmental influences on PEs (as measured by CAPE scores), controlling for season of birth, and *ii*) potential differences in the PEs–thickness relationship depending on season of birth. The Monte Carlo Null-Z simulation method, based on the AFNI's AlphaSim algorithm (Ward, 2000), was implemented to adjust for multiple comparisons. A cluster-forming threshold of $p < 0.01$ (vertex-z-threshold = 2.0) was applied, since it is the standard value in FreeSurfer's QDEC tool.

To determine the relationship between cortical features and familial and unique environmental components of CAPE scores, a standard General Linear Model (GLM) is implemented in the FreeSurfer software, using a regression procedure described elsewhere (Begg and Parides, 2003). Specifically, the linear regression model

$$Y_{ij} = \beta_0 + \beta_B \mu_{i.} + \beta_W (X_{ij} - \mu_i)$$

provides estimates of both *a*) familial factors (genetic plus shared environment, β_B) and *b*) unique environmental influences (from non-shared events within a pair, β_W) on CAPE scores. Subindex $i \in \{1,\ldots,n\}$ stands for pair number (here, n = 24 MZ pairs) and $j \in \{1, 2\}$ refers to co-twin number (randomly assigned). Y_{ij} represents the cortical thickness at a given vertex of co-twin j from the i-th pair. β_0 stands for intercept; $\mu_{i.} = (X_{i1} + X_{i2})/2$ is the mean CAPE score of the i-th pair, and $X_{ij} - \mu_{i.}$ denotes the deviation of co-twin j from the pair's mean score. When testing the impact of familial factors (i.e., $\mu_{i.}$), the term for unique environment (i.e., $X_{ij} - \mu_{i.}$) is included as covariate, and vice versa. In all regression models season of birth is coded as a discrete factor with two groups (Winter/Spring and Summer/Autumn); gender and age are controlled for by introducing them as predictors. I.e., in the first set of analyses, the model

$$Y_{ij} = \beta_0 + \beta_1(\text{gender}) + \beta_2(\text{age}) + \beta_3(\text{season}) + \beta_B \mu_{i.} + \beta_W (X_{ij} - \mu_{i.})$$

allows both controlling for covariates and testing the hypotheses of interest. Next, when testing for differences in the PEs–thickness relationship depending on season of birth, interaction effects between season of birth and either β_B or β_W are evaluated: i.e.,

$$Y_{ij} = \beta_0 + \beta_1(\text{gender}) + \beta_2(\text{age}) + \beta_3(\text{season}) + \beta_B \mu_{i.} + \beta_W (X_{ij} - \mu_{i.}) + \beta_4(\text{season} \times \mu_{i.})$$

for familial factors influencing PEs, and

$$Y_{ij} = \beta_0 + \beta_1(\text{gender}) + \beta_2(\text{age}) + \beta_3(\text{season}) + \beta_B \mu_{i.} + \beta_W (X_{ij} - \mu_{i.}) + \beta_5[\text{season} \times (X_{ij} - \mu_{i.})]$$

for unique environmental effects on PEs.

3. Results

As a preliminary step, differences in cortical thickness depending on season of birth were assessed in all individuals, covarying for gender and age. No association was found. Unadjusted vertex-wise *p*-maps for this test can be found in Supplementary Figure S1. While this finding may seem in contrast with findings from Pantazatos (2013), the current (null) result may be due either to a small effect size of birth seasonality on cortical phenotypes (i.e., a lack of statistical power to detect this association) or to the different imaging analysis methods employed: Pantazatos (2013) performed a Voxel-Based Morphometry (VBM) analysis, whereas here authors examined cortical thickness.

A positive association between the familial component (genes plus shared environment) of negative PEs and cortical thickness was found in regions of both brain hemispheres (Fig. 1). Additionally, relationships between cortical thickness and the familial element of both depressive and negative PEs were different across season of birth groups (Fig. 2). Individuals born during Winter/Spring showed cortical thickening associated with higher scores in either depressive or negative PEs, while the association was in the opposite direction in subjects who were not exposed to this risk factor (more details on the direction of these effects can be found in Supplementary Figure S2).

Positive PEs scores were not related to cortical thickness, and there was no differential effect on this brain feature depending on season of birth.

No effect of unique environmental influences of any PEs dimension was detected in cortical thickness. Similarly, no environmental component of PEs was differentially related to cortical thickness depending on seasonality.

Unadjusted vertex-wise *p*-maps for Figs. 1–2, and for all other associations evaluated can be found in Supplementary Figures S3–S5.

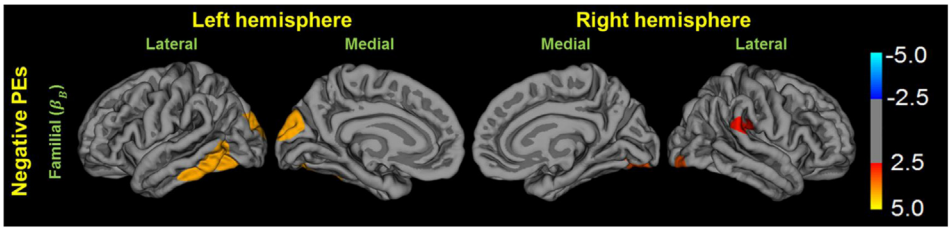


Fig. 1. Statistical maps of the association between the familial component of negative PEs and cortical thickening in both brain hemispheres. Regions in red/yellow indicate proportional increases of CAPE scores for negative PEs and cortical thickness. Absolute values of numbers in the scale represent -$\log_{10}(p)$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

This work tested the relationship between dimension-specific PEs and cortical thickness in a non-clinical population, accounting for the effect of birth season and using a genetically informative design.

Localized thickness increases in both brain hemispheres were found correlated with greater familial components of negative PEs. This is in agreement with a previous study detecting higher cortical thickness in healthy individuals with high schizotypal personality traits, whose results were interpreted under the hypothesis of a protective role of such brain morphology trait (Kuhn et al., 2012). Despite the positive correlations between schizotypy and PEs (Barrantes-Vidal et al., 2013), dissimilarities across studies in the identified brain regions could be attributable to the use of different instruments, which indeed assess distinct psychometric constructs. In addition, a previous report found larger gray matter volumes in subjects from the general population with high scores in the CAPE (Modinos et al., 2010), probably also suggesting that brain compensatory features may correlate with higher psychopathology scores measured by this questionnaire.

More specifically, the previous report of schizotypy and cortical morphology indicated that high schizotypal scorers display cortical thickening in specific regions across the right prefrontal and occipital cortex (Kuhn et al., 2012). In these brain regions, current results suggest that a similar right occipital thickness increase may correlate with high depressive PEs scores in Winter/Spring born individuals, whereas Summer/Autumn subjects could show the opposite correlation. Also, it is worth noticing that, using a different MRI processing approach, high CAPE scores were previously linked to larger regional volumes in the medial posterior cingulate cortex and precuneus (Modinos et al., 2010). Notably, results shown here suggest high negative or depressive PEs (as measured with the CAPE) could be associated with cortical thickening of these two regions in individuals born during Winter/Spring.

Afterward, widespread differential seasonal effects were identified for the relationships between either depressive or negative PEs on cortical thickness. While individuals born during Winter/Spring showed thickening associated with higher PEs, participants from the Summer/Autumn group displayed an association in the opposite direction: similar to SZ patients, higher rates of either depressive or negative PEs were related to decreases in cortical thickness. Interestingly, epidemiological evidence suggests that there could be different etiological pathways for SZ. Whereas the excess of SZ births during Winter/Spring may be due to manifestations of the disease with strong environmental sources (obstetric insults altering neurodevelopment), a different SZ subpopulation would have high familial/genetic risk but may not be affected by season of birth in the same way (Franzek and Beckmann, 1996). Results found here somehow suggest that these etiological differences within the SZ phenotype may be paralleled in subclinical psychopathological traits such as PEs.

By using a genetically informative (twin) design, the present study allowed determining that the relationship between either negative or depressive PEs and cortical thickness may be led by familial influences (genes plus shared environment) that determine these phenotypes; besides, season of birth seemed to moderate these associations. These results are feasible in view of previous reports demonstrating a considerable genetic load for both cortical measures and subclinical psychotic traits (Panizzon et al., 2009; Lataster et al., 2009); our results also suggest that some familial influences shared by cortical thickness and PEs are jointly modified by birth seasonality. More precisely, the familial component of PEs in individuals born during Summer/Autumn showed cortical thinning correlates similar to those found in SZ, but in the subset exposed to the environmental risk factor (Winter/Spring birth),

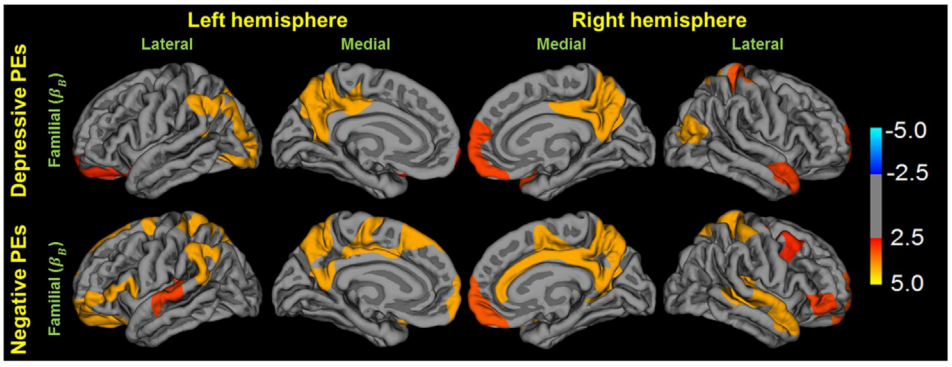


Fig. 2. Statistical maps showing differential effects for the relationship between PEs and cortical thickness, depending on season of birth. Uppermost maps refer to depressive PEs and lowermost ones denote negative PEs. Red/yellow colors specify regions where individuals born during Winter/Spring manifested positive correlations between CAPE scores and cortical thickness, whereas those born in Summer/Autumn showed a negative correlation. Absolute values of numbers in the scale represent−$\log_{10}(p)$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

familial effects enhancing PEs also seem to underpin a cortical thickening mechanism.

Accordingly, a recent protective/compensatory SZ model has integrated findings from the widely studied neurodevelopmental, multifactorial-oligogenic and gene-environment perspectives of SZ to propose that developmental adaptation reactions may be greater in individuals having severe early impairments, and that disease would arise only in the absence or failure of such a compensatory response (Maziade and Paccalet, 2013). Hence, cortical thickness increases were detected in individuals of this study who were under early environmental risk (the Winter/Spring subset) and later showed high PEs scores. This thickening may perhaps have sheltered them against transitions to definite psychosis.

The fact that statistically significant results were found for depressive and negative (but not positive) PEs may be related with different etiological pathways underlying each psychopathological dimension. In effect, it has been proposed that positive PEs may be originated by environmental exposures such as trauma, cannabis use and urbanicity, whereas negative/disorganized PEs could be driven by developmental disruptions (Dominguez et al., 2010). Besides, it has been described that cortical features may be different across schizophrenia subgroups classified according to symptom profiles (Nenadic et al., 2013), which could be hypothesized to parallel (in non-affected individuals) distinct relationships between dimension-specific PEs and cortical thickness.

Finally, it is important mentioning that a previous work found cortical thickness increases in offspring of mothers who suffered mild prenatal infections (Willette et al., 2011). Similarly, regarding participants of the current study, the fact of being born during Winter/Spring probably made them prone to just slightly altered immune reactions. One could also speculate that, in response to this mild exposure, familial factors influencing both cortical thickness and PEs may activate a similar thickening mechanism.

Some remarks should be stated concerning methodological limitations of this study. First, the sample size was modest; nevertheless, associations found here would support the presence of strong effects. Besides, further research using similar psychometrically-assessed subclinical psychotic traits may clarify potential dimension-specific effects of PEs in the general population. Finally, in the whole community twin sample ($n = 230$) from which participants of this MRI study ($n = 48$) where extracted, lifetime incidence of depressive/anxiety pathology was detected in some individuals. Presence of depression/anxiety was clearly associated with higher values of dimension-specific PEs scores across the 230 participants of this sample (in line with results by Wigman et al. (2012); data not shown). Thus, higher PEs scores may well be overrepresented in those 20 (out of 48) individuals (of this MRI sample) who showed liability for depression/anxiety.

Conflict of interest

Authors have no conflict of interest to declare.

Contributors

Córdova-Palomera A contributed to study design, data management, MRI post-processing, statistical analyses and writing of the manuscript. Alemany S contributed to data collection, psychometrical evaluations, data management, MRI acquisition and writing of the manuscript. Falcón C contributed to MRI acquisition and post-processing, statistical analyses and writing of the manuscript. Goldberg X contributed to data collection, psychometrical evaluations, data management and critical reading and writing of the manuscript. Bargalló N, Crespo-Facorro B and Nenadic I contributed to critical reading and writing of the manuscript. Fañanás L contributed to original study design, data collection supervision and writing of the manuscript.

Role of funding source

The current study was supported by the Spanish SAF2008-05674 and PIM2010ERN-00642, European Twins Study Network on Schizophrenia Research Training Network (grant number EUTwinsS; MRTN-CT-2006-035987) and the Catalan 2014-SGR1636. A. Córdova-Palomera was funded by CONACyT (Mexico). Funding sources had no further role in study design, analysis and interpretation of the data, writing, or decision to submit the manuscript for publication. Authors are responsible for statements and assertions, which do not constitute the views of the funding sources.

Acknowledgments

Special thanks are given to all twin pairs who participated in this study. The current study was supported by the Spanish SAF2008-05674-C03-01, European Twins Study Network on Schizophrenia Research Training Network (grant number EUTwinsS; MRTN-CT-2006-035987) and the Catalan SGR1636-2014. Thanks to Ministry of Science and Innovation (PIM2010ERN-00642) in frame of ERA-NET NEURON. We are indebted to the Medical Image core facility of the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) for the technical help. MRI technicians César Garrido and Santi Sotés contributed to this work. A. Córdova-Palomera was funded by CONACyT (Mexico, ID: 215418).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jpsychires.2014.05.014.

References

Barrantes-Vidal N, Gross GM, Sheinbaum T, Mitjavila M, Ballespi S, Kwapil TR. Positive and negative schizotypy are associated with prodromal and schizophrenia-spectrum symptoms. Schizophr Res 2013;145:50–5.
Begg MD, Parides MK. Separation of individual-level and cluster-level covariate effects in regression analysis of correlated data. Stat Med 2003;22:2591–602.
Boksa P. Effects of prenatal infection on brain development and behavior: a review of findings from animal models. Brain Behav Immun 2010;24:881–97.
Carpentier PA, Haditsch U, Braun AE, Cantu AV, Moon HM, Price RO, et al. Stereotypical alterations in cortical patterning are associated with maternal illness-induced placental dysfunction. J Neurosci 2013;33:16874–88.
Cohen AS, Najolia GM. Birth characteristics and schizotypy: evidence of a potential"second hit". J Psychiatr Res 2011;45:955–61.
d'Amato T, Rochet T, Dalery J, Chauchat JH, Martin JP, Marie-Cardine M. Seasonality of birth and ventricular enlargement in chronic schizophrenia. Psychiatry Res 1994;55:65–73.
Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis. I. Segmentation and surface reconstruction. Neuroimage 1999;9:179–94.
Davies G, Welham J, Chant D, Torrey EF, McGrath J. A systematic review and meta-analysis of Northern Hemisphere season of birth studies in schizophrenia. Schizophr Bull 2003;29:587–93.
Demjaha A, MacCabe JH, Murray RM. How genes and environmental factors determine the different neurodevelopmental trajectories of schizophrenia and bipolar disorder. Schizophr Bull 2012;38:209–14.
Dominguez MD, Saka MC, Lieb R, Wittchen HU, van Os J. Early expression of negative/disorganized symptoms predicting psychotic experiences and subsequent clinical psychosis: a 10-year study. Am J Psychiatry 2010;167:1075–82.
Eyles D, Brown J, Mackay-Sim A, McGrath J, Feron F. Vitamin D3 and brain development. Neuroscience 2003;118:641–53.
Eyles DW, Burne TH, McGrath JJ. Vitamin D, effects on brain development, adult brain function and the links between low levels of vitamin D and neuropsychiatric disease. Front Neuroendocrinol 2013;34:47–64.
Fatemi SH, Emamian ES, Kist D, Sidwell RW, Nakajima K, Akhter P, et al. Defective corticogenesis and reduction in reelin immunoreactivity in cortex and hippocampus of prenatally infected neonatal mice. Mol Psychiatry 1999;4:145–54.

First MB. Structured clinical interview for DSM-IV axis I disorders: SCID–I: clinician version: administration booklet. Washington, D.C.: American Psychiatric Press; 1997.
Fischl B, Liu A, Dale AM. Automated manifold surgery: constructing geometrically accurate and topologically correct models of the human cerebral cortex. IEEE Trans Med Imaging 2001;20:70–80.
Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 2002;33:341–55.
Fischl B, Salat DH, van der Kouwe AJ, Makris N, Segonne F, Quinn BT, et al. Sequence-independent segmentation of magnetic resonance images. Neuroimage 2004;23(Suppl. 1):S69–84.
Fischl B, Sereno MI, Tootell RB, Dale AM. High-resolution intersubject averaging and a coordinate system for the cortical surface. Hum Brain Mapp 1999;8:272–84.
Franzek E, Beckmann H. Gene-environment interaction in schizophrenia: season-of-birth effect reveals etiologically different subgroups. Psychopathology 1996;29:14–26.
Garbett KA, Hsiao EY, Kalman S, Patterson PH, Mirnics K. Effects of maternal immune activation on gene expression patterns in the fetal brain. Transl Psychiatry 2012;2:e98.
Gejman PV, Sanders AR, Kendler KS. Genetics of schizophrenia: new findings and challenges. Annu Rev Genomics Hum Genet 2011;12:121–44.
Giezendanner S, Walther S, Razavi N, Van Swam C, Fisler MS, Soravia LM, et al. Alterations of white matter integrity related to the season of birth in schizophrenia: a DTI study. PLoS One 2013;8:e75508.
Goldman AL, Pezawas L, Mattay VS, Fischl B, Verchinski BA, Chen Q, et al. Widespread reductions of cortical thickness in schizophrenia and spectrum disorders and evidence of heritability. Arch Gen Psychiatry 2009;66:467–77.
Guilherme R, Drunat S, Delezoide AL, Oury JF, Luton D. Zygosity and chorionicity in triplet pregnancies: new data. Hum Reprod 2009;24:100–5.
Hatfield T, Wing DA, Buss C, Head K, Muftuler LT, Davis EP. Magnetic resonance imaging demonstrates long-term changes in brain structure in children born preterm and exposed to chorioamnionitis. Am J Obstet Gynecol 2011;205(4):384.e1–8.
Hazlett EA, Buchsbaum MS, Haznedar MM, Newmark R, Goldstein KE, Zelmanova Y, et al. Cortical gray and white matter volume in unmedicated schizotypal and schizophrenia patients. Schizophr Res 2008;101:111–23.
Kaasinen V, Jokinen P, Joutsa J, Eskola O, Rinne JO. Seasonality of striatal dopamine synthesis capacity in Parkinson's disease. Neurosci Lett 2012;530:80–4.
Kelleher I, Cannon M. Psychotic-like experiences in the general population: characterizing a high-risk group for psychosis. Psychol Med 2011;41:1–6.
Kuhn S, Schubert F, Gallinat J. Higher prefrontal cortical thickness in high schizotypal personality trait. J Psychiatr Res 2012;46:960–5.
Lataster T, Myin-Germeys I, Derom C, Thiery E, van Os J. Evidence that self-reported psychotic experiences represent the transitory developmental expression of genetic liability to psychosis in the general population. Am J Med Genet B Neuropsychiatr Genet 2009;150B(8):1078–84.
Lewis S, Owen M, Murray R. Obstetric complications and schizophrenia: methodology and mechanisms. New York: Oxford University Press; 1989.
Matheson SL, Shepherd AM, Laurens KR, Carr VJ. A systematic meta-review grading the evidence for non-genetic risk factors and putative antecedents of schizophrenia. Schizophr Res 2011;133:133–42.
Maziade M, Paccalet T. A protective-compensatory model may reconcile the genetic and the developmental findings in schizophrenia. Schizophr Res 2013;144:9–15.
Modinos G, Mechelli A, Ormel J, Groenewold NA, Aleman A, McGuire PK. Schizotypy and brain structure: a voxel-based morphometry study. Psychol Med 2010;40:1423–31.
Moore PB, El-Badri SM, Cousins D, Shepherd DJ, Young AH, McAllister VL, et al. White matter lesions and season of birth of patients with bipolar affective disorder. Am J Psychiatry 2001;158:1521–4.
Nenadic I, Yotter RA, Sauer H, Gaser C. Cortical surface complexity in frontal and temporal areas varies across subgroups of schizophrenia. Hum Brain Mapp 2013;35(4):1691–9.
Nesvag R, Lawyer G, Varnas K, Fjell AM, Walhovd KB, Frigessi A, et al. Regional thinning of the cerebral cortex in schizophrenia: effects of diagnosis, age and antipsychotic medication. Schizophr Res 2008;98:16–28.
Panizzon MS, Fennema-Notestine C, Eyler LT, Jernigan TL, Prom-Wormley E, Neale M, et al. Distinct genetic influences on cortical surface area and cortical thickness. Cereb Cortex 2009;19:2728–35.
Pantazatos SP. Prediction of individual season of birth using MRI. Neuroimage 2013;88C:61–8.
Rakic P. Confusing cortical columns. Proc Natl Acad Sci U S A 2008;105:12099–100.
Rapoport JL, Giedd JN, Gogtay N. Neurodevelopmental model of schizophrenia: update 2012. Mol Psychiatry 2012;17:1228–38.
Rimol LM, Nesvag R, Hagler Jr DJ, Bergmann O, Fennema-Notestine C, Hartberg CB, et al. Cortical volume, surface area, and thickness in schizophrenia and bipolar disorder. Biol Psychiatry 2012;71:552–60.
Sacchetti E, Calzeroni A, Vita A, Terzi A, Pollastro F, Cazzullo CL. The brain damage hypothesis of the seasonality of births in schizophrenia and major affective disorders: evidence from computerised tomography. Br J Psychiatry 1992;160:390–7.
Schultz CC, Koch K, Wagner G, Roebel M, Nenadic I, Schachtzabel C, et al. Complex pattern of cortical thinning in schizophrenia: results from an automated surface based analysis of cortical thickness. Psychiatry Res 2010;182:134–40.
Schwartz PJ. Season of birth in schizophrenia: a maternal-fetal chronobiological hypothesis. Med Hypotheses 2011;76:785–93.
Smith SE, Elliott RM, Anderson MP. Maternal immune activation increases neonatal mouse cortex thickness and cell density. J Neuroimmune Pharmacol 2012;7:529–32.
Sowell ER, Peterson BS, Thompson PM, Welcome SE, Henkenius AL, Toga AW. Mapping cortical change across the human life span. Nat Neurosci 2003;6:309–15.
Stefanis NC, Hanssen M, Smirnis NK, Avramopoulos DA, Evdokimidis IK, Stefanis CN, et al. Evidence that three dimensions of psychosis have a distribution in the general population. Psychol Med 2002;32:347–58.
Suzuki M, Zhou SY, Takahashi T, Hagino H, Kawasaki Y, Niu L, et al. Differential contributions of prefrontal and temporolimbic pathology to mechanisms of psychosis. Brain 2005;128:2109–22.
Tochigi M, Nishida A, Shimodera S, Okazaki Y, Sasaki T. Season of birth effect on psychotic-like experiences in Japanese adolescents. Eur Child Adolesc Psychiatry 2013;22:89–93.
Walshe M, McDonald C, Boydell J, Zhao JH, Kravariti E, Touloupoulou T, et al. Long-term maternal recall of obstetric complications in schizophrenia research. Psychiatry Res 2011;187:335–40.
Ward BD. Simultaneous inference for fMRI data. In: AFNI 3dDeconvolve documentation. Milwaukee: Medical College of Wisconsin; 2000.
Wigman JT, van Nierop M, Vollebergh WA, Lieb R, Beesdo-Baum K, Wittchen HU, et al. Evidence that psychotic symptoms are prevalent in disorders of anxiety and depression, impacting on illness onset, risk, and severity–implications for diagnosis and ultra-high risk research. Schizophr Bull 2012;38:247–57.
Willette AA, Lubach GR, Knickmeyer RC, Short SJ, Styner M, Gilmore JH, et al. Brain enlargement and increased behavioral and cytokine reactivity in infant monkeys following acute prenatal endotoxemia. Behav Brain Res 2011;219:108–15.
Zammit S, Odd D, Horwood J, Thompson A, Thomas K, Menezes P, et al. Investigating whether adverse prenatal and perinatal events are associated with non-clinical psychotic symptoms at age 12 years in the ALSPAC birth cohort. Psychol Med 2009;39:1457–67.

Advisor's report on the contribution of the Ph.D. candidate to the article

Prof. Dr. Lourdes Fañanás Saura, associate professor at the Faculty of Biology, University of Barcelona, and supervisor of the present doctoral thesis by Aldo Córdova Palomera, hereby certifies that the participation of the Ph.D. candidate in the article "Cortical thickness correlates of psychotic experiences: examining the effect of season of birth using a genetically informative design" included the following tasks:

- MRI data pre- and post-processing.
- Participation in study design.
- Statistical analyses.
- Writing of the first manuscript draft.
- Critical revision of the article.

Prof. Dr. Lourdes Fañanás Saura

Barcelona, June 30th 2015.

Polymorphic variation in the epigenetic gene **DNMT3B** *modulates the environmental impact on cognitive ability: a twin study*

Córdova-Palomera A, Fatjó-Vilas M, Kebir O, Gastó C, Krebs MO, Fañanás L

European Psychiatry (2015), 30(2), 303-308

DOI: http://dx.doi.org/10.1016/j.eurpsy.2014.11.011

European Psychiatry 30 (2015) 303–308

Contents lists available at ScienceDirect

European Psychiatry

journal homepage: http://www.europsy-journal.com

Original article

Polymorphic variation in the epigenetic gene *DNMT3B* modulates the environmental impact on cognitive ability: A twin study

A. Córdova-Palomera [a,b], M. Fatjó-Vilas [a,b], O. Kebir [c,d,e], C. Gastó [b,f,g], M.O. Krebs [c,d,e], L. Fañanás [a,b,*]

[a] *Unitat d'Antropologia, Departament de Biologia Animal, Facultat de Biologia and Institut de Biomedicina (IBUB), Universitat de Barcelona, avenue Diagonal, 643, 08028 Barcelona, Spain*
[b] *Centro de Investigaciones Biomédicas en Red de Salud Mental (CIBERSAM), Instituto de Salud Carlos III, Madrid, Spain*
[c] *Inserm, UMR 894, laboratoire de physiopathologie des maladies psychiatriques, centre de psychiatrie et neurosciences, université Paris-Descartes, PRES Paris Sorbonne Cité, 75014 Paris, France*
[d] *Service hospitalo-universitaire, faculté de médecine Paris-Descartes, hôpital Sainte-Anne, 75014 Paris, France*
[e] *GDR3557-institut de psychiatrie, 75014 Paris, France*
[f] *Instituto de Investigaciones Biomédicas August Pi i Sunyer (IDIBAPS), Barcelona, Spain*
[g] *Departamento de Psiquiatría, Instituto Clínico de Neurociencias (ICN), Hospital Clínico, Barcelona, Spain*

ARTICLE INFO

Article history:
Received 9 October 2014
Received in revised form 19 November 2014
Accepted 19 November 2014
Available online 16 December 2014

Keywords:
Gene-environment
Cognitive plasticity
DNMT3B
Monozygotic twins

ABSTRACT

Background: Though cognitive abilities in adulthood are largely influenced by individual genetic background, they have also been shown to be importantly influenced by environmental factors. Some of these influences are mediated by epigenetic mechanisms. Accordingly, polymorphic variants in the epigenetic gene *DNMT3B* have been linked to neurocognitive performance. Since monozygotic (MZ) twins may show larger or smaller intrapair phenotypic differences depending on whether their genetic background is more or less sensitive to environmental factors, a twin design was implemented to determine if particular polymorphisms in the *DNMT3B* gene may be linked to a better (worse) response to enriched (deprived) environmental factors.
Methods: Applying the variability gene methodology in a sample of 54 healthy MZ twin pairs (108 individuals) with no lifetime history of psychopathology, two *DNMT3B* polymorphisms were analyzed in relation to their intrapair differences for either intellectual quotient (IQ) or working memory performance.
Results: MZ twin pairs with the CC genotype for rs406193 SNP showed statistically significant larger intrapair differences in IQ than CT pairs.
Conclusions: Results suggest that *DNMT3B* polymorphisms may explain variability in the IQ response to either enriched or impoverished environmental conditions. Accordingly, the applied methodology is shown as a potentially valuable tool for determining genetic markers of cognitive plasticity. Further research is needed to confirm this specific result and to expand on other putative genetic markers of environmental sensitivity.

1. Introduction

Interindividual differences in adult cognitive abilities are highly influenced by genetic background [19,21]. However, environmental influences and gene-environment interactions have also been suggested to play an important role in shaping cognition [30,38,47,62]. Of note, the popular concept of cognitive plasticity refers to changes in cognitive performance in response to experience, rising from either structured education/training or other environmental factors [31,44].

In this context, determining whether individuals with a given genetic background are more sensitive to external influences which modify cognitive traits can be achieved by the variability gene methodology. The concept of "variability gene" [8,9] was first

* Corresponding author. Unitat d'Antropologia, Departament de Biologia Animal, Facultat de Biologia and Institut de Biomedicina (IBUB), Universitat de Barcelona, avenue Diagonal, 643, 08028 Barcelona, Spain. Tel.: +34 93 402 1461; fax: +34 93 403 5740.
E-mail address: lfananas@ub.edu (L. Fañanás).

http://dx.doi.org/10.1016/j.eurpsy.2014.11.011
0924-9338/

introduced in the field of human genetics to label genes contributing to phenotypic variation (individual differences) rather than affecting the mean level of a trait in a population.

Variability genes as a proxy for cognitive plasticity may constitute an important issue in epidemiological research. Their study may allow not only identifying people with high capability of response to training or other interventions, but at the same time determining which individuals are particularly prone to cognitive deficits after potential environmental deprivation/insults. Also, research findings have been reported on the relationship between polymorphic genetic variants and response to cognitive training [5,61,76]. In this regard, the variability gene approach has (in principle) the additional advantage of testing gene-environment interactions through recognizing polymorphic variants which may be associated to either better or worse cognition depending on the environment. To the knowledge of authors, the use of this methodology in cognitive research is still lacking, even though similar genetically informative designs have shown a role for genetic variants in supporting cognitive plasticity and/or flexibility [29,46,77].

The main conceptual and methodological approach in variability gene studies is the assessment of intrapair differences in monozygotic (MZ) twins in relation to their genotype for a particular polymorphic variant. Since MZ twins of a pair have almost identical DNA sequences, this approach postulates that duos having a specific genetic background would display larger intrapair differences than other pairs with distinct genes: the environment would have a larger or smaller effect depending on the genes with which it interacts. In general, intrapair differences for a phenotype in a MZ twin are assumed to be caused by uniquely environmental influences and stochastic events [80].

The parallel concept of "phenotypic plasticity" has largely been used in disciplines like Biology or Ecology to explain the ability of a single genome/genotype to produce more than one phenotypical trait in response to environmental conditions and hence, give rise to interindividual differences [23,71,84]. The analogous "plasticity gene" term has similarly been underscored [6] in behavioral science since it posits a novel conceptual framework to study gene-environment interactions: rather than engendering susceptibility to deficit, a "plasticity gene" would be part of a genetic structure with high likelihood of either enrichment (from supportive experiences) or impoverishment (from adverse external inputs).

In particular, feasible candidate genetic loci for investigating variability genes in relation to cognitive plasticity may be at genes from the epigenetic machinery. Epigenetics can be defined as the study of heritable changes in gene expression occurring through modulation of the chromatin structure rather than by changes in the DNA sequence [2]. Despite their almost identical DNA sequence, epigenetic differences resulting from exposure to distinct environments can be found within members of a MZ twin pair [41,69]. One of the most widely studied epigenetic mechanisms is DNA methylation. In response to environmental clues, DNA (cytosine-5-)-methyltransferases (DNMT) enzymes catalyze the addition of methyl groups, typically in CpG dinucleotides of DNA regions, thus altering gene expression and cell function [45]. Importantly, there is consistent evidence linking epigenetic processes such as DNA methylation with brain and cognitive plasticity [4,10,39,48]. In this regard, DNA methyltransferase 3B (DNMT3B) is of particular significance, due to its active role in modulating global methylation dynamics and central nervous system development in mammals [24,40,60]. Accordingly, previous neuroscience research has suggested an important role for the DNMT3B protein activity in cognitive plasticity, mainly via covalent modifications to DNA leading to synaptic changes [53,56,78].

Since several reports have been published relating polymorphic variation in genes coding for proteins that regulate epigenetic processes and both neuropsychiatric and neurocognitive disruptions [57], the present study takes as starting point the likewise plausible hypothesis that DNA polymorphisms in the *DNMT3B* gene may contribute to cognitive differences between subjects in response to similar external experiences.

Of note, the *DNMT3B* gene has largely been studied in relation to psychopathology, neurocognition and associated epidemiological variables [16,17,15,34,35,58,64,73,86]. These studies provide some evidence linking *DNMT3B* polymorphisms and risk for cognitive performance deficits and mental health problems. Complementarily, the current work is aimed at determining whether some of these polymorphic variants are likely to cause more permeable cognitive performance (either intellectual quotient (IQ) or working memory (WM)) in healthy individuals. IQ and WM are particularly important in this setting, since both of them have been shown to be permeable to environmental influences such as training [43,50,55]; this is likely influenced by the genetic background [11,75]. It is worth noting that, despite the genetic link between IQ and WM [28], previous research indicates that stimulating better performance in one of them does not necessarily imply improvements in the other [68,74], probably indicating distinct plasticity pathways.

To evaluate the potential role of *DNMT3B* polymorphisms as modulators of the cognitive response to environmental factors, informative single nucleotide polymorphisms (SNPs) were genotyped for a group of 108 MZ twins (54 pairs) from the general population who did not show a lifetime history of (DSM-IV) mental disorders. Intrapair differences in neuropsychological test scores for either IQ or WM were estimated and studied in relation to *DNMT3B* genotypes of each pair.

2. Materials and methods

2.1. Sample description

Twins included in this study were drawn from a larger ongoing twin sample consisting of 242 Caucasian Spanish adult twins (UB Twin Registry) from the general population who gave permission to be contacted for research purposes. Exclusion criteria applied for that sample included age under 17 and over 65, a medical history of neurological disturbance, presence of sensory or motor alterations and current substance misuse or dependence. Written informed consent was obtained from all participants after a detailed description of the study aims and design, approved by the local Ethics Committee.

Peripheral blood or saliva samples were obtained, and zygosity of the pairs was determined by genotyping 16 highly polymorphic microsatellite loci from DNA samples (SSRs; PowerPlex® 16 System Promega Corporation). Identity on all the markers can be used to assign monozygosity with greater than 99% accuracy [33]. From this sample, 186 individuals were members of MZ twin pairs (i.e., there were 93 MZ pairs).

A battery of psychological and neurocognitive tests and medical records and were completed for all participants in face-to-face interviews by trained psychologists. The Structural Clinical Interview for DSM-IV Axis I Disorders (SCID-I) [25] was administered in a face-to-face interview to screen for presence of any lifetime mental disorder. Twin pairs where one or both co-twins met diagnostic criteria for any current or past psychiatric disorder were excluded from the larger 186-MZ-subject sample. Accordingly, 108 healthy twins (54 MZ pairs) were included in all statistical analyses described below. Further recruiting and demographic details of this sample can be found elsewhere [1].

2.2. Neurocognitive assessment

Intelligence quotient (IQ) was estimated from five subtests (block design, digit span, matrix reasoning, information and

vocabulary) of the Wechsler Adult Intelligence Scale (WAIS-III) [70,82] by trained psychologists. Also using the WAIS-III, WM performance was estimated from two subtests (digit span and letter number sequencing).

2.3. Selection of SNPs

A literature search for SNPs spanning the *DNMT3B* and potentially related to neuropsychiatric and neurocognitive phenotypes was performed in PubMed, by crossing the search terms *DNMT3B*, DNA methyltransferase, psychiatry, psychopathology, intelligence, cognition and neurocognition. Afterwards, a complementary manual search was performed across references cited in the previously retrieved manuscripts. Six studies of this gene and neuropsychiatric/neurocognitive conditions and traits were retrieved [16,17,34,58,73,86]. The SNP with more positive statistical associations (i.e., presented as linked to cognition/psychopathology outcomes by independent researchers) was rs2424913 (46359C > T), in an intronic *DNMT3B* region.

Other SNPs previously reported as associated with phenotypes were rs2424908 and rs6119954 (early onset schizophrenia [86]), rs2424932 (suicide attempt [58]), rs1569686 (late onset Alzheimer's disease [16]), rs406193 (cognitive performance in psychotic patients [73]). From the previous list, rs2424908 and rs2424932 were located near to rs2424913. Likewise, there was high genotype similarity between rs1569686 and rs2424913 in the 1000 Genomes' CEU population [26]. Accordingly, rs2424908, rs1569686 and rs2424932 were discarded as candidate SNPs since their information was somehow redundant with that of rs2424913 (in effect, genotypes of these SNPs were correlated [26], suggesting some linkage disequilibrium). Additionally, in recognition that rs406193 (*DNMT3B*'s 3′ UTR) has directly been associated with a cognitive phenotype, it was selected as informative and genotyped in the current sample. rs6119954 was discarded to reduce the number of tests, since it was reported just once within the spanned literature field, in relation to early onset psychosis risk. Thus, only rs2424913 and rs406193 were analyzed afterwards.

2.4. Genotyping

Genotypes for rs2424913 and rs406193 were obtained for all participants with Illumina Infinium iSelect HD Custom Genotyping BeadChips®. Allele and genotype frequencies were estimated for the current 54 MZ pair sample. Hardy-Weinberg equilibrium was calculated using one genotype from each twin pair, by means of standard Pearson's Chi-squared tests with simulated *P*-values based on 10,000 replicates, from R's genetics package [81].

2.5. Statistical analyses

All analyses were performed with R [65]. In recognition that some adjustment is needed to account for the fact that epigenetically-driven differences in MZ twins are largely affected by age and gender of pairs [79], these two variables were included as covariates in the analyses.

Preliminary tests evaluated the relationship between genotypes in the *DNMT3B* gene and either WM or IQ, by analyzing each co-twin as an independent participant (i.e., 108 individuals). Ordinary least squares linear regression tests were performed and the Huber-White method was used to adjust the variance-covariance matrix to account for correlated responses from twin pairs [37]. Since previous reports indicate odds ratio for SNPs in *DNMTs* ranging from small (∼1.5) [34] to large (> 4) [16], a statistical power assessment was conducted, using R's pwr package [13,12]. With the current sample size, linear models adjusting for gender and age, and using two predictor SNPs would have a statistical power of 82% of detecting odds ratio around 1.5; if only one SNP is included in the model, the statistical power goes up to 86%.

Finally, a linear regression model was implemented to test for putative variability genes. Specifically, associations between genotypes and intrapair differences in cognition were evaluated by means of:

$$|Y_{i1} - Y_{i2}| = \beta_0 + \beta_1 \text{gender}_i + \beta_2 \text{age}_i + \beta_3 rs2424913_i + \beta_4 rs406193_i$$

In the regression equation, subindex $i \in \{1, \ldots, n\}$ stands for pair number (here, $n = 54$ MZ pairs) and Y_{ij} designates the psychometric score (IQ or WM) of co-twin 1 or 2 (i.e., a randomly assigned $j \in \{1, 2\}$) from the i-th pair. As inferred, only two different linear regression tests were initially performed (one for IQ and another for WM). Gender, age and SNPs only take one value from each pair (they are shared by both co-twins). An intercept (β_0) is included to account for average intrapair differences present across all pairs, regardless of gender, age or genetic background. While the reduced number of observations may be an issue at this point (54 entries, one per each healthy MZ pair), statistical power estimations revealed that linear models adjusted for gender and age, and with one SNP had a power of 52%, whereas removing the covariates the power raised up to > 70%. Hence, models with and without covariates – and their respective r^2 discrimination indexes – are compared when appropriate.

P-values for these regression models were obtained from permutation tests, with the *lmPerm* package for *R* [85]. Rather than using the conventional evaluation of standard errors to compute *P*-values, this software estimates statistical significance by calculating a permutation distribution. Permutation-based *P*-values are particularly advantageous for saturated experimental designs, datasets from non-normal populations or with apparent outliers.

There is previous evidence of slight changes in the significance and effect sizes obtained of *DNMT*'s SNPs in cognition depending on the choice of additive or dominant models [34]. Accordingly, preliminary exploratory analyses were conducted to determine whether this could considerably alter the current results for the rs2424913. The results obtained did not show large differences. Since additive models have been shown to produce more conservative results in similar analyses (i.e., lower effect sizes and larger *P*-values, with a correspondingly lower chance of false positives) [34], they were selected to be shown in the next sections.

When statistically significant nominal *P*-values were retrieved from a given statistical test, multiple comparisons corrections were implemented with the False Discovery Rate (FDR) adjustment; the type-I error rate correction adopted here was based on previous literature of statistical analysis for biological and behavioural data [7,14,27,49,59,63]. When applicable, FDR-adjusted *P*-values from a test are shown and results are discussed accordingly.

3. Results

The sample included in all analyses was composed of 108 healthy MZ twins, as shown in Table 1. As mentioned above, none of these co-twins had a history of lifetime DSM-IV psychopathology.

In the whole sample ($n = 108$), the psychometric scores were in good agreement with previous population-based studies [52] (Table 1). Within MZ twin pairs, intrapair correlation in WM scores was 0.722 ($P = 7 \times 10^{-10}$), and correlation in IQ was 0.721 ($P = 8 \times 10^{-10}$). These correlations are similar to other values reported in MZ twin samples [3,20,51]. Of note, their similarity (0.722 and 0.721) does not imply here a within-subject correlation

Table 1
Demographic and cognitive variables of the twin sample of this study, including data sorted by *DNMT3B* genotypes of rs2424913 and rs406193.

	Whole sample 54 MZ pairs: 108 subjects	DNA (cytosine-5-)-methyltransferase 3 beta (*DNMT3B*) gene				
		rs2424913 SNP genotype			rs406193 SNP genotype	
		CC 7 MZ pairs: 14 subjects	CT 30 MZ pairs: 60 subjects	TT 17 MZ pairs: 34 subjects	CC 38 MZ pairs: 76 subjects	CT 16 MZ pairs: 32 subjects
Age (mean ± SD)	33.1 ± 12.2	40.8 ± 11.4	33.3 ± 12.1	29.6 ± 11.6	32.9 ± 13.2	33.8 ± 9.6
Gender (male/female)	46/62	6/8	24/36	16/18	38/38	8/24
IQ (mean ± SD)	105.4 ± 10.5	108.3 ± 12.2	105.6 ± 8.1	103.7 ± 13.2	105.6 ± 11.8	104.9 ± 6.6
WM (mean ± SD)	105.1 ± 13.6	112.6 ± 10.9	103.7 ± 12.4	104.4 ± 15.8	105.6 ± 14.1	103.8 ± 12.2
Intrapair difference in IQ (mean ± SD)	6.7 ± 5	6.6 ± 4	5.9 ± 4.9	8.2 ± 5.6	7.8 ± 5.2	4 ± 3.4
Intrapair difference in WM (mean ± SD)	8.2 ± 6.5	5.4 ± 3.2	8.1 ± 7.4	9.4 ± 5.6	7.7 ± 5.7	9.4 ± 8.2

MZ: monozygotic; SD: standard deviation; IQ: intellectual quotient; WM: working memory; SNP: single nucleotide polymorphisms.
As mentioned in the text, due to the low TT genotype frequency for the rs406193 SNP across the general population, TT carriers were not identified in the present sample.

for the two phenotypes. Namely, for a given individual, having a high/low IQ score does not necessarily mean having a high/low WM score. This argument is derived from the fact that, within an individual, there was only a moderate correlation between IQ and WM ($r = 0.683$, $P = 0.029$), as expected from previous reports [28].

Genotype distribution of none of the analysed SNPs in this sample departed significantly from Hardy-Weinberg equilibrium (rs2424913: X-squared = 1.22, $P = 0.392$; rs406193: X-squared = 1.63, $P = 0.338$). Also, genotype frequencies were very similar to those reported for the 1000 Genomes' CEU population [26] (rs2424913: X-squared = 0.76, $P = 0.727$; rs406193: X-squared = 0.64, $P = 1$). Of note, rs406193 T allele and TT genotype frequencies are very low across general population samples. For instance, the 1000 Genomes' CEU population was constituted by 59 CC, 25 CT and 1 TT genotype carrier individuals; in the rest of 1000 Genomes populations, TT genotype frequencies ranged from 0% to 3.4%. This is consistent with the fact that, across the present 54-genotype sample (one genotype per MZ twin pair), there were no TT carriers.

Preliminary tests assessing the potential link between *DNMT3B* genotypes and either WM or IQ across the 108 participants did not reveal statistically significant associations (WM: rs2424913's $\beta = -1.96$, SE = 2.8, $t = -0.7$, $P = 0.485$, rs406193's $\beta = 2.01$, SE = 3.27, $t = 0.61$, $P = 0.541$; IQ: rs2424913's $\beta = -0.83$, SE = 2.31, $t = -0.36$, $P = 0.719$, rs406193's $\beta = 1.39$, SE = 2.47, $t = 0.56$, $P = 0.575$).

Finally, further linear regression analyses were performed in order to determine whether intrapair differences in cognitive performance (IQ and WM) were related to *DNMT3B* genotypes.

Differences in WM performance were not related to these two polymorphisms (rs2424913's $\beta = 1.55$, $P = 0.245$, rs406193's $\beta = -1.23$, $P = 0.342$; adjusted R-squared: 0.009, model's $P = 0.36$). However, as depicted in Fig. 1, C homozygotes for the rs406193 SNP showed statistically significant larger intrapair differences in IQ than CT genotype pairs (rs2424913's $\beta = 1.09$, $P = 0.748$, rs406193's $\beta = 3.52$, $P = 0.019$; adjusted r-squared: 0.106, model's $P = 0.049$). Significance of this statistical association survived FDR multiple testing adjustment (rs406193's FDR-adjusted $P = 0.038$).

Post-hoc tests evaluated the association between rs406193 genotype and intrapair differences in IQ, removing the other covariates. The association remained statistically significant when removing either the rs2424913 genotype (rs406193's $\beta = 3.34$, $P = 0.018$, adjusted r-squared: 0.106), or in a univariate model (i.e., removing gender, age and rs2424913) (rs406193's $\beta = 3.84$, $P = 0.006$, adjusted r-squared: 0.107).

Briefly, Fig. 1 depicts a summary of results for the *DNMT3B*'s rs406193 genotype. Each symbol represents one twin pair. Mean IQ of a pair (vertical axis) is an average measure of intelligence of that pair. As illustrated, symbols in the plot do not sort according to genotype through the vertical axis, indicating this paired-average IQ measure is not affected by the rs406193 SNP. However, rs406193's CC genotype pairs often display large intrapair differences in IQ (circles in the rightmost part of the plot are mostly twin pairs with CC genotype).

4. Discussion

This work evaluated whether genetic background related to epigenetic processes is more responsive to environmental influences on cognitive performance. It was found that MZ twins with the CC genotype for rs406193 SNP (in the *DNMT3B* gene) display (statistically significant) larger intrapair differences in IQ than twin pairs with other genotypes. Accordingly, the IQ of CC carriers would be more variable in response to either potentially enriched or deprived environmental factors altering cognition. This finding may be in line with previous reports suggesting that the modulation of DNA methylation dynamics by the DNMT3B protein is related to brain and cognitive plasticity [53,56,78].

The finding of an association between a specific genotype and variability in IQ – but not in WM – may also have relevance considering previous reports on cognitive plasticity and training. Recent evidence indicates that IQ is not generally improved by means of WM training, partly because distinct cognitive features respond to particular training protocols [54,68,72]. The current results indirectly expand on this issue, and support the idea that

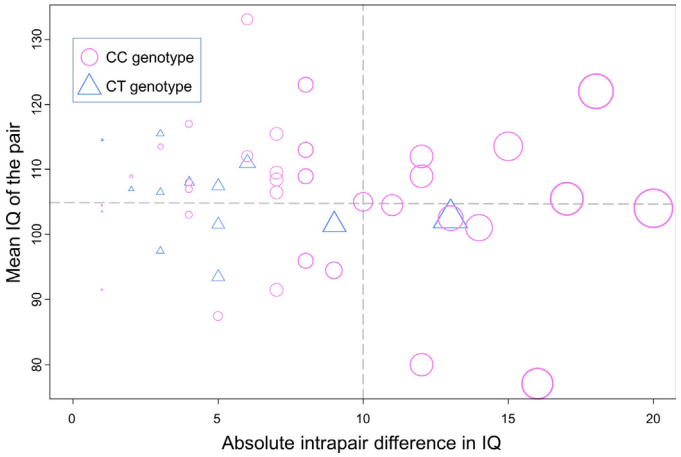


Fig. 1. Schematic of the 54 MZ pairs (each figure symbol), depicting their DNMT3B's rs406193 genotype, absolute intrapair difference in IQ and mean IQ score of the same pair. Each symbol in the plot (circles: CC genotype; triangles: CT genotype) represents each of the 54 MZ twin pairs. Gray dotted lines through axes centers divide quadrants.

genetically-driven plasticity – and response to environmental factors – may be different across cognitive domains, in agreement with research on the genetics of cognitive training response [5,76].

Remarkably, *DNMT3B* rs406193's C allele has been previously associated with worse cognitive performance (as measured by a comprehensive neuropsychological battery) in patients with psychotic disorders [73]. Though using a different conceptual and methodological approach which does not directly evaluate the same association, current results are somehow in line with that report. Simons et al. [73] suggest that within a group of patients who, by definition, were likely exposed to environmental risk factors, the C allele for rs406193 confers risk for worse cognition. The variability gene approach implemented herein suggests that, within a MZ twin pair with the CC genotype, the co-twin suffering environmental insults or lack of stimulation may have a low IQ score, whereas his co-twin, who was likely exposed to enriched environmental experiences, would perform better in intelligence tests.

It is likewise worth noticing that rs406193's T allele– which was associated here with reduced cognitive plasticity –has a relatively low frequency across the population, suggesting that cognitive flexibility may possibly be boosted by selective mechanisms. Of note, there is some previous evidence suggesting that adaptive cognitive plasticity may be rooted on evolution and have a genetic basis [22]. As previously noticed, DNA sequence changes of the *DNMT3B* may have enormous consequences on the regulation of epigenetic signaling throughout an organism, probably by reducing its phenotypic plasticity– defined as the ability to modify a behaviour in response to environmental cues –[23,36]. The present results are in line with this idea, by indicating that individuals with the *DNMT3B*'s rs406193 T allele may have less cognitive plasticity in front of variable environments.

In the same way, it is important mentioning a recently reported association between rs406193 genotype and DNA methylation levels of the insulin-like growth factor binding protein 3 (*IGFBP3*) gene in neonatal cord blood [64]. Such finding anywise indicates the significance of rs406193 genotype in shaping the human response to early environment, through epigenetic modulation of biological mechanisms. Other evidence points out that environmental insults occurring early in life may have long-lasting effects on cognitive performance [66,67]. Furthermore, the insulin-like growth factor family of proteins has been recognized to have a role in brain functioning and related processes [18,83], which may probably underlie biological mechanisms linking environmental factors, DNA methyltransferases activity and later cognitive outcomes.

Additionally, it is worth mentioning the fact that IQ but not WM was influenced by the rs406193 SNP. Importantly, heritability of these two phenotypes varies around 87% and 40%, respectively [21,32]. Hence, noticing that i) IQ has a much larger heritability estimate and ii) recent evidence points that gene-environment interactions may account for an important fraction of heritability estimates [42], it is feasible speculating that IQ is more likely affected by phenomena as described here. More precisely, the increased sensibility in response to the environment observed in rs406193 CC genotype individuals would constitute a gene-environment interaction.

Regarding the other *DNMT3B* polymorphic variant analyzed here (rs2424913), it should be noted that current preliminary results of no association between this SNP and either WM or IQ in adults are consistent with the (null) result by Haggarty et al. [34], who found rs2424913 related to general cognitive abilities neither in childhood nor adulthood. Nevertheless, rs2424913 may likely be a "level genotype" with relevant cognitive effects within neuropsychiatric patient populations, as found by Simons et al. [73]. Namely, rs2424913 could affect the mean level of cognitive traits in psychotic individuals, but currently available data suggest it is not related neither to cognitive performance in healthy adults.

Though confirmatory research and explanations of underlying epigenetic mechanisms are pendant, overall findings of this report seem biologically plausible on the basis of previous literature.

Finally, authors acknowledge some limitations of the present study, such as the modest sample size and the small number of SNPs assessed. Nevertheless, having found an association in this sample, even after adjusting for multiple potential confounders, suggests relevant effect sizes. Also, further research is invited to evaluate other relevant SNPs in both *DNMT3B* and distinct genes with relevance for epigenetically-mediated response to non-genetic factors and cognitive processing.

Disclosure of interest

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

Acknowledgements

Supported by the Spanish Ministry of Science and Innovation (PIM2010ERN-00642) in frame of ERA-NET NEURON, the Spanish SAF2008-05674-C03-01, the European Twins Study Network on Schizophrenia Research Training Network (EUTwinsS; MRTN-CT-2006-035987), and the Catalan 2014SGR1636. Authors are indebted to X. Goldberg and S. Alemany for their contribution in sample data collection.

References

[1] Alemany S, Goldberg X, van Winkel R, Gasto C, Peralta V, Fananas L. Childhood adversity and psychosis: examining whether the association is due to genetic confounding using a monozygotic twin differences approach. Eur Psychiatry 2013;28:207–12.
[2] Allis CD, Jenuwein T, Reinberg D. Epigenetics. Cold Spring Harbor, N.Y: Cold Spring Harbor Laboratory Press; 2007.
[3] Ando J, Ono Y, Wright MJ. Genetic structure of spatial and verbal working memory. Behav Genet 2001;31:615–24.
[4] Bavelier D, Levi DM, Li RW, Dan Y, Hensch TK. Removing brakes on adult brain plasticity: from molecular to behavioral interventions. J Neurosci 2010;30:14964–71.
[5] Bellander M, Brehmer Y, Westerberg H, Karlsson S, Furth D, Bergman O, et al. Preliminary evidence that allelic variation in the LMX1A gene influences training-related working memory improvement. Neuropsychologia 2011;49: 1938–42.
[6] Belsky J, Jonassaint C, Pluess M, Stanton M, Brummett B, Williams R. Vulnerability genes or plasticity genes? Mol Psychiatry 2009;14:746–54.
[7] Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B 1995;289–300.
[8] Berg K. Variability gene effect on cholesterol at the Kidd blood group locus. Clin Genet 1988;33:102–7.
[9] Berg K. Gene-environment interaction: variability gene concept. Genetic factors in coronary heart disease. Springer 1994;373–83.
[10] Bohacek J, Farinelli M, Mirante O, Steiner G, Gapp K, Coiret G, et al. Pathological brain plasticity and cognition in the offspring of males subjected to postnatal traumatic stress. Mol Psychiatry 2014.
[11] Brans RG, Kahn RS, Schnack HG, van Baal GC, Posthuma D, van Haren NE, et al. Brain plasticity and intellectual ability are influenced by shared genes. J Neurosci 2010;30:5519–24.
[12] Champely S. pwr: basic functions for power analysis; 2012 [computer program. Version 1.1.1; 2012. Available from: http://CRAN.R-project.org/package=pwr].
[13] Cohen J. Statistical power analysis for the behavioral sciences, 2nd ed., Hillsdale, N.J: L. Erlbaum Associates; 1988.
[14] Cook RJ, Farewell VT. Multiplicity considerations in the design and analysis of clinical trials. J R Stat Soc Ser A 1996;93–110.
[15] Coppede F, Zitarosa MT, Migheli F, Lo Gerfo A, Bagnoli S, Dardano A, et al. DNMT3B promoter polymorphisms and risk of late onset Alzheimer's disease. Curr Alzheimer Res 2012;9:550–4.
[16] Coppede F, Bosco P, Tannorella P, Romano C, Antonucci I, Stuppia L, et al. DNMT3B promoter polymorphisms and maternal risk of birth of a child with Down syndrome. Hum Reprod 2013;28:545–50.
[17] Coppede F, Grossi E, Buscema M, Migliore L. Application of artificial neural networks to investigate one-carbon metabolism in Alzheimer's disease and healthy matched individuals. PLoS One 2013;8:e74012.
[18] Cordova-Palomera A, Alemany S, Fatjo-Vilas M, Goldberg X, Leza JC, Gonzalez-Pinto A, et al. Birth weight, working memory and epigenetic signatures in IGF2 and related genes: a MZ Twin Study. PLoS One 2014;9:e103639.

[19] Davies G, Tenesa A, Payton A, Yang J, Harris SE, Liewald D, et al. Genome-wide association studies establish that human intelligence is highly heritable and polygenic. Mol Psychiatry 2011;16:996–1005.
[20] Deary IJ, Spinath FM, Bates TC. Genetics of intelligence. Eur J Hum Genet 2006;14:690–700.
[21] Deary IJ, Penke L, Johnson W. The neuroscience of human intelligence differences. Nat Rev Neurosci 2010;11:201–11.
[22] Dukas R. Evolutionary biology of animal cognition. Annu Rev Ecol Evol Syst 2004;347–74.
[23] Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. Nature 2007;447:433–40.
[24] Feng J, Chang H, Li E, Fan G. Dynamic expression of de novo DNA methyltransferases Dnmt3a and Dnmt3b in the central nervous system. J Neurosci Res 2005;79:734–46.
[25] First MB. In: Clinician, editor. SCID-I: structured clinical interview for DSM-IV axis I disorders. New York: American Psychiatric Press; 1997.
[26] Genomes Project C, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, et al. An integrated map of genetic variation from 1092 human genomes. Nature 2012;491:56–65.
[27] Glickman ME, Rao SR, Schultz MR. False discovery rate control is a recommended alternative to Bonferroni-type adjustments in health studies. J Clin Epidemiol 2014;67:850–7.
[28] Goldberg X, Alemany S, Rosa A, Picchioni M, Nenadic I, Owens SF, et al. Substantial genetic link between iq and working memory: Implications for molecular genetic studies on schizophrenia. the european twin study of schizophrenia (EUTwinsS). Am J Med Genet B Neuropsychiatr Genet 2013;162:413–8.
[29] Goldberg X, Fatjo-Vilas M, Alemany S, Nenadic I, Gasto C, Fananas L. Gene-environment interaction on cognition: a twin study of childhood maltreatment and COMT variability. J Psychiatr Res 2013;47:989–94.
[30] Graff J, Mansuy IM. Epigenetic codes in cognition and behaviour. Behav Brain Res 2008;192:70–87.
[31] Greenwood PM, Parasuraman R. Neuronal and cognitive plasticity: a neurocognitive framework for ameliorating cognitive aging. Front Aging Neurosci 2010;2:150.
[32] Greenwood TA, Braff DL, Light GA, Cadenhead KS, Calkins ME, Dobie DJ, et al. Initial heritability analyses of endophenotypic measures for schizophrenia: the consortium on the genetics of schizophrenia. Arch Gen Psychiatry 2007;64:1242–50.
[33] Guilherme R, Drunat S, Delezoide AL, Oury JF, Luton D. Zygosity and chorionicity in triplet pregnancies: new data. Hum Reprod 2009;24:100–5.
[34] Haggarty P, Hoad G, Harris SE, Starr JM, Fox HC, Deary IJ, et al. Human intelligence and polymorphisms in the DNA methyltransferase genes involved in epigenetic marking. PLoS One 2010;5:e11329.
[35] Haggarty P, Hoad G, Horgan GW, Campbell DM. DNA methyltransferase candidate polymorphisms, imprinting methylation, and birth outcome. PLoS One 2013;8:e68896.
[36] Hansen RS, Wijmenga C, Luo P, Stanek AM, Canfield TK, Weemaes CM, et al. The DNMT3B DNA methyltransferase gene is mutated in the ICF immunodeficiency syndrome. Proc Natl Acad Sci U S A 1999;96:14412–7.
[37] Harrel F. rms: regression modeling strategies; 2013 [computer program. Version 4.2-1; 2013. Available from: http://CRAN.R-project.org/package=rms].
[38] Haworth CM, Wright MJ, Luciano M, Martin NG, de Geus EJ, van Beijsterveldt CE, et al. The heritability of general cognitive ability increases linearly from childhood to young adulthood. Mol Psychiatry 2010;15:1112–20.
[39] Hong EJ, West AE, Greenberg ME. Transcriptional control of cognitive development. Curr Opin Neurobiol 2005;15:21–8.
[40] Jurkowska RZ, Jurkowski TP, Jeltsch A. Structure and function of mammalian DNA methyltransferases. Chembiochem 2011;12:206–22.
[41] Kaminsky ZA, Tang T, Wang SC, Ptak C, Oh GH, Wong AH, et al. DNA methylation profiles in monozygotic and dizygotic twins. Nat Genet 2009;41:240–5.
[42] Kaprio J. Twins and the mystery of missing heritability: the contribution of gene-environment interactions. J Intern Med 2012;272:440–8.
[43] Klingberg T. Training and plasticity of working memory. Trends Cogn Sci 2010;14:317–24.
[44] Kramer AF, Willis SL. Cognitive plasticity and aging. In: Ross BH, editor. The psychology of learning and motivation. San Diego, CA: Academic Press; 2003.
[45] Kumar S, Cheng X, Klimasauskas S, Mi S, Posfai J, Roberts RJ, et al. The DNA (cytosine-5) methyltransferases. Nucleic Acids Res 1994;22:1–10.
[46] Lamb YN, Thompson JM, Murphy R, Wall C, Kirk IJ, Morgan AR, et al. Perceived stress during pregnancy and the catechol-O-methyltransferase (COMT) rs165599 polymorphism impacts on childhood IQ. Cognition 2014;132: 461–70.
[47] Lehto K, Akkermann K, Parik J, Veidebaum T, Harro J. Effect of COMT Val158-Met polymorphism on personality traits and educational attainment in a longitudinal population representative study. Eur Psychiatry 2013;28:492–8.
[48] Lillard AS, Erisir A. Old dogs learning new tricks: neuroplasticity beyond the juvenile period. Dev Rev 2011;31:207–39.
[49] Liu W, Jamshidian M, Zhang Y. Multiple comparison of several linear regression models. J Am Statist Assoc 2004;99:395–403.
[50] Lovden M, Backman L, Lindenberger U, Schaefer S, Schmiedek F. A theoretical framework for the study of adult cognitive plasticity. Psychol Bull 2010;136:659–76.
[51] Luciano M, Wright M, Smith GA, Geffen GM, Geffen LB, Martin NG. Genetic covariance among measures of information processing speed, working memory, and IQ. Behav Genet 2001;31:581–92.
[52] Lynn R, Meisenberg G. National IQs calculated and validated for 108 nations. Intelligence 2010;38:353–60.
[53] MacDonald JL, Roskams AJ. Epigenetic regulation of nervous system development by DNA methylation and histone deacetylation. Prog Neurobiol 2009;88:170–83.
[54] Melby-Lervåg M, Hulme C. Is working memory training effective? A meta-analytic review. Dev Psychol 2013;49:270.
[55] Mercado 3rd E. Neural and cognitive plasticity: from maps to minds. Psychol Bull 2008;134:109–37.
[56] Miller CA, Sweatt JD. Covalent modification of DNA regulates memory formation. Neuron 2007;53:857–69.
[57] Murgatroyd C, Spengler D. Genetic variation in the epigenetic machinery and mental health. Curr Psychiatry Rep 2012;14:138–49.
[58] Murphy TM, Mullins N, Ryan M, Foster T, Kelly C, McClelland R, et al. Genetic variation in DNMT3B and increased global DNA methylation is associated with suicide attempts in psychiatric patients. Genes Brain Behav 2013;12:125–32.
[59] Nakagawa S. A farewell to Bonferroni: the problems of low statistical power and publication bias. Behav Ecol 2004;15:1044–5.
[60] Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 1999;99:247–57.
[61] Panizzutti R, Hamilton SP, Vinogradov S. Genetic correlate of cognitive training response in schizophrenia. Neuropharmacol 2013;64:264–7.
[62] Perneczky R, Alexopoulos P, Wagenpfeil S, Bickel H, Kurz A. Head circumference, apolipoprotein E genotype and cognition in the Bavarian School Sisters Study. Eur Psychiatry 2012;27:219–22.
[63] Perneger TV. What's wrong with Bonferroni adjustments. BMJ 1998;316:1236–8.
[64] Potter C, McKay J, Groom A, Ford D, Coneyworth L, Mathers JC, et al. Influence of DNMT genotype on global and site specific DNA methylation patterns in neonates and pregnant women. PLoS One 2013;8:e76506.
[65] R Development Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2011.
[66] Rahu K, Rahu M, Pullmann H, Allik J. Effect of birth weight, maternal education and prenatal smoking on offspring intelligence at school age. Early Hum Dev 2010;86:493–7.
[67] Raznahan A, Greenstein D, Lee NR, Clasen LS, Giedd JN. Prenatal growth in humans and postnatal brain maturation into late adolescence. Proc Natl Acad Sci U S A 2012;109:11366–71.
[68] Redick TS, Shipstead Z, Harrison TL, Hicks KL, Fried DE, Hambrick DZ, et al. No evidence of intelligence improvement after working memory training: a randomized, placebo-controlled study. J Exp Psychol Gen 2013;142:359.
[69] Saffery R, Morley R, Foley DL. The utility of twins for epigenetic analysis. In: Michels KB, editor. Epigenet Epidemiol. Dordrecht, Netherlands: Springer; 2012. p. 161–83.
[70] Sattler JM. Assessment of children: cognitive applications, 4th ed., San Diego: J.M. Sattler; 2001.
[71] Schlichting CD. Origins of differentiation via phenotypic plasticity. Evol Dev 2003;5:98–105.
[72] Shipstead Z, Redick TS, Engle RW. Is working memory training effective? Psychol Bull 2012;138:628.
[73] Simons CJ, van Winkel R, Group. Intermediate phenotype analysis of patients, unaffected siblings, and healthy controls identifies VMAT2 as a candidate gene for psychotic disorder and neurocognition. Schizophr Bull 2013;39:848–56.
[74] Slagter HA. Conventional working memory training may not improve intelligence. Trends Cogn Sci 2012;16:582–3.
[75] Soderqvist S, Bergman Nutley S, Peyrard-Janvid M, Matsson H, Humphreys K, Kere J, et al. Dopamine, working memory, and training induced plasticity: implications for developmental research. Dev Psychol 2012;48:836–43.
[76] Soderqvist S, Matsson H, Peyrard-Janvid M, Kere J, Klingberg T. Polymorphisms in the dopamine receptor 2 gene region influence improvements during working memory training in children and adolescents. J Cogn Neurosci 2014;26:54–62.
[77] Stromswold K. Why aren't identical twins linguistically identical? Genetic, prenatal and postnatal factors. Cognition 2006;101:333–84.
[78] Sweatt JD. Experience-dependent epigenetic modifications in the central nervous system. Biol Psychiatry 2009;65:191–7.
[79] Tan Q. Epigenetic epidemiology of complex diseases using twins. Med Epigenet 2013;1:46–51.
[80] Tan Q, Christiansen L, Thomassen M, Kruse TA, Christensen K. Twins for epigenetic studies of human aging and development. Ageing Res Rev 2013;12:182–7.
[81] Warnes G. Genetics: population genetics; 2013 [computer program. Version 1.3.8.1; 2013. Available from: http://CRAN.R-project.org/package=genetics].
[82] Wechsler D, Cordero Pando A, Yela Granizo M, Zimmerman IL, Woo-Sam JM, Glasser AJ. WAIS escala de inteligencia de Wechsler para adultos, 12th ed., Barcelona: TEA Ediciones; 1997.
[83] Werner H, Leroith D. Insulin and insulin-like growth factor receptors in the brain: physiological and pathological aspects. Eur Neuropsychopharmacol 2014.
[84] West-Eberhard MJ. Phenotypic plasticity and the origins of diversity. Annu Rev Ecol Evol Syst 1989;249–78.
[85] Wheeler B. lmPerm: permutation tests for linear models; 2010 [computer program. Version 1.1-2; 2010. Available from: http://CRAN.R-project.org/package=lmPerm].
[86] Zhang C, Fang Y, Xie B, Cheng W, Du Y, Wang D, et al. DNA methyltransferase 3B gene increases risk of early onset schizophrenia. Neurosci Lett 2009;462: 308–11.

Advisor's report on the contribution of the Ph.D. candidate to the article

Prof. Dr. Lourdes Fañanás Saura, associate professor at the Faculty of Biology, University of Barcelona, and supervisor of the present doctoral thesis by Aldo Córdova Palomera, hereby certifies that the participation of the Ph.D. candidate in the article "Polymorphic variation in the epigenetic gene *DNMT3B* modulates the environmental impact on cognitive ability: a twin study" included the following tasks:

- Participation in study design.
- Statistical analyses.
- Writing of the first manuscript draft.
- Critical revision of the article.

Prof. Dr. Lourdes Fañanás Saura

Barcelona, June 30th 2015.

Further evidence of **DEPDC7** *DNA hypomethylation in depression: a study in adult twins*

Córdova-Palomera A, Fatjó-Vilas M, Palma-Gudiel H, Blasco-Fontecilla H, Kebir O, Fañanás L

European Psychiatry (2015), *In press*

DOI: http://dx.doi.org/10.1016/j.eurpsy.2015.04.001

European Psychiatry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

European Psychiatry

journal homepage: http://www.europsy-journal.com

Original article

Further evidence of *DEPDC7* DNA hypomethylation in depression: A study in adult twins

A. Córdova-Palomera [a,b], M. Fatjó-Vilas [a,b], H. Palma-Gudiel [a], H. Blasco-Fontecilla [b,c], O. Kebir [d,e,f], L. Fañanás [a,*,b]

[a] *Unitat d'Antropologia, Departament de Biologia Animal, Facultat de Biologia and Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain*
[b] *Centro de Investigaciones Biomédicas en Red de Salud Mental (CIBERSAM), Instituto de Salud Carlos III, Madrid, Spain*
[c] *Department of Psychiatry, IDIPHIM-Puerta de Hierro University Hospital, Madrid, Spain*
[d] *Inserm, centre de psychiatrie et neurosciences, UMR 894, laboratoire de physiopathologie des maladies psychiatriques, université Paris Descartes, PRES Paris Sorbonne Cité, Paris, France*
[e] *Service hospitalo-universitaire, faculté de médecine Paris Descartes, hôpital Sainte-Anne, Paris, France*
[f] *GDR3557, institut de psychiatrie, Paris, France*

ARTICLE INFO

Article history:
Received 27 February 2015
Received in revised form 30 March 2015
Accepted 1st April 2015
Available online xxx

Keywords:
DNA methylation
Depression
MZ twins
Brief Symptom Inventory
DEPDC7

ABSTRACT

Late and early stressful factors have widely been recognized to play a role in the aetiology of depression. Recent research indicates that such adverse environmental stimuli may alter gene expression in humans via epigenetic modifications. While epigenetic changes such as DNA methylation are likely involved in these processes, it is still unknown what specific genomic loci may be hyper- or hypo-methylated in depression. The association between depressive symptoms during the last 30 days (Brief Symptom Inventory [BSI]) and peripheral-blood DNA methylation levels at genomic loci previously reported as epigenetically altered in saliva and brain of depressive patients was evaluated in a community sample of 34 adult Caucasian MZ twins (17 pairs). Intrapair DNA methylation differences in an intron of *DEPDC7* (chr11:33040743) were associated with intrapair differences in current depressive symptoms. Accordingly, a site-specific 10% DNA hypomethylation in a co-twin would correlate with a current depressive symptom score around 3.1 BSI points above the score of his/her less-depressed co-twin. These findings indicate that *DEPDC7* hypomethylation in peripheral blood DNA may be associated with recent depressive symptomatology, in line with previous results.

1. Background

Several studies have evidenced that some epigenetic alterations following adverse environmental stimuli may considerably alter gene expression patterns to induce depression and related psychopathology [10,15,21].

One of the best candidate epigenetic alterations in psychiatric epidemiology research is DNA methylation, due to its accessibility and its relevance for the biology of gene expression [23]. Methylation of DNA happens at position 5 of the cytosine pyrimidine ring of CpG dinucleotides, and it is closely related to the gene expression modifications. Accordingly, previous research has indicated that late and early stressful factors may get embedded into the genome via DNA methylation changes, which may ultimately lead to gene expression disruptions that increase the risk for depressive psychopathology [21,24]. However, the precise genomic loci at which DNA methylation alterations occur in depression remain still not identified with certainty.

A recent study showed intrapair DNA methylation differences at two CpG sites across the genome in the saliva of 18 monozygotic (MZ) twin pairs discordant for adolescent depression, and later replicated these findings in post-mortem cerebellum of subjects with major depressive disorder (MDD) [12]. While these are certainly robust results, replication using blood, and in samples with different demographics and depression-related phenotypes is needed to determine the extent to which the previous relationships remain valid. This may also serve to identify potential biological mechanisms mediating these associations.

Therefore, this work aims to expand on previous findings to determine whether the above-mentioned epigenetic signatures,

* Corresponding author. Tel.: +34 9340 21461; fax: +34 9340 35740.
E-mail address: lfananas@ub.edu (L. Fañanás).

http://dx.doi.org/10.1016/j.eurpsy.2015.04.001
0924-9338/

which have been found associated with clinically-relevant depression in saliva of adolescents and in MDD cerebellum, can likewise be detected in peripheral blood DNA of adult MZ twins with less-severe depressive phenotypes (psychopathological symptoms) manifested during the last month.

2. Methods

2.1. Subjects

Participants of this study were part of a larger ongoing twin sample consisting of 242 Caucasian Spanish adult twins from the general population who gave permission to be contacted for research purposes. For that sample, exclusion criteria applied included age under 18 and over 65, a medical history of neurological disturbance, presence of sensory or motor alterations and current substance misuse or dependence. Written informed consent was obtained from all participants after a detailed description of the study aims and design, approved by the local Ethics Committee.

Medical records and a battery of psychological and neurocognitive tests were obtained in face-to-face interviews by trained psychologists. Additionally, peripheral blood or saliva samples were obtained from all participants, and zygosity of the pairs was determined by genotyping 16 highly polymorphic microsatellite loci from DNA samples (SSRs; PowerPlex® 16 System Promega Corporation). Identity on all the markers can be used to assign monozygosity with greater than 99% accuracy [17].

Additional research protocols were administered to a smaller MZ twin group from this 242-twin set. Briefly, neuroimaging and epigenome-wide data was collected for a group of 34 middle-aged participants (17 MZ twin pairs; age range 22–56, median age 38; 47% female) who were informative for psychopathology, neurocognition and early stress factors. The choice of these 34 participants from the larger UB Twin Registry was mainly based on three criteria:

- both members of each pair were willing to collaborate in upcoming appointments;
- there was good-quality information on neurocognitive performance and both prenatal and childhood neurodevelopment for both twins from retrospective questionnaires;
- some of the pairs had lifetime liability for anxious-depressive psychopathology, as indexed by the SCID interview (see Section 2.2 Psychopathology assessment) [16].

Although the SCID interview indicated that some of these twins had had lifetime liability for anxiety and depression, only five out of the thirty-four participants showed psychopathology symptoms above the SCID thresholds by the time of blood extraction. These five individuals scored moderately higher than the other twins for the current depression symptoms assessment; however, since this is a community sample that was not collected from a clinical setting, the symptom severity was not extreme. It is also important noticing that, due to the transversal character of the present design, the focus is on current symptoms – by the time of blood extraction and epigenetic marker assessment – rather than on lifetime psychopathological liability.

Peripheral blood was available for all members of this 34-twin group. All analyses described below refer to this 34-individual subset. Further information on the sample can be found in Table 1 and elsewhere [9].

2.2. Psychopathology assessment

Current psychiatric symptoms were evaluated with the Brief Symptom Inventory (BSI) [13,29]. The BSI is a self-administered 46-item screening instrument aimed at identifying the experience of psychopathological symptoms during the last 30 days. It is composed by six subscales (depression, phobic anxiety, paranoid ideation, obsession-compulsion, somatization and hostility) conceived for use in both clinical and non-clinical samples. Items are rated on a 5-point scale of distress, according to self-perception of symptoms. Descriptive information of the BSI scores in this sample can be found in Table 1.

Of note, previous research has consistently demonstrated a relationship between the BSI depression scores and the clinical phenotype of depression [13,14]. Although the specificity of the BSI to detect clinical depression varies depending on score cut-offs [31], the "healthy" baseline for the BSI depression items used here would be around 1.8 points (raw score) [25], and psychiatric diagnoses are typically found when scoring about six times [13] the "healthy" rating (i.e., about 10.8 points in this case).

2.3. Methylation data

The Illumina Infinium HumanMethylation450 (450K) BeadChip [4,30] was employed. Specifically, by genotyping sodium bisulfite treated DNA, DNA methylation is assayed by this platform at >450,000 CpG sites across the genome at single base resolution; next, bisulfite-converted DNA undergoes whole-genome amplification, before being fragmented and hybridized to microarray probes. DNA methylation fraction of each CpG site is estimated as $\beta = M/(M + U + \alpha)$; M and U stand for methylated and unmethylated fluorescence intensities, and α is an arbitrary offset applied to stabilize β values with low intensities.

Infinium methylation data was processed with Methylation Module of GenomeStudio software using HumanMethylation450 manifest v1.1 following the instructions published by Bibikova et al. [4]. CpG sites with poor detection quality ($P > 10^{-4}$) were removed from further analysis.

2.4. CpG probes selection

Genomic loci previously reported by Dempster, Wong [12] as hyper- or hypo-methylated in depressed individuals – in both

Table 1
Descriptive data for variables included in the analysis.

Sample description $n = 34$ (17 MZ twin pairs, 47% female)	Individual-level description		Intrapair differences (absolute values)	
	Mean (SD)	Range	Mean (SD)	Range
Age (years)	35.5 (11)	19–54	–	–
Total BSI score	18.3 (14.2)	1–57	13.2 (10.7)	0–35
BSI depression score	3.6 (4.1)	0–20	3.5 (3.8)	0–13
cg07080019 methylation fraction (%)	35.1 (9)	15.5–74.7	6 (5.9)	0–23.4
cg09090376 methylation fraction (%)	64.8 (5.2)	47.2–75.1	4.7 (4.4)	0.2–17.4

MZ: monozygotic; SD: standard deviation; BSI: Brief Symptom Inventory.

Please cite this article in press as: Córdova-Palomera A, et al. Further evidence of *DEPDC7* DNA hypomethylation in depression: A study in adult twins. European Psychiatry (2015), http://dx.doi.org/10.1016/j.eurpsy.2015.04.001

peripheral and brain tissue samples – were selected for assessment. Specifically, the CpG sites cg07080019 (chr10:134036804, hg19) and cg09090376 (chr11:33040743, hg19) were evaluated.

This previous report validated the biological relevance of both cg07080019 and cg09090376 using three different assessment steps: genome-wide methylation measurements in saliva of MZ twins, confirmation through bisulfate pyrosequencing and replication in MDD post-mortem cerebellum samples. However, the effect sizes shown in that study for these two probes were relatively small (<8% methylation difference between depressed and healthy samples).

In view of this, the present study focused on these two CpG sites rather than on the larger (epi)genome-wide context, since:

- including more CpG probes here would increase the likelihood of other larger-effect and/or false-positive probes showing up as more relevant than cg07080019 and cg09090376;
- the number of statistical tests required to independently assess all CpG probes in the Illumina array makes very unlikely finding an association between 8% methylation changes and depression after multiple testing adjustments. Namely, even in the presence of a true biological effect, it may not seem relevant when examining all the genome-wide data available, thus hampering the current replication attempts.

2.5. Statistical analyses

To evaluate whether intrapair differences in DNA methylation were associated with intrapair differences in current depressive symptoms, a regression procedure [6,8] was implemented. Briefly, the method adopted here consisted in the estimation of the expected value $E[D_i^Y] = \beta_W D_i^X$, where $D_i^X = X_{i1} - X_{i2}$, and $D_i^Y = Y_{i1} - Y_{i2}$. Here, X corresponds to the predictor variable (DNA methylation fraction) and Y represents the outcome (BSI depressive score). The index $i \in \{1,\ldots,n\}$ stands for the randomly-assigned pair number (here, $n = 17$: the total number of pairs), and sub-indices "1" and "2" in the expressions for D_i^X and D_i^Y stand for a randomly-assigned co-twin number. The regression results provided below assess the statistical significance and the magnitude of β_W to determine the potential biological implications of the findings.

These analyses were conducted using the R software, estimating the P-values with a permutation-based procedure [27,37]. These permutation-based P-values are advantageous for non-normal populations and for saturated study designs. Briefly, linear regression model fitting and testing using this methodology instead of normal theory tests consisted in running all necessary iterations (up to 5000) until the estimated standard error of the estimated proportion P was smaller than P times 0.1. These criteria follow standard guidelines from previous literature [37,1]. Since two pairs exhibited outlier intrapair differences on depressive symptom scores (>2 standard deviations from the mean), analyses were conducted with and without them for comparison.

3. Results

In the present sample, intrapair DNA methylation differences in cg09090376 – but not in cg07080019 – were associated with intrapair differences in current depressive symptoms (cg07080019's $\beta = -9.15$, $P = 0.516$, adjusted R-squared = −0.04; cg09090376's $\beta = -31.23$, $P = 0.008$, adjusted R-squared = 0.38). Hence, a 10% hypomethylation in a co-twin would correlate with an increase in his/her current depressive symptom score of about 3.1 points with respect to his/her co-twin. This result remained statistically significant at $P < 0.05$ even when including outlier pairs.

Including both outlier pairs made the regression coefficient for cg09090376 increase ($\beta = -35.81$), though it reduced the overall model fitting statistics to less than half the model without outliers (adjusted R-squared = 0.15) and increased β's P-value ($P = 0.048$). Importantly, removal of only one of the two outliers – a twin pair with an 11-point difference in BSI score, with the most depressed co-twin showing a hypermethylation of 3.7% with respect to the less depressed co-twin – led to statistical model fitting parameters very similar to those in the first case (cg09090376's $\beta = -42.4$, $P = 0.01$, adjusted R-squared = 0.35). The former two analysis results suggest that the inclusion of a particular outlier observation from this sample – a twin pair with a 13-point difference in BSI score, where the most depressed co-twin displayed a 7.1% hypomethylation with respect to the less depressed co-twin – considerably alters the regression parameters, producing the above mentioned marginally-significant P-value and low model fitting parameters ($P = 0.048$; adjusted R-squared = 0.15).

4. Conclusions

The current analysis shows an association between hypomethylation of chr11:33040743 (hg19; *DEPDC7*) and depressive symptomatology, in line with the results obtained by Dempster et al. [12], who showed hypomethylation in co-twins affected by adolescent depression as well as in postmortem brain tissue of subjects with MDD.

This CpG site is located in an intronic region of *DEPDC7*, whose function has previously been linked to brain-related phenotypes [3,22]. For instance, research has indicated that *DEPDC7* could participate in epigenetic processes of striatal neurons [22]. Likewise, it may regulate neural cell development and physiology [3]. This finding may be in line with models of epigenetically-mediated neurodevelopmental influences on depression [2]. Hence, studies should now lend particular attention to the epigenetic functioning of this genomic locus as it may play a significant role in the biological mechanisms of depressive psychopathology.

The hypothesis of a system-wide epigenetic disruption in psychopathology proposes that some gene expression changes, and their consequent protein activity alterations, are not limited to the central nervous system, but show cross-tissue validity [34]. This subject has several implications, since cross-tissue epigenetic changes may constitute the very biological basis of some peripheral blood biomarkers of depression [5,28,35].

The present findings may support theories of system-wide epigenetic disruptions in psychopathology [34], by expanding on the cross-tissue validity of the locus-specific *DEPDC7* hypomethylation in depression. In this sense, while the work of several independent laboratories has shown large cross-tissue differences in DNA methylation [11,19,20,36], results mainly from Szyf et al. show a correlation between DNA methylation levels across peripheral lymphocytes and a number of brain regions; they also suggest that it should reflect environmental factors leading to psychopathology [26,32,33]. The above-mentioned result somehow points in this direction. Further biological interpretation of how some similar epigenetic changes occur in different tissues in depressed individuals, as well as their potential biomarker validity, remain still in progress.

The lack of association obtained here for the cg07080019 site may probably be explained by the distinct depressive conditions and the time-scale of the phenotype considered here. While Dempster et al. [12] used a psychometric instrument based on DSM-III criteria for depression, and found large depression discordance in at least two independent time stages during adolescence, the depressive symptomatology of these participants refers to the last month before the DNA sample extraction. Thus, it might be hypothesized that methylation of cg07080019 is related

Please cite this article in press as: Córdova-Palomera A, et al. Further evidence of *DEPDC7* DNA hypomethylation in depression: A study in adult twins. European Psychiatry (2015), http://dx.doi.org/10.1016/j.eurpsy.2015.04.001

both to causal factors leading to psychopathology and to trait depression, rather than to actual symptom manifestation (state). However, the positive association found here indicates that hypomethylation of cg09090376 is probably associated mainly with the expression of depressive symptoms (state). Also, caution should be taken when interpreting the association of cg07080019 DNA methylation in postmortem brain as described by Dempster et al. [12], since cg07080019 contains the rs145220785 SNP [7,18], which may influence results in comparisons of genetically diverse samples (i.e., the brains of genetically independent MDD patients and controls).

In summary, this is the third independent sample evidencing a potential relationship between cg09090376 (chr11:33040743) DNA hypomethylation and depression-related phenotypes. Of note, all three results are very consistent despite the use of moderate sample sizes and different biological tissues. This consistency may be indicative of considerable effect sizes.

5. Limitations

Some limitations of the present study deserve mention. First, the modest sample size may have dampened the possibility of detecting an association between cg07080019 DNA methylation and depression. Nevertheless, both the previous report [12] and the present study were conducted using similar sample sizes (18 and 17 MZ pairs), and the association shown here for the other CpG site (cg09090376) indicates that a lack of statistical power may not be the main explanation for cg07080019's null result.

Besides, larger phenotypic discordances (i.e., higher intrapair differences in depression) in the MZ co-twins may allow detecting greater effect sizes. Due to the community-based character of this sample, severe depressive disorders are not included. This, however, may increase the ecological validity of the results: they may suggest that DNA methylation changes are present even in not-severe phenotypes.

Disclosure of interest

In the last three years, Dr. Hilario Blasco-Fontecilla has received lecture fees from Eli Lilly, AB-Biotics, Janssen, Rovi, and Shire. The rest of the authors declare that they have no conflicts of interest concerning this article.

Acknowledgements

Supported by the Spanish SAF2008-05674-C03-01, European Twins Study Network on Schizophrenia Research Training Network (grant number EUTwinsS; MRTN-CT-2006-035987) and the Ministry of Science and Technology (PIM2010ERN-00642) as part of the ERA-NET NEURON network. Thanks to the Comissionat per a Universitats i Recerca del DIUE (2014SGR1636). A. Córdova-Palomera was funded by CONACyT (Mexico). Authors are indebted to J. Ignacio Martín-Subero for technical advice, and to Ximena Goldberg and Silvia Alemany for their contribution to data collection. We also thank the Genotype service at Centro Nacional de Investigaciones Oncológicas (CNIO Madrid, Spain) for performing array hybridization and providing technical support.

References

[1] Anscombe FJ. Sequential estimation. J R Stat Soc 1953;15:1–29.
[2] Ansorge MS, Hen R, Gingrich JA. Neurodevelopmental origins of depressive disorders. Curr Opin Pharmacol 2007;7:8–17.
[3] Berezovsky AD, Poisson LM, Cherba D, Webb CP, Transou AD, Lemke NW, et al. Sox2 promotes malignancy in glioblastoma by regulating plasticity and astrocytic differentiation. Neoplasia 2014;16. 193–206, e19–25.
[4] Bibikova M, Barnes B, Tsan C, Ho V, Klotzle B, Le JM, et al. High density DNA methylation array with single CpG site resolution. Genomics 2011;98:288–95.
[5] Carlberg L, Scheibelreiter J, Hassler MR, Schloegelhofer M, Schmoeger M, Ludwig B, et al. Brain-derived neurotrophic factor (BDNF)-epigenetic regulation in unipolar and bipolar affective disorder. J Affect Disord 2014;168:399–406.
[6] Carlin JB, Gurrin LC, Sterne JA, Morley R, Dwyer T. Regression models for twin studies: a critical review. Int J Epidemiol 2005;34:1089–99.
[7] Chen YA, Lemire M, Choufani S, Butcher DT, Grafodatskaya D, Zanke BW, et al. Discovery of cross-reactive probes and polymorphic CpGs in the Illumina Infinium HumanMethylation450 microarray. Epigenetics 2013;8:203–9.
[8] Córdova-Palomera A. mztwinreg: regression models for monozygotic twin data; 2015.
[9] Cordova-Palomera A, Alemany S, Fatjo-Vilas M, Goldberg X, Leza JC, Gonzalez-Pinto A, et al. Birth weight, working memory and epigenetic signatures in IGF2 and related genes: a MZ twin study. PLoS One 2014;9:e103639.
[10] Dalton VS, Kolshus E, McLoughlin DM. Epigenetics and depression: return of the repressed. J Affect Disord 2014;155:1–12.
[11] Davies MN, Volta M, Pidsley R, Lunnon K, Dixit A, Lovestone S, et al. Functional annotation of the human brain methylome identifies tissue-specific epigenetic variation across brain and blood. Genome Biol 2012;13:R43.
[12] Dempster EL, Wong CC, Lester KJ, Burrage J, Gregory AM, Mill J, et al. Genome-wide methylomic analysis of monozygotic twins discordant for adolescent depression. Biol Psychiatry 2014;76:977–83.
[13] Derogatis LR, Melisaratos N. The Brief Symptom Inventory: an introductory report. Psychol Med 1983;13:595–605.
[14] Derogatis LR, Savitz KL. The SCL-90-R, Brief Symptom Inventory, and Matching Clinical Rating Scales. In: Maurish ME, editor. The use of psychological testing for treatment planning and outcomes assessment. 2nd ed., Mahwah, NJ, US: Lawrence Erlbaum Associates Publishers; 1999. p. 679–724.
[15] Fiori LM, Turecki G. Gene expression profiling of suicide completers. Eur Psychiatry 2010;25:287–90.
[16] First MB. Structured clinical interview for DSM-IV axis I disorders: SCID – I: clinician version: administration booklet. Washington, D.C.: American Psychiatric Press; 1997.
[17] Guilherme R, Drunat S, Delezoide AL, Oury JF, Luton D. Zygosity and chorionicity in triplet pregnancies: new data. Hum Reprod 2009;24:100–5.
[18] Heyn H, Li N, Ferreira HJ, Moran S, Pisano DG, Gomez A, et al. Distinct DNA methylomes of newborns and centenarians. Proc Natl Acad Sci U S A 2012;109:10522–7.
[19] Iwamoto K, Bundo M, Ueda J, Oldham MC, Ukai W, Hashimoto E, et al. Neurons show distinctive DNA methylation profile and higher interindividual variations compared with non-neurons. Genome Res 2011;21:688–96.
[20] Ladd-Acosta C, Pevsner J, Sabunciyan S, Yolken RH, Webster MJ, Dinkins T, et al. DNA methylation signatures within the human brain. Am J Hum Genet 2007;81:1304–15.
[21] Mann JJ, Currier DM. Stress, genetics and epigenetic effects on the neurobiology of suicidal behavior and depression. Eur Psychiatry 2010;25:268–71.
[22] McFarland KN, Das S, Sun TT, Leyfer D, Kim MO, Xia E, et al. Genome-wide increase in histone H2A ubiquitylation in a mouse model of Huntington's disease. J Huntingtons Dis 2013;2:263–77.
[23] Mill J, Heijmans BT. From promises to practical strategies in epigenetic epidemiology. Nat Rev Genet 2013;14:585–94.
[24] Mill J, Petronis A. Molecular studies of major depressive disorder: the epigenetic perspective. Mol Psychiatry 2007;12:799–814.
[25] Mohammadkhani P, Dobson KS, Amiri M, Ghafari FH. Psychometric properties of the Brief Symptom Inventory in a sample of recovered Iranian depressed patients. Int J Clin Health Psychol 2010;10:541–51.
[26] Provencal N, Suderman MJ, Guillemin C, Massart R, Ruggiero A, Wang D, et al. The signature of maternal rearing in the methylome in rhesus macaque prefrontal cortex and T cells. J Neurosci 2012;32:15626–42.
[27] R., Development Core Team. R: a language and environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2011.
[28] Rotter A, Asemann R, Decker A, Kornhuber J, Biermann T. Orexin expression and promoter-methylation in peripheral blood of patients suffering from major depressive disorder. J Affect Disord 2011;131:186–92.
[29] Ruiperez M, Ibáñez MI, Lorente E, Moro M, Ortet G. Psychometric properties of the Spanish version of the BSI: contributions to the relationship between personality and psychopathology. Eur J Psychol Assess 2001;17:241.
[30] Sandoval J, Heyn H, Moran S, Serra-Musach J, Pujana MA, Bibikova M, et al. Validation of a DNA methylation microarray for 450,000 CpG sites in the human genome. Epigenetics 2011;6:692–702.
[31] Stukenberg KW, Dura JR, Kiecolt-Glaser JK. Depression screening scale validation in an elderly, community-dwelling population. Psychol Assess J Consult Clin Psychol 1990;2:134.
[32] Szyf M. DNA methylation, behavior and early life adversity. J Genet Genomics 2013;40:331–8.
[33] Szyf M, Bick J. DNA methylation: a mechanism for embedding early life experiences in the genome. Child Dev 2013;84:49–57.
[34] Tylee DS, Kawaguchi DM, Glatt SJ. On the outside, looking in: a review and evaluation of the comparability of blood and brain "-omes". Am J Med Genet B Neuropsychiatr Genet 2013;162B:595–603.
[35] Uher R, Weaver IC. Epigenetic traces of childhood maltreatment in peripheral blood: a new strategy to explore gene-environment interactions. Br J Psychiatry 2014;204:3–5.
[36] Varley KE, Gertz J, Bowling KM, Parker SL, Reddy TE, Pauli-Behn F, et al. methylation across diverse human cell lines and tissues. Genome Res 2013;23:555–67.
[37] Wheeler B. lmPerm: permutation tests for linear models; 2010.

Please cite this article in press as: Córdova-Palomera A, et al. Further evidence of *DEPDC7* DNA hypomethylation in depression: A study in adult twins. European Psychiatry (2015), http://dx.doi.org/10.1016/j.eurpsy.2015.04.001

Advisor's report on the contribution of the Ph.D. candidate to the article

Prof. Dr. Lourdes Fañanás Saura, associate professor at the Faculty of Biology, University of Barcelona, and supervisor of the present doctoral thesis by Aldo Córdova Palomera, hereby certifies that the participation of the Ph.D. candidate in the article "Further evidence of *DEPDC7* DNA hypomethylation in depression: a study in adult twins" included the following tasks:

- Participation in study design.
- Statistical analyses.
- Writing of the first manuscript draft.
- Critical revision of the article.

Prof. Dr. Lourdes Fañanás Saura

Barcelona, June 30th 2015.

Genome-wide methylation study on depression: differential methylation and variable methylation in monozygotic twins

Córdova-Palomera A, Fatjó-Vilas M, Gastó C, Navarro V, Krebs MO, Fañanás L

Translational Psychiatry (2015), 5, e557

DOI: http://dx.doi.org/10.1038/tp.2015.49

OPEN

Citation: Transl Psychiatry (2015) 5, e557; doi:10.1038/tp.2015.49

www.nature.com/tp

ORIGINAL ARTICLE

Genome-wide methylation study on depression: differential methylation and variable methylation in monozygotic twins

A Córdova-Palomera[1,2], M Fatjó-Vilas[1,2], C Gastó[2,3,4], V Navarro[2,3,4], M-O Krebs[5,6,7] and L Fañanás[1,2]

Depressive disorders have been shown to be highly influenced by environmental pathogenic factors, some of which are believed to exert stress on human brain functioning via epigenetic modifications. Previous genome-wide methylomic studies on depression have suggested that, along with differential DNA methylation, affected co-twins of monozygotic (MZ) pairs have increased DNA methylation variability, probably in line with theories of epigenetic stochasticity. Nevertheless, the potential biological roots of this variability remain largely unexplored. The current study aimed to evaluate whether DNA methylation differences within MZ twin pairs were related to differences in their psychopathological status. Data from the Illumina Infinium HumanMethylation450 Beadchip was used to evaluate peripheral blood DNA methylation of 34 twins (17 MZ pairs). Two analytical strategies were used to identify (a) differentially methylated probes (DMPs) and (b) variably methylated probes (VMPs). Most DMPs were located in genes previously related to neuropsychiatric phenotypes. Remarkably, one of these DMPs (cg01122889) was located in the *WDR26* gene, the DNA sequence of which has been implicated in major depressive disorder from genome-wide association studies. Expression of *WDR26* has also been proposed as a biomarker of depression in human blood. Complementarily, VMPs were located in genes such as *CACNA1C*, *IGF2* and the p38 MAP kinase *MAPK11*, showing enrichment for biological processes such as glucocorticoid signaling. These results expand on previous research to indicate that both differential methylation and differential variability have a role in the etiology and clinical manifestation of depression, and provide clues on specific genomic loci of potential interest in the epigenetics of depression.

Translational Psychiatry (2015) **5,** e557; doi:10.1038/tp.2015.49; published online 28 April 2015

INTRODUCTION

Depressive psychopathology has been shown to be highly influenced by environmental factors, some of which are believed to exert stress on human brain functioning via epigenetic modifications.[1–3] Accordingly, the search for precise molecular epigenetic signatures underlying this environmental impact is a current trend in the field.

Among several different epigenetic marks, DNA methylation is particularly interesting in this context, as previous evidence indicates that depressed individuals exhibit particular profiles of both methylation levels (that is, hyper- and hypomethylation at some loci) and methylation variance (that is, increased variance in affected subjects).[4–6] The number of publications relating DNA methylation to these conditions has been increasing in recent years; overall, they suggest an association, even when typically studying DNA samples from peripheral tissues of unrelated individuals.[6]

Notably, a substantial degree of the DNA methylation profile is determined by the underlying DNA sequence of the organism,[7,8] suggesting that some adjustment for inter-individual sequence differences is required when associating this epigenetic mark with other phenotypes. As pairs of monozygotic (MZ) twins have almost identical DNA sequences.[9,10] studies of their phenotypic discordance provide a valuable tool in epigenetic research.[11] Methylation profiles of members of a MZ twin pair may be very similar not only due to their DNA sequence resemblance, but also as a consequence of shared zygotic epigenetic features and a common (pre- and post-natal) environment, among other issues.[8,11] Hence, differences in their DNA methylation levels arise in response to unique environmental factors and stochastic influences.[12,13]

In this sense, a consistent finding in the literature is the increased variance of genome-wide DNA methylation profiles of affected co-twins in depression-discordant pairs.[4,5,14] As intrapair differences in MZ co-twins are related not only to environmental but also to stochastic epigenetic factors,[12,13] a feasible hypothesis is that mean DNA methylation level differences—measured as differentially methylated probes (DMPs)—could be linked to environmental factors related to the etiology and clinical manifestation of a disease; complementarily, the changes in methylation variance—measured as variably methylated probes (VMPs)—may be associated with stochasticity.

Although this idea of epigenetic stochasticity has been little explored in relation to psychiatric disorders, research mainly on cancer shows that stochastic epigenetic processes have a dear role in the difference between health and disease phenotypes.[15–19] Notably, some methodological tools based on second-moment statistics (that is, variance) of genome-wide DNA

[1]Unitat d´Antropologia, Departament de Biologia Animal, Facultat de Biologia and Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain; [2]Centro de Investigaciones Biomédicas en Red de Salud Mental (CIBERSAM), Instituto de Salud Carlos III, Madrid, Spain; [3]Instituto de Investigaciones Biomédicas August Pi i Sunyer (IDIBAPS), Barcelona, Spain; [4]Departamento de Psiquiatría and Instituto Clínico de Neurociencias (ICN), Hospital Clínico, Barcelona, Spain; [5]Université Paris Descartes, PRES Paris Sorbonne Cité, INSERM, Laboratoire de Physiopathologie des Maladies Psychiatriques, Centre de Psychiatrie et Neurosciences, Paris, France; [6]Hôpital Sainte-Anne, Service Hospitalo-Universitaire, Faculté de Médecine Paris Descartes, Paris, France and [7]GDR3557-Institut de Psychiatrie, Paris, France. Correspondence: Professor L Fañanás, Unitat d'Antropologia, Departament de Biologia Animal, Facultat de Biologia and Institut de Biomedicina (IBUB), Universitat de Barcelona, Avenida Diagonal, 645, Barcelona 08028, Spain.
E-mail: lfananas@ub.edu
Received 1 December 2014; revised 2 March 2015; accepted 9 March 2015

methylation profiles have recently been introduced to discriminate between affected and unaffected samples;[20,21] their biological significance largely relies on the effects of stochastic epigenetic factors. Broadly speaking, these tools have been developed in recognition that most genomic regions do not exhibit DNA methylation variability and, thus, small numbers of VMPs across the genome are typically identified in complex diseases.[20,21] Interestingly, it has recently been proposed that similar analytical approaches may be useful to study depressive psychopathology.[22]

To our knowledge, no previous study has indicated specific genomic loci at which methylation variability may have relevance for psychopathology. This is particularly important in light of the three previous reports showing increased genome-wide methylation variance in depressed MZ co-twins from discordant pairs.[4,5,14]

Accordingly, the present study aimed to identify epigenetic differences in depressive psychopathology using two distinct analytical strategies. First, a widely known genome-wide methylation approach that detects DMPs on the basis of both the statistical significance and the magnitude of DNA methylation differences was implemented. This approach has proven to be useful in DNA methylation studies in psychiatry.[5,23,24] Second, the genomic loci of the CpG probes exhibiting DNA methylation variance that could be associated with disease were obtained using an analytic methodology proposed herein; this approach is especially suited to identify VMPs in samples consisting of disease-concordant, discordant and healthy MZ twins. This method assumes that stochastic epigenetic variance among diagnostic-concordant pairs is related to etiological and symptomatic differences within pairs,[25,26] whereas epigenetic variability found only in diagnostic-discordant pairs would relate to a more homogeneous core of the disease.

MATERIALS AND METHODS

Sample description

Participants in this study were part of a larger ongoing twin sample (UB-Twin Registry) consisting of 242 Caucasian Spanish adult twins from the general population who gave permission to be contacted for research purposes. For that sample, the exclusion criteria included age under 18 and over 65 years, a medical history of neurological disturbance, presence of sensory or motor alterations and current substance misuse or dependence. Written informed consent was obtained from all participants after a detailed description of the study aims and design, as approved by the local Ethics Committee.

Medical records and a battery of psychological and neurocognitive tests were obtained in face-to-face interviews by trained psychologists. In addition, peripheral blood or saliva samples were obtained from all 242 participants. The zygosity of the pairs was determined by genotyping 16 highly polymorphic microsatellite loci from DNA samples (SSRs; PowerPlex 16 System; Promega, Madison, WI, USA). Identity on all the markers can be used to assign monozygosity with greater than 99% accuracy.[27]

A final group of 34 middle-aged participants (17 MZ twin pairs; age range 22–56 years, median age 38 years; 47% female) who were representative and informative for psychopathology, neurocognition and early stress factors was extracted from the above-described sample, to be investigated for brain function and genome-wide epigenetic signatures. Similar MZ twin sample sizes have previously been used in comparable literature reports.[5,14] Peripheral blood was available from all members of this group. All analyses described below refer to this 34-individual subset.

Clinical evaluation

A trained clinical psychologist applied the Structural Clinical Interview for DSM-IV Axis I Disorders (SCID-I)[28] in a face-to-face interview to screen for the presence of any lifetime depression (F32.x) or related anxiety spectrum disorder (F40.x and F41.x). This apparently broad category of outcomes was used in conjunction with evidence on the comorbidity, shared etiopathology and diagnostic criteria overlap between depressive and anxious disorders,[6,29–32] as well as taking into account evidences of some shared DNA methylation mechanisms in these diagnoses.[6]

Individuals meeting the diagnostic criteria for at least one lifetime diagnosis of (DSM-IV) anxious or depressive disorder were classified as affected by a stress-related disorder, and 'concordant', 'discordant' and 'healthy' statuses of twin pairs were defined accordingly. Specifically, there were seven healthy pairs, six discordant and four concordant pairs for lifetime DSM-IV diagnoses. In addition, on the day of blood sampling, current psychiatric symptoms were evaluated using the Brief Symptom Inventory (BSI).[33,34] The BSI is a self-administered 46-item screening instrument aimed at identifying the experience of psychopathological symptoms during the last 30 days. It is composed by six subscales (depression, phobic anxiety, paranoid ideation, obsession-compulsion, somatization and hostility) conceived for use in both clinical and non-clinical samples. Items are rated on a five-point scale of distress, according to self-perception of symptoms. Descriptive data from the current sample are summarized in Table 1.

Overall, there were four concordant, six discordant and seven healthy MZ twin pairs (Table 1). Briefly, in the eight diagnostic-concordant-participant subset (four pairs), there were four individuals with depression (F32.x) and four diagnoses of anxiety disorders (F40.x and F41.x); half of these diagnoses were experienced by the individuals at the moment of blood extraction, and the rest of the individuals had met diagnostic criteria for psychopathology some years before that date (estimated mean (s.d.) elapsed time since last remission: 13.8 (9.2) years; a right-tailed skewed distribution ranging from 2 to 28 years). Regarding the six affected co-twins from the diagnostic-discordant pairs, there were four depression (F32.x) and two anxiety (F41.0) diagnoses; one of them fulfilled diagnostic criteria at the moment of peripheral blood sampling, and the rest of them had met such criteria before. Importantly, despite the apparent clinical heterogeneity, there were statistically significant intergroup differences in the current psychopathological assessment (that is, last-month symptoms measured by the BSI), at the level of both general symptomatology (total BSI score: $P = 0.013$) and ongoing depression (depressive BSI subscale: $P = 0.04$). Namely, twins with no lifetime history of DSM-IV diagnosis had lower BSI scores—fewer self-reported symptoms—in both the depressive subscale and the whole questionnaire than diagnostic-discordant pairs, whereas the diagnostic-concordant twins had greater BSI scores than discordant and healthy twin groups. Likewise, a logistic regression model was performed to evaluate the relationship between (current) BSI depressive scores and categorical (DSM-IV) diagnoses in the 34-twin sample. After adjusting for the correlated nature of twin data (heteroskedasticity), higher (current depression) BSI scores predicted a greater risk of a clinical diagnosis ($\beta = 0.362$, $P = 0.013$, $R^2 = 0.295$). Similarly, in the six diagnostic-discordant pairs, the DSM-IV-affected co-twins had higher BSI

Table 1. Demographic, psychopathological and neurocognitive data for DSM-IV diagnostic concordant, discordant and healthy MZ twin pairs

	Concordant (8 subjects, 8 females)		*Discordant (12 subjects, 4 females)*		*Healthy (14 subjects, 4 females)*		*Group comparison*
	Mean (s.d.)	*Range*	*Mean (s.d.)*	*Range*	*Mean (s.d.)*	*Range*	X^2;[a] P
Age	42.5 (13)	22–54	37 (10.9)	20–50	30.3 (7.3)	19–39	5.9; 0.052
IQ	105.1 (12.5)	87–127	108.1 (11.8)	87–131	110.5 (5.5)	103–118	1.9; 0.393
Current psychopathology (total BSI)	27.9 (16.5)	6–57	20.9 (13.3)	4–45	10.6 (9.3)	1–33	8.7; 0.013[b]
Current depressive symptoms (BSI subscale)	6.9 (6.5)	1–20	3.5 (2.7)	0–9	1.7 (1.8)	0–6	6.4; 0.04[b]

Abbreviations: BSI, Brief Symptom Inventory; IQ, intellectual quotient; MZ, monozygotic. [a]Kruskal–Wallis X^2, as these variables were continuous. [b]Statistically significant *P*-value.

scores than their healthy co-twins (mean/median score in affected: 4.2/4.5; mean/median score in healthy: 2.8/2.5); however, due to some properties of this small data set, the nonparametric Wilcoxon–Mann–Whitney test could not estimate exact *P*-values due to ties and zeroes.

All participants were asked to report if they had received pharmacological or psychological treatment or had consulted a psychiatrist or psychologist since they first participated in the study. Only one of the 34 participants had lifetime exposure to drug treatment for anxiety or depression by the time of this study.

Methylation data

The Illumina Infinium HumanMethylation450 (450 K) BeadChip[35,36] was employed with peripheral blood DNA samples for all 34 participants. Specifically, by genotyping sodium bisulfite-treated DNA, DNA methylation is assayed by this platform at >450,000 CpG sites across the genome at single-base resolution; next, bisulfite-converted DNA undergoes whole-genome amplification, before being fragmented and hybridized to microarray probes. The DNA methylation fraction of each CpG site is estimated as $\beta = M/(M+U+\alpha)$; M and U stand for methylated and unmethylated fluorescence intensities, and α is an arbitrary offset applied to stabilize β-values with low intensities.

Statistical analyses

DMPs. In order to find DMPs, a previously described analytical approach[5,23,24] was conducted using, initially, data from the six depression-discordant twin pairs. This method aims to rank all CpG probes in the array. The highest-ranked probes are those with a combination of low *P*-value and high mean difference ($\Delta\beta$). Briefly, the first step consists in conducting a paired *t*-test for every CpG probe in the array; a score is assigned to every probe depending on its *P*-value: the lower the *P*-value, the higher the score. Afterward, the absolute mean intrapair difference is estimated for each CpG probe, and a second score is assigned to every probe: the larger the methylation difference (absolute $\Delta\beta$), the higher the score. The two scores are combined (that is, added) for every probe, and all probes are ranked from high to low scoring. Namely, probes with both a low *P*-value and a relatively large methylation difference are in the top of the rank. From this general rank for the >450,000 probes, a list of the top 10 probes (that is, those with the best arrangements of low *P*-value and high $\Delta\beta$) was extracted.

To further validate these CpG sites, an additional step was undertaken using the information from diagnostic-concordant and healthy pairs. The mean absolute differences ($|\Delta\beta|$) in DNA methylation were retrieved for the three groups to test the null hypotheses that these top 10 CpG probes found in the discordant co-twins that have also large methylation differences in either concordant and healthy pairs. This additional test allowed assessing whether the top 10 DMP probes often display methylation differences within MZ pairs, regardless of their phenotypic statuses. Large intrapair methylation differences across all pairs would indicate that a given CpG site may be environmentally sensitive, but not linked to the etiopathology of depression. Thus, by performing Wilcoxon–Mann–Whitney tests, it was evaluated whether DNA methylation differences within discordant pairs are larger than those found in either concordant or healthy pairs ($|\Delta\beta_{discordant}| > |\Delta\beta_{concordant}|$ and $|\Delta\beta_{discordant}| > |\Delta\beta_{healthy}|$). Additional information about this procedure can be found in Supplementary Table 1.

VMPs. A data-driven analytical approach using information from all concordant, discordant and healthy MZ twins was used. Initially, absolute intrapair differences in DNA methylation levels were computed for all >450,000 CpG sites across the genome for all 17 MZ twin pairs. From this information, the median value of absolute DNA methylation (intrapair) differences is computed for each diagnostic group (concordant, discordant and healthy) at each of the >450,000 probes. These median values are used as centrality measures since they are more robust to outliers than conventional arithmetic means.

After this step, an $m \times 3$ matrix is retrieved, where m stands for the number of CpG sites considered (>450,000) and 3 is the number of diagnostic groups (here, concordant, discordant and healthy). Each cell contains the median value of the intrapair absolute methylation difference observed for a given diagnostic group at a specific CpG site.

Note that information introduced to the previous matrix does not contain any clue about direction of the differences. Some assumptions are used to further interpret this information: (i) probes with large intrapair methylation differences across only discordant co-twins—that is, with no intrapair differences in concordant and healthy twins—could have arisen from stochastic factors altering the methylation level of the affected co-twins' (in discordant pairs); due to this stochasticity, the affected co-twin may have transitioned from the normal methylation level of his/her healthy co-twin to either hyper- or hypomethylation. (ii) CpG sites with large methylation differences only in diagnostic-concordant MZ pairs should be linked to symptom heterogeneity of a pair[26,37] and (iii) probes with large intrapair differences only in healthy pairs should be relatively less frequent—methylation stochasticity is typically associated with disease—[15–18] and may index processes that are either not related to the specific physiopathological conditions studied here, or normally activated in health (but dynamically constrained in disease).

The next step consists in determining a methylation difference threshold above which the observed variability for each diagnostic group and at each CpG site (the median value of the absolute intrapair differences) can be considered large. As previous reports indicate that methylation differences above 10% in Illumina assays have biological significance and have a low probability of being technical artifacts,[38–40] a CpG site was considered 'variable' if above this $\Delta\beta \geq |0.1|$ threshold. Of note, as shown in the Results section, CpG probes with an intrapair $\Delta\beta \geq |0.1|$ are highly infrequent in this data set, suggesting this may be a proper threshold.

Then, after the previous step, all >450,000 CpG sites are examined to determine which of them show variability only within concordant, discordant or healthy pairs; they are later examined via pathway analysis to evaluate the potential biological relevance of their stochastic disruptions.

Pathway analysis. Relevant sets of genes from whole-genome analyses indicated in the Results section were uploaded to Cytoscape (version 3.0.2)[41], using Reactome FI Cytoscape Plugin 4,[42] network version 2013, to obtain data on underlying reactions, pathways and biological processes.

All the outcome assessment steps (that is, statistical analyses) described in this section were conducted by an investigator (AC-P) who did not participate in sample collection, clinical evaluation, zygosity determination or DNA methylation measurement and data pre-processing.

RESULTS

DMPs

With data from the six diagnostic-discordant MZ pairs, an analytic approach previously reported in Psychiatric Epigenetics studies[5,23,24] was used to identify the top 10 CpG sites with the largest methylation differences and smallest *P*-values. Table 2 contains information on these 10 CpG sites across the genome. $\Delta\beta$- and *P*-values were in the range of those reported previously in similar study designs.[5] Remarkably, as shown in Table 2, most probes were located in genes previously reported in neuropsychiatric studies.

Of particular interest in this 10-site list are cg11433980, cg17798944, cg00567749 and cg01122889. They are respectively located in *CBR3* (carbonyl reductase 3), *RPL3* (ribosomal protein L3), *VCAN* (versican) and *WDR26* (WD repeat domain 26). These genes have previously been associated with depressive phenotypes.[43–46] Further information on this finding can be found in the Discussion.

As indicated by the superindices of the probe names (first column of Table 2), neither concordant nor healthy twin pairs displayed intrapair differences in four out of the initial 10-site list: cg06493080 (*HOXB7*), cg18974921, cg14747903 (*LSR*) and cg01122889 (*WDR26*). This indicates greater robustness of the findings for these four CpG sites than for the other six probes. Namely, in this twin sample, these four CpG sites appeared to be modulated by the environment only in depression-discordant MZ pairs; co-twins from neither concordant nor healthy pairs differed in their methylation levels.

VMPs

As a whole, DNA methylation profiles were highly correlated across twin pairs, regardless of the diagnostic status of co-twins. A

Table 2. Top-ranked differentially methylated probes (DMPs) in six adult MZ twin pairs discordant for depression, and potential neuropsychiatric relevance of their associated genes

Probe name (target ID)[a]	P-*value*	*Δβ*	*Coordinates (hg19)*	*Gene name (UCSC)*	*Gene region feature category (UCSC)*	*Potential relevance of the gene in neuropsychiatric disorders*
cg06493080[b,c]	0.000574	−0.085	Chr17: 46688310	HOXB7; HOXB7	5′UTR; 1st exon	Target of *FOXP2,* a gene linked to neurodevelopment.[82] *HOXB7* may diminish cancer tumor risk in schizophrenia.[83]
cg00567749[b]	0.000818	−0.105	Chr5: 82767908	VCAN; VCAN; VCAN; VCAN	5′UTR; 5′UTR; 5′UTR; 5′UTR	Prospective blood transcriptomic marker for depression.[43] Axonal growth.[84–86] Potential genetic overlap between herpes simplex and depression.[87] Altered expression in the olfactory epithelium in schizophrenia.[88]
cg18974921[b,c]	0.000565	−0.076	Chr11: 78131895	—	—	—
cg14747903[b,c]	0.000571	−0.072	Chr19: 35740509	LSR; LSR; LSR	Body; body; body	Probable role in prosopagnosia and visual agnosia.[d]
cg15696634	0.000682	−0.072	Chr18: 19746953	—	—	—
cg11433980[b]	0.001425	−0.075	Chr21: 37510727	CBR3	Body	Altered hippocampal gene expression by antidepressant treatment in adult rats.[44]
cg01122889[b,c]	0.000806	−0.071	Chr1: 224620779	WDR26; WDR26	Body; body	GWAS hit in major depressive disorder (meta-analysis of three independent samples).[45] Prospective blood transcriptomic marker for depression.[43]
cg10550693[b]	0.001554	−0.073	Chr11: 64902189	SYVN1; SYVN1	TSS200; TSS200	Potential role in autism.[89,90]
cg23004466[b]	0.000854	−0.071	Chr7: 106815478	HBP1	5′UTR	Epigenetics of neurodegeneration and Alzheimer's disease.[91–94]
cg17798944[b]	0.001679	−0.072	Chr22: 39715225	SNORD43; RPL3; RPL3	TSS200; body; body	Hypothalamic–pituitary–adrenal regulation of stress response.[46] Potential role in the glycobiology of schizophrenia.[95]

Abbreviations: 1st exon, first exon; MZ, monozygotic; TSS, transcription start site; TSS200, within 200 bp of a TSS; 5′UTR, 5′ untranslated region. Target ID, Illumina identifier; body, within gene body; *Δβ*, mean methylation fraction difference in discordant pairs (co-twin with depression minus co-twin without depression). [a]Superindices next to each probe name indicate whether or not there were (absolute) methylation differences in concordant and healthy pairs. [b]Absolute intrapair differences in the four diagnostic-concordant MZ pairs were significantly smaller than in the discordant pairs. [c]Absolute intrapair differences in the seven healthy MZ pairs were significantly smaller than in the discordant pairs. [d]Information for the *LSR* gene was extracted from http://www.genecards.org/cgi-bin/carddisp.pl?gene = LSR.

detailed description of these correlations can be found in Supplementary Table 2.

Briefly, based on previous reports,[38–40] a median absolute intrapair difference $\geq$10% within each twin group (concordant, discordant or healthy) was chosen in this study as a threshold to determine which CpG probes could be considered variable. Only 1.7% of the whole data set of absolute intrapair differences (17 intrapair differences at 485,512 CpG sites: a matrix with 8,055,688 cells) showed values equal to or larger than 10% of the total methylation fraction. In recognition that large intrapair differences are likely to have major biological relevance, the next step consisted of the identification of probes with variability only between each twin set (concordant, discordant or healthy) (Figure 1).

Each of the three diagnostic groups (concordant, discordant and healthy controls) had a specific set of CpG probes with large intrapair differences in methylation fraction; specifically, healthy pairs showed variability at 85 CpG sites that were not variable in the other groups; similarly, discordant and concordant pairs had, respectively, 175 and 221 variable probes that did not show variability in the other diagnostic groups of twins (Figure 1). Of note, healthy co-twins had the least variable genome-wide DNA methylation profile, seemingly in agreement with findings of increased variability in disease.[4,5,14] The distribution of gene region feature categories (UCSC) of each of the three sets of CpG sites was very similar for all three groups (concordant, discordant and healthy). Identifiers and additional data for these individual CpG sites are available in Supplementary Table 3. Owing to the design of the DNA methylation array employed here, even genes with the largest numbers of CpG probes would not be likely to appear by randomly sampling 85, 175 or 221 probes. Further information and discussion about these sampling probabilities can be found in Supplementary Table 4.

Interestingly, pathway analysis using the lists of genes with highly variable probes (from variable CpG sites exclusive of concordant, discordant or healthy twin pairs) generated relatively large functional interaction networks (>10 interacting proteins) only in discordant and concordant pairs (Figure 2). Accordingly, gene lists from the concordant and discordant groups showed enrichment for several processes, some of which are of relevance in neuropsychiatry (Figure 2 and Table 3). For instance, processes such as 'rapid glucocorticoid signaling', 'dopaminergic synapse' and 'interleukin-2-mediated signaling events' were enriched in the VMPs of discordant pairs, whereas 'nervous system development' was enriched in both concordant and discordant groups. In contrast, the epigenetic variability of the healthy pairs was enriched only for 'HIF-1-alpha transcription factor network' (Table 3). A detailed list of the VMPs and the full gene lists from which these networks were generated can be found in Supplementary Table 3.

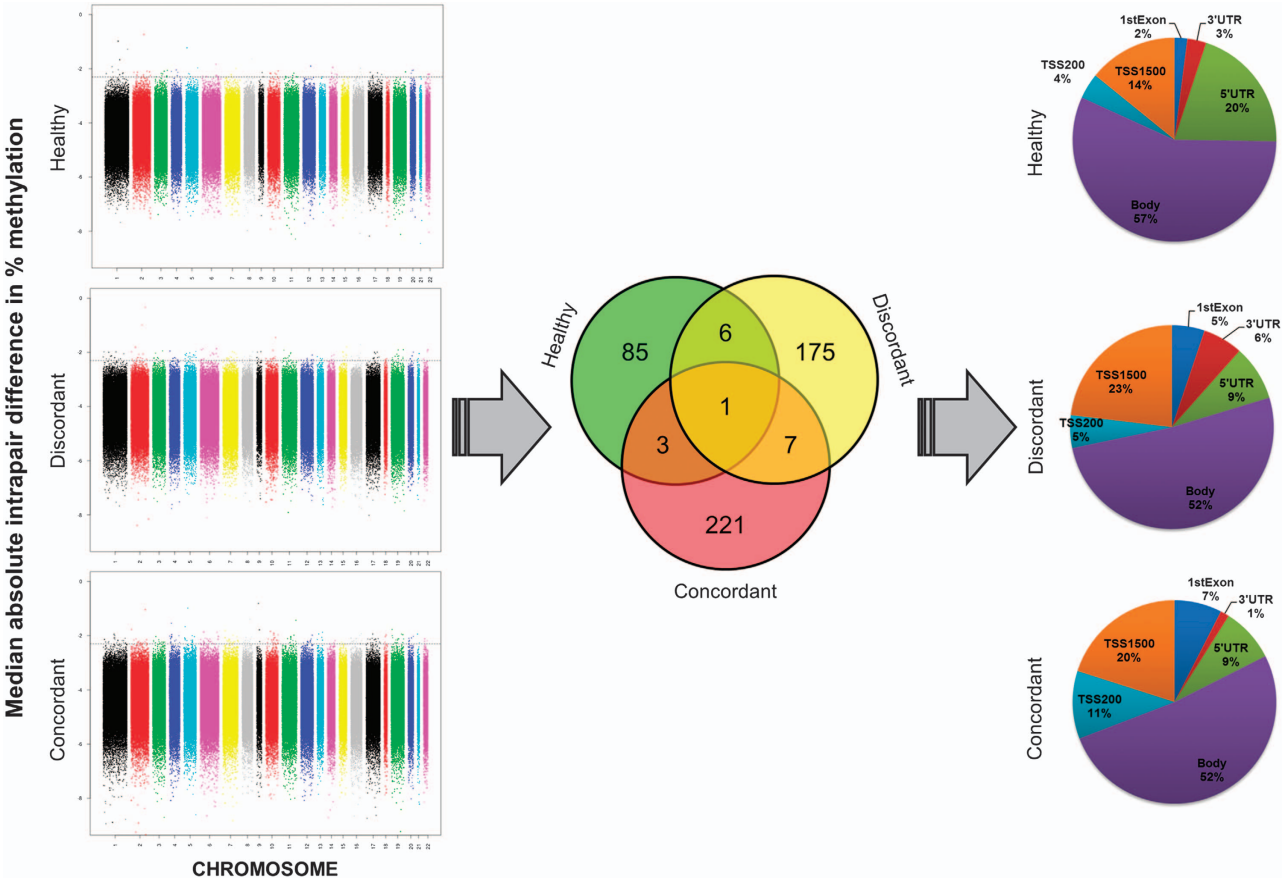


Figure 1. Selection of highly variable CpG sites across DSM-IV diagnostic groups based on large intrapair differences in whole-genome percentage methylation. Left**:** after estimating absolute intrapair differences for each CpG region in every twin pair, median values of these differences were calculated across diagnostic groups and are plotted in a logarithmic scale, according to genomic location. Individual CpGs (dots) above the 10% threshold (dashed line, at log(0.1) = − 2.3) methylation differences were identified and separated for the next step. Centre**:** Venn diagram showing intersections and disjunctions of the highly variable CpG probes obtained before. Right**:** gene region feature categories (UCSC) of the CpG sites showing large intrapair differences only in each of the diagnostic groups. Inset numbers represent percentage of CpG sites. 1st exon, first exon; 3′UTR, 3′ untranslated region; 5′UTR, 5′ untranslated region; TSS, transcription start site; TSS1500, within 1.5 kb of a TSS; TSS200, within 200 bps of a TSS.

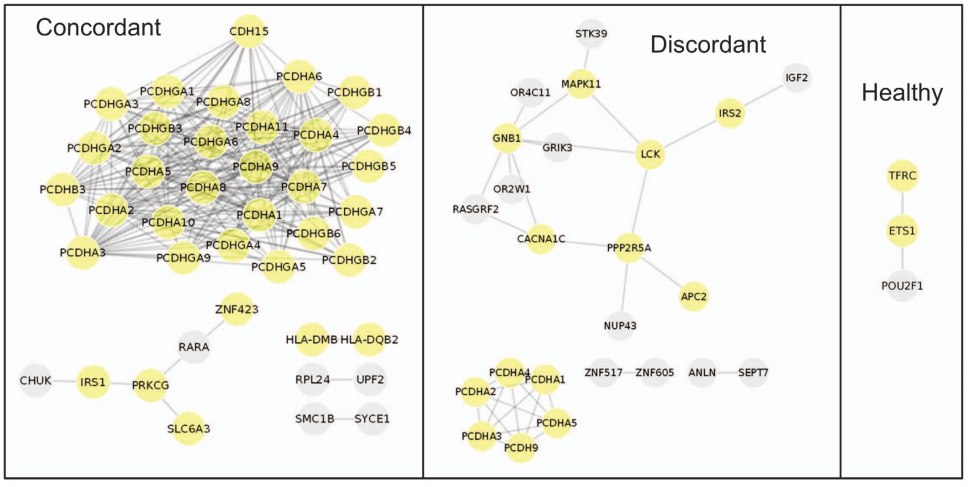


Figure 2. Molecular interaction networks from the lists of genes showing large intrapair differences between each of the diagnostic groups. Left: concordant pairs; center: discordant pairs; right: healthy pairs. Proteins enriched for biological pathways (Table 3) are highlighted in the network diagrams.

Table 3. Results of pathway analysis

Gene set	P-*value*	*FDR*	*Nodes*
Concordant			
Wnt signaling pathway	0	< 5.000e − 04	PCDH family, PRKCG, CDH15
Cadherin signaling pathway	0	< 5.000e − 04	PCDH family, CDH15
Integral to membrane	0	< 1.000e − 03	HLA-DQB2, SLC6A3, HLA-DMB, PCDH family, CDH15
Integral to plasma membrane	0	0.0005	PCDH family, SLC6A3
Plasma membrane	0.0007	0.03967	PCDH family, IRS1, CDH15
Homophilic cell adhesion	0	< 1.000e − 03	PCDH family, CDH15
Nervous system development	0	< 5.000e − 04	PCDH family, ZNF423
Cell adhesion	0	< 3.333e − 04	PCDH family, CDH15
Calcium ion binding	0	< 1.000e − 03	PCDH family, CDH15
Discordant			
Wnt signaling pathway	0	< 1.000e − 03	APC2, PCDH family, PPP2R5A, GNB1
Cadherin signaling pathway	0	< 5.000e − 04	PCDH family
Rapid glucocorticoid signaling	0.0001	0.01167	MAPK11, GNB1
Dopaminergic synapse	0.0002	0.0115	PPP2R5A, MAPK11, GNB1, CACNA1C
IL-2-mediated signaling events	0.0002	0.009833	IRS2, MAPK11, LCK
Thromboxane A2 receptor signaling	0.0002	0.009833	MAPK11, GNB1, LCK
Beta1 adrenergic receptor signaling pathway	0.0017	0.04944	GNB1, CACNA1C
Homophilic cell adhesion	0	< 1.000e −03	PCDH family
Nervous system development	0.0001	0.0475	PCDH family
Healthy			
HIF-1-alpha transcription factor network (N)	0.0067	0.019	TFRC, ETS1

Abbreviations: FDR, false discovery rate; IL-2, interleukin-2; PCDH, protocadherin. Gene lists from loci of CpG sites with large intrapair differences in each set of twins (concordant, discordant and healthy) were enriched for the processes shown. For the underlying gene networks, see Figure 1. The full gene lists uploaded for pathway analysis can be found in Supplementary Table 3.

DISCUSSION

This work evaluated the potential relationship between DNA methylation levels and depressive psychopathology using genome-wide data. Two distinct approaches, each of which has a particular biological meaning, were used: (i) differential methylation (DMPs) and (ii) variable methylation (VMPs). The current design, including healthy, concordant and discordant MZ twin pairs, allowed us to search for potential environmentally induced and pathology-specific DNA methylation differences. Previous reports had shown increased variability in the DNA methylation profiles of affected MZ co-twins from depression-discordant pairs.[4,14,23] However, to the best of our knowledge, this is the first study aimed at unraveling specific genomic loci at which methylation variability may be a marker of depression.

DMPs

One of the most relevant outcomes of the current study is the association between hypomethylation of cg01122889 in *WDR26* and a lifetime diagnosis of depression. Notably, neither healthy nor diagnostic-concordant MZ pairs exhibited intrapair differences in DNA methylation of this CpG site, indicating that it could be a marker of environmental influences leading to depression. Remarkably, one of the largest meta-analytic studies conducted to date of major depressive disorder (MDD) genome-wide association study data suggests a role for *WDR26*'s rs11579964 single-nucleotide polymorphism—about 80 kbp from cg01122889—in the causality of depression.[45] Of note, a mega-analysis of MDD genome-wide association study data also reported this and other single-nucleotide polymorphisms close to *WDR26* (rs2088619: Chr1:222825183) as probably predisposing for MDD, although confirmation is needed.[47] In addition, there is evidence suggesting an association between decreased blood transcript levels of *WDR26* and depression-related phenotypes.[43] Although using DNA methylation data to derive conclusions about gene expression could be somehow speculative, hypomethylation of gene bodies may be related to lower gene expression.[48,49] Accordingly, as cg01122889 is located in *WDR26*'s gene body, its hypomethylation in depressed individuals may be associated with lower gene expression levels, consistent with the findings of Pajer *et al.*[43] Nevertheless, confirmation is needed, as there is evidence of distinct relationships between *WDR26*'s body DNA methylation and expression (that is, hypomethylation can be associated with either lower or greater gene expression) across a number of distinct healthy and pathological tissues.[50]

Other DMPs found herein were located in genes previously related to depressive phenotypes, such as *CBR3, RPL3* and *VCAN*.[43,44,46] For instance, hippocampal upregulation of CBR3 enzymes has been found after antidepressant treatment in adult rats.[44] As with *WDR26*, the current association between hypomethylation of the *CBR3*'s gene body in adult depressed individuals may be related to lower expression of CBR3, which may be compensated by antidepressant treatment. A similar argument could be proposed for *VCAN*: the hypomethylation in depressed individuals of the present sample somehow resembles the decreased gene expression in an animal model of depression proposed by Pajer *et al.*[43] Note that current results for both *VCAN* and *WDR26* are consistent with those proposed by Pajer *et al.*[43] Likewise, Lee *et al.*[46] reported downregulation of *RPL3* hippocampal gene expression in an animal model of stress, in agreement with the current DNA methylation finding.

The above results were obtained by following a sound methodological procedure.[4,5,23] However, it is worth noting that, along with *WDR26*'s cg01122889, methylation changes in cg06493080 (*HOXB7*), cg18974921 and cg14747903 (*LSR*) seem to be cross-validated by comparison of the diagnostic-discordant group of twins with concordant and healthy pairs. This follows from the observation that diagnostic-concordant and healthy co-twins have very similar methylation levels at these four sites. Namely, probably only through a differential environmental influence (which would have taken place in affected co-twins from discordant pairs) may a co-twin differ from his/her healthy counterpart. cg06493080 is located in *HOXB7*, a target of the neurodevelopmental gene *FOXP2*. A different functional role has

been described for *LSR*, which may predispose to neurocognitive disorders.

VMPs

As MZ twins are very similar at the epigenome-wide level,[8] they offer an opportunity to search for markers of stochasticity. Accordingly, an analytic approach was undertaken to determine whether epigenetic instability—as indexed by large and non-systematic DNA methylation differences—within MZ pairs could be related to depressive psychopathology.

In this sample, healthy co-twins had less variable probes than concordant and discordant pairs. It was found that highly variable probes in depression-discordant MZ pairs were located in genes within enriched biological pathways and previously associated with depression, such as *CACNA1C*, *IGF2* and *MAPK11* (Figure 2 and Table 3; additional information in Supplementary Table 3).

In this respect, DNA sequence variation of *CACNA1C* has been widely recognized as a susceptibility factor for depressive psychopathology,[45] and methylation changes of *CACNA1C* have likewise been associated with early-life stress,[51,52] a risk factor for depressive disorders.[53] Similarly, depressive behavior is likely modulated by *IGF2*.[54,55] Also, MAPK11 is one of the four p38 mitogen-activated protein kinases, the activity of which has been linked to depression and related phenotypes.[56,57]

It is also worth mentioning some findings from the VMP analysis in diagnostic-concordant twin pairs, as one could speculate that stochastic variability within these MZ twins may correlate with etiological and symptomatic heterogeneity. Namely, even though depression-concordant MZ pairs show considerable symptom similarity,[25] previous research has shown an important role for unique environmental differences in fostering psychopathological heterogeneity within these MZ twin pairs.[58,59] Thus, it is also worth noting that genes with large DNA methylation intrapair differences across diagnostic-concordant pairs were enriched for 'nervous system development', by the interaction of protocadherin members and ZNF423 (Table 3 and Figure 2). Protocadherin encodes a family of proteins that are expressed in neurons and are relevant in synaptic functions, and whose DNA methylation may be altered in response to early-life stress;[60–63] the *ZNF423* gene has been associated with the cerebellum-related Joubert syndrome.[64] Also, the dopamine transporter gene *SLC6A3*, whose DNA sequence and methylation levels have been found to correlate with depressive psychopathology,[65–67] was within the enriched pathways (Table 3).

The current findings, which are indicative of stochastic variation in depression, are particularly important in view of recent publications from Feinberg *et al*.[68,69] They postulated the existence of variably methylated regions as a mechanism to explain developmentally regulated epigenetic plasticity; they have likewise provided evidence of stochastic DNA methylation variation across a population, even when analyzing samples from the same tissue and the same DNA sequence (that is, isogenic mice). These findings in genetically identical individuals (MZ twins) are in line with their reports, as they also described the abundance of variably methylated regions at loci important for neural and immune system development.

Importantly, a novel data-driven strategy was employed here to detect VMPs. Although other statistically sound tools to compare methylation variance between healthy and affected population samples have recently been introduced;[20,70] the present strategy is particularly suited for samples consisting of concordant, discordant and healthy MZ twin pairs. It is worth mentioning that the potential biological significance of the VMPs detected here—as suggested by the pathway analysis—confirms the feasibility of this analytical approach to provide new insights about biological mechanisms underlying some pathologies.

Overlap in findings from DMPs and VMPs

The fact that no top DMP was found within a gene exhibiting variable methylation is consistent with the notion that different epigenetic mechanisms influence DMPs and VMPs.[68,69] However, some interplay may exist between these forms of epigenetic regulation. For instance, it is worth noting that WDR26 (encoded by a gene containing a DMP) suppresses the MAPK signaling pathway,[71] and that a VMP was found in *MAPK11*. As a member of the p38 family, MAPK11 may participate in the stress response,[72] and was found here to be enriched within pathways such as 'glucocorticoid signaling' and 'interleukin-2-mediated signaling', both of which are likely related to depressive psychopathology. Importantly, this suggestion is derived from data from diagnostic-discordant pairs.

Hence, the current findings may be indicative of the interplay between different epigenetic regulation mechanisms, and suggest that the combination of distinct differentially and variably methylated loci may have an important role in the biological signatures of depression.

Limitations of the study

Three main limitations of this exploratory study deserve consideration. Namely, the generalizability of the present findings may be limited by (i) the sample size employed, (ii) the clinical heterogeneity of the individuals and (iii) the lack of confirmatory DNA methylation analysis using bisulfite pyrosequencing.

These limitations should be understood in the wider context of the current study design. First, the sample size is small. Although replication using larger independent samples is definitely required, it is worth noting that the current findings are consistent with previous literature on the biological mechanisms underlying depression, probably suggesting the presence of strong effect sizes for the above mentioned relationships between methylation and depression (Table 2 and Table 3). Likewise, the use of three different groups of twin pairs (concordant, discordant and healthy) allowed contrasting some putative epigenetic signatures of environmental stress in diagnostic-discordant co-twins with corresponding measurements in concordant and healthy pairs, which has rarely been done in former studies. As shown in Table 2 and Table 3, the comparison of epigenetic signatures in discordant twins with the methylation profiles of healthy and concordant pairs added robustness to some findings on both DMPs and VMPs.

Another potential limitation of this work is the phenotypical (that is, clinical) diversity of the participants. Although comparing the present results with independent data sets from individuals with a narrower phenotypic distribution (that is, a more clinically homogeneous population) would definitely be useful, two features of the present sample should be noted. First, although there were some individuals with predominantly anxious psychopathology, Table 1 shows that, as a group, concordant co-twins have greater scores in depressive symptomatology during the last month than discordant pairs, and that discordant pairs had more (current) depressive psychopathology than healthy controls. Thus, in contrast to the apparent diagnostic heterogeneity of the participants, the sample has an acceptable distribution with regard to current depression. It is important mentioning that this fact implies that the associations found here can probably be interpreted only in relation to depressive symptoms but not as linked to early etiological mechanisms.

Similarly, as noticed in the Clinical evaluation subsection, it is worth observing that previous research on the epidemiological features and the epigenetic signatures of internalizing disorders (that is, depression and anxiety) indicate important overlaps in etiopathology, diagnostic criteria and DNA methylation profiles across these diagnoses.[6,29–32] Of note, these overlaps may have allowed detecting epigenetically altered genes and pathways with biological relevance for depression.

An additional limitation of this work is the lack of confirmatory DNA methylation analysis by techniques such as bisulfite pyrosequencing. However, it should be noted that previous technical research has pointed out that the sensitivity of Illumina DNA methylation microarrays increases with β-value differences.[73] Accordingly, it has been suggested that, on average, values of $\Delta\beta \geq 0.136$ can be detected with 95% confidence. Although the methylation assessment conducted here would definitely be more robust if confirmed by bisulfite pyrosequencing, having used methodologies focused on relatively large methylation differences —for both DMPs and VMPs—adds some soundness to the findings.

Additional implications and future directions

Some points raised by this study deserve further discussion. First, it is important recalling that a common assumption of several psychiatric epigenetic studies is that DNA methylation of blood and brain may correlate and explain variability in psychopathology.[74] Although the work from several independent laboratories has demonstrated broad cross-tissue differences in methylation profiles,[75–78] there is also evidence of a correlation between DNA methylation levels across peripheral lymphocytes and a number of brain regions in response to environmental events.[79–81] Likewise, similar cross-tissue DNA methylation profiles —at specific genomic loci—have been found in independent samples of individuals with psychopathology.[5]

To the best of our knowledge, this is the fourth study using an epigenome-wide approach in MZ twins to evaluate the relationship between environmentally induced DNA methylation changes and depressive psychopathology. Regarding VMPs, the former three reports[4,5,14] consistently found increased DNA methylation variance in the affected co-twins of discordant pairs. Although they evaluated the overall statistical variance of the methylomic profiles, the present study expands on this topic by assessing which specific genomic loci could underlie this statistical feature. Of note, the data-driven analytical approach developed here to detect VMPs is specifically suited for samples including diagnostic-concordant and healthy pairs, as it is based on contrasting their epigenetic variability with that of discordant co-twins. It is important noting that, due to the novelty of the topic, some statistical protocols to perform genome-wide VMP assessment have just recently been introduced in cancer research[20,21,70] and thus may need additional adjustments for psychiatric phenotypes. This is particularly important in light of recent findings highlighting specific statistical properties of the genome-wide DNA methylation profiles in depression,[22] which may certainly differ from cancer data sets. Overall, the current biologically plausible findings suggest that the adopted strategy may have statistical and conceptual feasibility.

As regards to DMPs, the above mentioned previous reports have suggested that methylation of different genomic loci may be associated with depression. Namely, there is no unanimous agreement between these studies, probably due to the different clinical and demographic composition of the samples, as well as to the DNA methylation assessment techniques used. For instance, Byrne *et al.*[14] analyzed *P*-values obtained from the Infinium HumanMethylation450 Beadchip; in contrast, the assessment of potentially DMPs was based on another statistical approach in this and two other studies.[4,5] Likewise, inter-study differences between the present work and the report by Dempster *et al.*[5] may have arisen from clinical and demographic sample differences, as well as from the use of distinct biological tissues (they analyzed saliva DNA samples from severely depressed adolescents). Also, the *ZBTB20* gene-coding region reported by Davies *et al.*[4] (Chr3:114618751-114619251) is not evaluated by the Illumina assay employed here, and there may also be a lack of statistical power in the present sample.

From the above mentioned observations, some suggestions for further research of DNA methylation in depression can be derived: (i) cross-sample validations in larger MZ twin samples is needed; (ii) selecting the best informative peripheral tissue (probably either blood or saliva) may allow clearer findings; and (iii) finding proper statistical protocols for epigenetic analyses in depression, especially for the examination of VMPs, could likewise allow research advances (despite relatively small samples, the available literature suggests a role for methylation variability in depression).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Supported by the Spanish SAF2008-05674-C03-01, European Twins Study Network on Schizophrenia Research Training Network (grant number EUTwinsS; MRTN-CT-2006-035987), the Catalan 2014SGR1636 and the Ministry of Science and Innovation (PIM2010ERN-00642) in frame of ERA-NET NEURON. AC-P was funded by CONACyT (Mexico). We are indebted to X Goldberg and S Alemany for their collaboration in sample collection, and to J Ignacio Martín-Subero for his critical reading of the manuscript. We are indebted to the Genotype service at Centro Nacional de Investigaciones Oncologicas (CNIO Madrid, Spain) for performing array hybridization and providing technical support.

REFERENCES

1 Kendler KS, Myers JM, Maes HH, Keyes CL. The relationship between the genetic and environmental influences on common internalizing psychiatric disorders and mental well-being. *Behav Genet* 2011; **41**: 641–650.
2 McEwen BS. Brain on stress: how the social environment gets under the skin. *Proc Natl Acad Sci USA* 2012; **109**: 17180–17185.
3 Nugent NR, Tyrka AR, Carpenter LL, Price LH. Gene-environment interactions: early life stress and risk for depressive and anxiety disorders. *Psychopharmacology (Berl)* 2011; **214**: 175–196.
4 Davies MN, Krause L, Bell JT, Gao F, Ward KJ, Wu H *et al.* Hypermethylation in the ZBTB20 gene is associated with major depressive disorder. *Genome Biol* 2014; **15**: R56.
5 Dempster EL, Wong CC, Lester KJ, Burrage J, Gregory AM, Mill J *et al.* Genome-wide methylomic analysis of monozygotic twins discordant for adolescent depression. *Biol Psychiatry* 2014; **76**: 977–983.
6 Klengel T, Pape J, Binder EB, Mehta D. The role of DNA methylation in stress-related psychiatric disorders. *Neuropharmacology* 2014; **80**: 115–132.
7 Gertz J, Varley KE, Reddy TE, Bowling KM, Pauli F, Parker SL *et al.* Analysis of DNA methylation in a three-generation family reveals widespread genetic influence on epigenetic regulation. *PLoS Genet* 2011; **7**: e1002228.
8 Kaminsky ZA, Tang T, Wang SC, Ptak C, Oh GH, Wong AH *et al.* DNA methylation profiles in monozygotic and dizygotic twins. *Nat Genet* 2009; **41**: 240–245.
9 Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. *Nat Rev Genet* 2002; **3**: 872–882.
10 van Dongen J, Slagboom PE, Draisma HH, Martin NG, Boomsma DI. The continuing value of twin studies in the omics era. *Nat Rev Genet* 2012; **13**: 640–653.
11 Saffery R, Morley R, Foley DL. The utility of twins for epigenetic analysis. In: *Epigenetic Epidemiology*. Springer Netherlands: The Netherlands, 2012, pp 161–183.
12 Haque FN, Gottesman II, Wong AH. Not really identical: epigenetic differences in monozygotic twins and implications for twin studies in psychiatry. *Am J Med Genet C Semin Med Genet* 2009; **151C**: 136–141.
13 Wong AH, Gottesman II, Petronis A. Phenotypic differences in genetically identical organisms: the epigenetic perspective. *Hum Mol Genet* 2005; **1**: R11–R18.
14 Byrne EM, Carrillo-Roa T, Henders AK, Bowdler L, McRae AF, Heath AC *et al.* Monozygotic twins affected with major depressive disorder have greater variance in methylation than their unaffected co-twin. *Transl Psychiatry* 2013; **3**: e269.
15 Feinberg AP. Epigenomics reveals a functional genome anatomy and a new approach to common disease. *Nat Biotechnol* 2010; **28**: 1049–1052.
16 Feinberg AP. Epigenetic stochasticity, nuclear structure and cancer: the implications for medicine. *J Intern Med* 2014; **276**: 5–11.
17 Feinberg AP, Irizarry RA, Fradin D, Aryee MJ, Murakami P, Aspelund T *et al.* Personalized epigenomic signatures that are stable over time and covary with body mass index. *Sci Transl Med* 2010; **2**: 49ra67.
18 Hansen KD, Timp W, Bravo HC, Sabunciyan S, Langmead B, McDonald OG *et al.* Increased methylation variation in epigenetic domains across cancer types. *Nat Genet* 2011; **43**: 768–775.

19 Timp W, Feinberg AP. Cancer as a dysregulated epigenome allowing cellular growth advantage at the expense of the host. *Nat Rev Cancer* 2013; **13**: 497–510.
20 Jaffe AE, Feinberg AP, Irizarry RA, Leek JT. Significance analysis and statistical dissection of variably methylated regions. *Biostatistics* 2012; **13**: 166–178.
21 Teschendorff AE, Widschwendter M. Differential variability improves the identification of cancer risk markers in DNA methylation studies profiling precursor cancer lesions. *Bioinformatics* 2012; **28**: 1487–1494.
22 Oh G, Wang SC, Pal M, Chen ZF, Khare T, Tochigi M *et al.* DNA Modification Study of Major Depressive Disorder: beyond locus-by-locus comparisons. *Biol Psychiatry* 2015; **77**: 246–255.
23 Dempster EL, Pidsley R, Schalkwyk LC, Owens S, Georgiades A, Kane F *et al.* Disease-associated epigenetic changes in monozygotic twins discordant for schizophrenia and bipolar disorder. *Hum Mol Genet* 2011; **20**: 4786–4796.
24 Wong CC, Meaburn EL, Ronald A, Price TS, Jeffries AR, Schalkwyk LC *et al.* Methylomic analysis of monozygotic twins discordant for autism spectrum disorder and related behavioural traits. *Mol Psychiatry* 2014; **19**: 495–503.
25 Kendler KS, Eaves LJ, Walters EE, Neale MC, Heath AC, Kessler RC. The identification and validation of distinct depressive syndromes in a population-based sample of female twins. *Arch Gen Psychiatry* 1996; **53**: 391–399.
26 Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ. Major depression and generalized anxiety disorder. Same genes, (partly) different environments? *Arch Gen Psychiatry* 1992; **49**: 716–722.
27 Guilherme R, Drunat S, Delezoide AL, Oury JF, Luton D. Zygosity and chorionicity in triplet pregnancies: new data. *Hum Reprod* 2009; **24**: 100–105.
28 First MB. *Structured Clinical Interview for DSM-IV Axis I Disorders: SCID - I: Clinician Version: Administration Booklet*. American Psychiatric Press: Washington, DC, USA, 1997.
29 Mosing MA, Gordon SD, Medland SE, Statham DJ, Nelson EC, Heath AC *et al.* Genetic and environmental influences on the co-morbidity between depression, panic disorder, agoraphobia, and social phobia: a twin study. *Depress Anxiety* 2009; **26**: 1004–1011.
30 Ressler KJ, Mayberg HS. Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. *Nat Neurosci* 2007; **10**: 1116–1124.
31 Wittchen HU, Kessler RC, Beesdo K, Krause P, Hofler M, Hoyer J. Generalized anxiety and depression in primary care: prevalence, recognition, and management. *J Clin Psychiatry* 2002; **63**: 24–34.
32 Zbozinek TD, Rose RD, Wolitzky-Taylor KB, Sherbourne C, Sullivan G, Stein MB *et al.* Diagnostic overlap of generalized anxiety disorder and major depressive disorder in a primary care sample. *Depress Anxiety* 2012; **29**: 1065–1071.
33 Derogatis LR, Melisaratos N. The Brief Symptom Inventory: an introductory report. *Psychol Med* 1983; **13**: 595–605.
34 Ruiperez M, Ibáñez MI, Lorente E, Moro M, Ortet G. Psychometric properties of the Spanish version of the BSI: contributions to the relationship between personality and psychopathology. *Eur J Psychol Assess* 2001; **17**: 241.
35 Bibikova M, Barnes B, Tsan C, Ho V, Klotzle B, Le JM *et al.* High density DNA methylation array with single CpG site resolution. *Genomics* 2011; **98**: 288–295.
36 Sandoval J, Heyn H, Moran S, Serra-Musach J, Pujana MA, Bibikova M *et al.* Validation of a DNA methylation microarray for 450,000 CpG sites in the human genome. *Epigenetics* 2011; **6**: 692–702.
37 Hettema JM. What is the genetic relationship between anxiety and depression? *Am J Med Genet C Semin Med Genet* 2008; **148C**: 140–146.
38 Nakamura K, Aizawa K, Nakabayashi K, Kato N, Yamauchi J, Hata K *et al.* DNA methyltransferase inhibitor zebularine inhibits human hepatic carcinoma cells proliferation and induces apoptosis. *PLoS One* 2013; **8**: e54036.
39 Yuen RK, Penaherrera MS, von Dadelszen P, McFadden DE, Robinson WP. DNA methylation profiling of human placentas reveals promoter hypomethylation of multiple genes in early-onset preeclampsia. *Eur J Hum Genet* 2010; **18**: 1006–1012.
40 Zeller C, Dai W, Steele NL, Siddiq A, Walley AJ, Wilhelm-Benartzi CS *et al.* Candidate DNA methylation drivers of acquired cisplatin resistance in ovarian cancer identified by methylome and expression profiling. *Oncogene* 2012; **31**: 4567–4576.
41 Smoot ME, Ono K, Ruscheinski J, Wang PL, Ideker T. Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics* 2011; **27**: 431–432.
42 Croft D, O'Kelly G, Wu G, Haw R, Gillespie M, Matthews L *et al.* Reactome: a database of reactions, pathways and biological processes. *Nucleic Acids Res* 2011; **39**: D691–D697.
43 Pajer K, Andrus BM, Gardner W, Lourie A, Strange B, Campo J *et al.* Discovery of blood transcriptomic markers for depression in animal models and pilot validation in subjects with early-onset major depression. *Transl Psychiatry* 2012; **2**: e101.
44 Karanges EA, Kashem MA, Sarker R, Ahmed EU, Ahmed S, Van Nieuwenhuijzen PS *et al.* Hippocampal protein expression is differentially affected by chronic paroxetine treatment in adolescent and adult rats: a possible mechanism of "paradoxical" antidepressant responses in young persons. *Front Pharmacol* 2013; **4**: 86.
45 Wray NR, Pergadia ML, Blackwood DH, Penninx BW, Gordon SD, Nyholt DR *et al.* Genome-wide association study of major depressive disorder: new results, meta-analysis, and lessons learned. *Mol Psychiatry* 2012; **17**: 36–48.
46 Lee HC, Chang DE, Yeom M, Kim GH, Choi KD, Shim I *et al.* Gene expression profiling in hypothalamus of immobilization-stressed mouse using cDNA microarray. *Brain Res Mol Brain Res* 2005; **135**: 293–300.
47 Major Depressive Disorder Working Group of the Psychiatric GC, Ripke S, Wray NR, Lewis CM, Hamilton SP, Weissman MM *et al.* A mega-analysis of genome-wide association studies for major depressive disorder. *Mol Psychiatry* 2013; **18**: 497–511.
48 LaSalle JM, Powell WT, Yasui DH. Epigenetic layers and players underlying neurodevelopment. *Trends Neurosci* 2013; **36**: 460–470.
49 Rauch TA, Wu X, Zhong X, Riggs AD, Pfeifer GP. A human B cell methylome at 100-base pair resolution. *Proc Natl Acad Sci USA* 2009; **106**: 671–678.
50 Huang WY, Hsu SD, Huang HY, Sun YM, Chou CH, Weng SL *et al.* MethHC: a database of DNA methylation and gene expression in human cancer. *Nucleic Acids Res* 2015; **43**: D856–D861.
51 Nieratschker V, Massart R, Gilles M, Luoni A, Suderman MJ, Krumm B *et al.* MORC1 exhibits cross-species differential methylation in association with early life stress as well as genome-wide association with MDD. *Transl Psychiatry* 2014; **4**: e429.
52 Oh JE, Chambwe N, Klein S, Gal J, Andrews S, Gleason G *et al.* Differential gene body methylation and reduced expression of cell adhesion and neurotransmitter receptor genes in adverse maternal environment. *Transl Psychiatry* 2013; **3**: e218.
53 Heim C, Binder EB. Current research trends in early life stress and depression: review of human studies on sensitive periods, gene-environment interactions, and epigenetics. *Exp Neurol* 2012; **233**: 102–111.
54 Cline BH, Steinbusch HW, Malin D, Revishchin AV, Pavlova GV, Cespuglio R *et al.* The neuronal insulin sensitizer dicholine succinate reduces stress-induced depressive traits and memory deficit: possible role of insulin-like growth factor 2. *BMC Neurosci* 2012; **13**: 110.
55 Luo Y-w, Xu Y, Cao W-y, Zhong X-l, Duan J, Wang X-q *et al.* Insulin-like growth factor 2 mitigates depressive behavior in a rat model of chronic stress. *Neuropharmacology* 2015; **89**: 318–324.
56 Bruchas MR, Schindler AG, Shankar H, Messinger DI, Miyatake M, Land BB *et al.* Selective p38alpha MAPK deletion in serotonergic neurons produces stress resilience in models of depression and addiction. *Neuron* 2011; **71**: 498–511.
57 Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol* 2006; **27**: 24–31.
58 Kendler KS, Eaves LJ, Loken EK, Pedersen NL, Middeldorp CM, Reynolds C *et al.* The impact of environmental experiences on symptoms of anxiety and depression across the life span. *Psychol Sci* 2011; **22**: 1343–1352.
59 Kendler KS, Heath AC, Martin NG, Eaves LJ. Symptoms of anxiety and symptoms of depression. Same genes, different environments? *Arch Gen Psychiatry* 1987; **44**: 451–457.
60 Kim SY, Mo JW, Han S, Choi SY, Han SB, Moon BH *et al.* The expression of non-clustered protocadherins in adult rat hippocampal formation and the connecting brain regions. *Neuroscience* 2010; **170**: 189–199.
61 Leung LC, Urbancic V, Baudet ML, Dwivedy A, Bayley TG, Lee AC *et al.* Coupling of NF-protocadherin signaling to axon guidance by cue-induced translation. *Nat Neurosci* 2013; **16**: 166–173.
62 McGowan PO, Suderman M, Sasaki A, Huang TC, Hallett M, Meaney MJ *et al.* Broad epigenetic signature of maternal care in the brain of adult rats. *PLoS One* 2011; **6**: e14739.
63 Anitha A, Thanseem I, Nakamura K, Yamada K, Iwayama Y, Toyota T *et al.* Protocadherin alpha (PCDHA) as a novel susceptibility gene for autism. *J Psychiatry Neurosci* 2013; **38**: 192–198.
64 Amberger J, Bocchini CA, Scott AF, Hamosh A. McKusick's online Mendelian inheritance in man (OMIM). *Nucleic Acids Res* 2009; **37**: D793–D796.
65 Chang SC, Koenen KC, Galea S, Aiello AE, Soliven R, Wildman DE *et al.* Molecular variation at the SLC6A3 locus predicts lifetime risk of PTSD in the Detroit Neighborhood Health Study. *PLoS One* 2012; **7**: e39184.
66 Dong C, Wong ML, Licinio J. Sequence variations of ABCB1, SLC6A2, SLC6A3, SLC6A4, CREB1, CRHR1 and NTRK2: association with major depression and antidepressant response in Mexican-Americans. *Mol Psychiatry* 2009; **14**: 1105–1118.
67 Uddin M, Koenen KC, Aiello AE, Wildman DE, de los Santos R, Galea S. Epigenetic and inflammatory marker profiles associated with depression in a community-based epidemiologic sample. *Psychol Med* 2011; **41**: 997–1007.
68 Feinberg AP, Irizarry RA. Evolution in health and medicine Sackler colloquium: Stochastic epigenetic variation as a driving force of development, evolutionary adaptation, and disease. *Proc Natl Acad Sci USA* 2010; **107**: 1757–1764.
69 Pujadas E, Feinberg AP. Regulated noise in the epigenetic landscape of development and disease. *Cell* 2012; **148**: 1123–1131.
70 Phipson B, Oshlack A. DiffVar: a new method for detecting differential variability with application to methylation in cancer and aging. *Genome Biol* 2014; **15**: 465.

71 Zhu Y, Wang Y, Xia C, Li D, Li Y, Zeng W *et al.* WDR26: a novel Gbeta-like protein, suppresses MAPK signaling pathway. *J Cell Biochem* 2004; **93**: 579–587.
72 Roux PP, Blenis J. ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. *Microbiol Mol Biol Rev* 2004; **68**: 320–344.
73 Bibikova M, Le J, Barnes B, Saedinia-Melnyk S, Zhou L, Shen R *et al.* Genome-wide DNA methylation profiling using Infinium(R) assay. *Epigenomics* 2009; **1**: 177–200.
74 Tylee DS, Kawaguchi DM, Glatt SJ. On the outside, looking in: a review and evaluation of the comparability of blood and brain "-omes". *Am J Med Genet B Neuropsychiatr Genet* 2013; **162B**: 595–603.
75 Davies MN, Volta M, Pidsley R, Lunnon K, Dixit A, Lovestone S *et al.* Functional annotation of the human brain methylome identifies tissue-specific epigenetic variation across brain and blood. *Genome Biol* 2012; **13**: R43.
76 Iwamoto K, Bundo M, Ueda J, Oldham MC, Ukai W, Hashimoto E *et al.* Neurons show distinctive DNA methylation profile and higher interindividual variations compared with non-neurons. *Genome Res* 2011; **21**: 688–696.
77 Ladd-Acosta C, Pevsner J, Sabunciyan S, Yolken RH, Webster MJ, Dinkins T *et al.* DNA methylation signatures within the human brain. *Am J Hum Genet* 2007; **81**: 1304–1315.
78 Varley KE, Gertz J, Bowling KM, Parker SL, Reddy TE, Pauli-Behn F *et al.* Dynamic DNA methylation across diverse human cell lines and tissues. *Genome Res* 2013; **23**: 555–567.
79 Provencal N, Suderman MJ, Guillemin C, Massart R, Ruggiero A, Wang D *et al.* The signature of maternal rearing in the methylome in rhesus macaque prefrontal cortex and T cells. *J Neurosci* 2012; **32**: 15626–15642.
80 Szyf M. DNA methylation, behavior and early life adversity. *J Genet Genomics* 2013; **40**: 331–338.
81 Szyf M, Bick J. DNA methylation: a mechanism for embedding early life experiences in the genome. *Child Dev* 2013; **84**: 49–57.
82 Spiteri E, Konopka G, Coppola G, Bomar J, Oldham M, Ou J *et al.* Identification of the transcriptional targets of FOXP2, a gene linked to speech and language, in developing human brain. *Am J Hum Genet* 2007; **81**: 1144–1157.
83 Huang KC, Yang KC, Lin H, Tsao Tsun-Hui T, Lee WK, Lee SA *et al.* Analysis of schizophrenia and hepatocellular carcinoma genetic network with corresponding modularity and pathways: novel insights to the immune system. *BMC Genomics* 2013; **14**: S10.
84 Klausmeyer A, Conrad R, Faissner A, Wiese S. Influence of glial-derived matrix molecules, especially chondroitin sulfates, on neurite growth and survival of cultured mouse embryonic motoneurons. *J Neurosci Res* 2011; **89**: 127–141.
85 Maeda N. Structural variation of chondroitin sulfate and its roles in the central nervous system. *Cent Nerv Syst Agents Med Chem* 2010; **10**: 22–31.
86 Wu Y, Sheng W, Chen L, Dong H, Lee V, Lu F *et al.* Versican V1 isoform induces neuronal differentiation and promotes neurite outgrowth. *Mol Biol Cell* 2004; **15**: 2093–2104.
87 Carter CJ. Susceptibility genes are enriched in those of the herpes simplex virus 1/host interactome in psychiatric and neurological disorders. *Pathog Dis* 2013; **69**: 240–261.
88 Pantazopoulos H, Boyer-Boiteau A, Holbrook EH, Jang W, Hahn CG, Arnold SE *et al.* Proteoglycan abnormalities in olfactory epithelium tissue from subjects diagnosed with schizophrenia. *Schizophr Res* 2013; **150**: 366–372.
89 Crider A, Pandya CD, Peter D, Ahmed AO, Pillai A. Ubiquitin-proteasome dependent degradation of GABAAalpha1 in autism spectrum disorder. *Mol Autism* 2014; **5**: 45.
90 Mohrmann I, Gillessen-Kaesbach G, Siebert R, Caliebe A, Hellenbroich Y. A de novo 0.57 Mb microdeletion in chromosome 11q13.1 in a patient with speech problems, autistic traits, dysmorphic features and multiple endocrine neoplasia type 1. *Eur J Med Genet* 2011; **54**: e461–e464.
91 Kaneko M, Koike H, Saito R, Kitamura Y, Okuma Y, Nomura Y. Loss of HRD1-mediated protein degradation causes amyloid precursor protein accumulation and amyloid-beta generation. *J Neurosci* 2010; **30**: 3924–3932.
92 Saito R, Kaneko M, Okuma Y, Nomura Y. Correlation between decrease in protein levels of ubiquitin ligase HRD1 and amyloid-beta production. *J Pharmacol Sci* 2010; **113**: 285–288.
93 Shen YX, Sun AM, Fang S, Feng LJ, Li Q, Hou HL *et al.* Hrd1 facilitates tau degradation and promotes neuron survival. *Curr Mol Med* 2012; **12**: 138–152.
94 Gillingwater TH, Wishart TM. Mechanisms underlying synaptic vulnerability and degeneration in neurodegenerative disease. *Neuropathol Appl Neurobiol* 2013; **39**: 320–334.
95 Narayan S, Head SR, Gilmartin TJ, Dean B, Thomas EA. Evidence for disruption of sphingolipid metabolism in schizophrenia. *J Neurosci Res* 2009; **87**: 278–288.

Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)

Advisor's report on the contribution of the Ph.D. candidate to the article

Prof. Dr. Lourdes Fañanás Saura, associate professor at the Faculty of Biology, University of Barcelona, and supervisor of the present doctoral thesis by Aldo Córdova Palomera, hereby certifies that the participation of the Ph.D. candidate in the article "Genome-wide methylation study on depression: differential methylation and variable methylation in monozygotic twins" included the following tasks:

- Participation in study design.
- Statistical analyses.
- Writing of the first manuscript draft.
- Critical revision of the article.

Prof. Dr. Lourdes Fañanás Saura

Barcelona, June 30th 2015.

Polymorphic variation in* FKBP5 *interacts with hippocampal connectivity to influence the risk for depression: a study in twins

Córdova-Palomera A, de Reus MA, Fatjó-Vilas M, Falcón C, Bargalló N, van den Heuvel M, Fañanás L

Under review

Polymorphic variation in *FKBP5* interacts with hippocampal connectivity to influence the risk for depression: a twin design study

Aldo Córdova-Palomera, MS[1,2]; Marcel A. de Reus, MS[3]; Mar Fatjó-Vilas, PhD[1,2]; Carles Falcón, PhD[4,5]; Nuria Bargalló, PhD[2,6,7]; Martijn P. van den Heuvel, PhD[3,*]; Lourdes Fañanás, PhD[1,2,*,**].

[1]Unidad de Antropología, Departamento de Biología Animal, Facultad de Biología and Instituto de Biomedicina (IBUB), Universitat de Barcelona; Av. Diagonal, 643, 08028. Barcelona, Spain. [2]Centro de Investigaciones Biomédicas en Red de Salud Mental (CIBERSAM); C/Doctor Esquerdo, 46, 28007. Madrid, Spain. [3]Brain Center Rudolf Magnus, Department of Psychiatry, University Medical Center Utrecht; Heidelberglaan 100, 3508 GA. Utrecht, the Netherlands. [4]BarcelonaBeta Brain Research Center, Pasqual Maragall Foundation; C/Dr Aiguader, 88, 08003. Barcelona, Spain. [5]Centro de Investigación Biomédica en Red en Bioingeniería, Biomedicina y Nanomedicina (CIBER-BBN); C/ Poeta Mariano Esquillor, s/n., 50018. Zaragoza, Spain. [6]Centro de Diagnóstico por Imagen, Hospital Clínic; C/Villarroel, 170. 08036 - Barcelona, Spain. [7]Medical Image core facility, the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS); C/Rosselló, 149-153, 08036. Barcelona, Spain.

Abstract

The hippocampus is a key modulator of stress responses underlying depressive behavior. Though both depression and hippocampal structure are influenced by genes and environment, the genetic or environmental causes of the hippocampal alterations in psychopathology remain only scarcely investigated. While the common functional variant rs1360780 in *FKBP5* has been found associated with a large number of stress-related outcomes and hippocampal features, its potential role in modifying the hippocampal communication transfer mechanisms with other brain regions remains largely unexplored.

The putative genetic or environmental roots of the association between depression and structural connectivity alterations of the hippocampus were evaluated combining diffusion weighted imaging with both a quantitative genetics approach and molecular information on the rs1360780 single nucleotide polymorphism, in a sample of 54 informative monozygotic twins (27 pairs).

Three main results were derived from the present analyses. First, graph-theoretical measures of hippocampal connectivity were altered in the depressed brain. Specifically, decreased connectivity strength and increased network centrality of the right hippocampus were found in depressed individuals. Second, these hippocampal alterations are potentially driven by familial factors (genes plus shared environment). Third, there is an additive interaction effect between *FKBP5*'s rs1360780 variant and the graph-theoretical metrics of hippocampal connectivity to influence depression risk.

Our data reveals alterations of the communication patterns between the hippocampus and the rest of the brain in depression, effects potentially driven by overall familial factors (genes plus shared twin environment) and modified by the *FKBP5* gene.

Introduction

Brain disorders such as depression are rapidly becoming one of the leading causes of disability worldwide, imposing severe burdens on public health systems (Murray *et al*, 2012). There is ample evidence showing that depression has a complex multifactorial etiology which can be traced back to genes, environment, and gene-environment interactions (Mandelli and Serretti, 2013). However, novel research methods are needed to determine the precise factors underlying this disorder (Saveanu and Nemeroff, 2012).

In this sense, studies combining neuroimaging and molecular genetic data provide a special opportunity to elicit the complex etiology of depression since brain phenotypes obtained with *in vivo* magnetic resonance imaging (MRI) techniques have consistently been linked to depressive phenotypes (Graham *et al*, 2013; Northoff, 2013), and some well-identified genetic variants may be linked to the activity of the brain, in both healthy and depressed individuals (Dick *et al*, 2015; Parasuraman and Jiang, 2012). Overall, neuroimaging genetic studies constitute a method to investigate how some genetic factors may alter brain activity and lead to behavioral and psychopathological outcomes (Parasuraman *et al*, 2012).

While one of the most recent approaches to study brain structure and function in psychopathology is the analysis of wiring patterns between different brain regions using graph theory and diffusion weighted imaging (DWI) (Bullmore and Sporns, 2009), to date there are only a few graph theoretical studies analyzing the potential role of brain network alterations in adult depression (Korgaonkar *et al*, 2014; Leow *et al*, 2013; Long *et al*, 2015; Qin *et al*, 2014). Having focused mainly on large-scale network analysis, some of them have found alterations in hippocampal connectivity (Leow *et al*, 2013; Long *et al*, 2015), in line with former biologically feasible evidence on the neurobiology of depression (Campbell *et al*, 2004; Eisch and Petrik, 2012; MacQueen *et al*, 2003).

The hippocampus is a highly sensitive brain region that has been identified as a key modulator of stress responses underlying depressive behavior (Chen *et al*, 2012; Snyder *et al*, 2011). Of note, the activity of the FK506 binding protein 5 (*FKBP5*) gene has been found associated with a large number of stress-related outcomes and hippocampal features (Binder *et al*, 2008; Fani *et al*, 2013; Guidotti *et al*, 2013; Klengel *et al*, 2013). The rs1360780 variant –one of the most studied single nucleotide polymorphisms (SNPs) in the *FKBP5* gene– has been linked to hippocampal volume and function in depression and stress (Fani *et al*, 2013; Fani *et al*, 2014; Pagliaccio *et al*, 2014). Though this may be related to genetic factors underlying a communicational deficit of the hippocampus in depression, to the best of our knowledge, no previous report has evaluated the (putative) association between this SNP and the organization of white matter tracts connecting the hippocampus to the rest of the brain.

With this background, the current study aimed to determine the role of genetic and environmental factors leading to depression via hippocampal alterations, and its potential modulation by the common functional *FKBP5*'s rs1360780 variant. To do so, whole brain structural data was obtained using DWI from a group of 54 monozygotic (MZ) twins (27 pairs) informative for depressive psychopathology. Since members of a MZ twin pair have almost identical DNA sequences, this work studied their phenotypic similarities and differences in order to obtain insights on familial and environmental influences. Various

centrality measures of hippocampal nodal connectivity were estimated by constructing whole-brain networks; also, putative interaction effects between hippocampal centrality and the rs1360780 single nucleotide polymorphism (SNP) in the *FKBP5* gene were explored.

Methods and Materials

Sample description. The present sample constitutes a subset extracted from a group of 115 Spanish Caucasian adult twin pairs (230 individuals) from the general population, who gave permission to be contacted for research purposes (UB Twin Registry). Written informed consent was obtained from all individuals after a detailed description of the study aims and design, approved by the local Ethics Committee. All procedures were conducted in accordance with the Declaration of Helsinki.

Zygosity of all twin pairs was assessed by genotyping 16 highly polymorphic microsatellite loci from DNA samples (SSRs; PowerPlex® 16 System Promega Corporation). Identity on all the markers can be used to assign monozygosity with over 99% precision (Guilherme *et al*, 2009). In the whole sample (115 twin pairs), 86 duos were MZ.

Using the previously collected data, a group of 54 individuals (27 MZ twin pairs) was selected from the set of MZ twins, as they were informative for obstetric and psychopathological traits and gave consent to participate in the MRI part of the present study.

Twins included in this 54-participant subset met the following criteria: *i*) age at scan between 21 and 53 years, *ii*) both twins right-handed, *iii*) none of the twins manifested liability for DSM-IV-R psychiatric diagnoses other than depression and/or anxiety, and *iv*) no twin had a medical history of neurological disturbance, sensory or motor alterations, or substance misuse or dependence. Pairs where one or both twins manifested either neurological or major medical illnesses were excluded as well (see *Measures*). Hence, the sample included in all statistical analyses discussed next consisted of 54 individuals (20 males, mean age: 34.8 years). Further information on this sample can be found elsewhere (Alemany *et al*, 2013).

Psychometric measures. Liability for (lifetime) psychopathology in this general population sample was screened in a face-to-face interview by a clinical psychologist, using the Structural Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (First, 1997). Participants were then asked to report if they had received pharmacological or psychological treatment or had consulted a psychiatrist or psychologist since they first participated in the study. While three individuals were likely exposed to life-time psychopharmacological treatment for depression, excluding them from the analyses did not change the significance of the results.

In the present sample, six individuals with a history of mainly anxious psychopathology were included in the psychopathology-affected group. This apparently broad category of outcomes was used in conjunction with evidence on the comorbidity, shared etiopathology and diagnostic criteria overlap between depressive and anxious disorders (Mosing *et al*, 2009; Ressler and Mayberg, 2007; Wittchen *et al*, 2002; Zbozinek *et al*, 2012), as well as taking into account evidences of shared hippocampal alterations across both diagnoses (Miller and Hen, 2015).

Repeating the statistical analyses removing predominantly anxious individuals produced very similar results.

Briefly, there were eleven healthy pairs, six concordant and ten discordant pairs for lifetime DSM-IV diagnoses. To further characterize this sample at the clinical level, current depression status and other psychiatric symptoms were evaluated using the Brief Symptom Inventory (BSI) (Derogatis and Melisaratos, 1983; Ruiperez *et al*, 2001). Descriptive data from the current sample is summarized in Table 1.

As shown, all diagnostic concordant pairs were females, and twins with no lifetime history of DSM-IV diagnosis had lower BSI scores –fewer self-reported symptoms– in both the depressive subscale and the whole questionnaire.

Genotyping. Genomic DNA was extracted from either saliva or blood samples from the total sample (*n* = 115 pairs) by using the Collection Kit BuccalAmp DNA extraction kit (Epicentre, ECOGEN, Spain) for saliva or a standard phenol-chloroform method for blood. The latter method was used for the 54 participants of this study, since peripheral blood samples were available. The common functional variant rs1360780, within the *FKBP5* gene, was genotyped using Applied Biosystems TaqMan technology (Applied Biosystems, California, USA). Applied Biosystems assay-on-demand service was used to order the probes. A random 10% of the total sample was selected to repeat the genotyping protocol for cross-validation. The reproducibility rate was 100%. Genotype determinations were performed blind to psychopathological status of the twin pairs. Departure from Hardy-Weinberg equilibrium was tested in both the whole sample (115 pairs) and the depression concordant, discordant and control subsets of twins (6, 11 and 10 pairs) by using one genotype from every pair, and following a recently introduced methodology that is particularly suited for small sample sizes with low minor allele counts (Graffelman and Moreno, 2013). The genotype distribution of the rs1360780 SNP was in Hardy-Weinberg equilibrium in all four cases; the *p*-values for equilibrium departure were 0.921 (whole UB sample), 0.136 (concordant), 0.068 (discordant) and 0.14 (healthy). There were no inter-group differences in genotype frequency distribution across concordant, discordant and healthy pairs (Table 1).

---------- Table 1 ----------

MRI acquisition and pre-processing. The images were acquired at the MRI Unit of the Image Platform (IDIBAPS, Hospital Clínic de Barcelona), using a TIM TRIO 3T scanner with an 8-channel head coil (Siemens, Erlangen, Germany). First, high resolution 3D structural datasets were obtained for anatomical reference, using a T1-weighted magnetization prepared rapid gradient echo, with the following parameters: 3D T1-weighted MPRAGE sequence, TR = 2300 ms, TE = 3.03 ms, TI = 900 ms, flip angle = 9°, 192 slices in the sagittal plane, matrix size = 256×256, 1 mm^3 isometric voxel. Diffusion weighted images were acquired by means of spin echo-planar imaging (TR = 7600 ms, TE = 98 ms, flip angle = 90°, slice thickness = 2.5 mm, matrix size = 192×192, voxel size = 1.25 × 1.25 × 2.5 mm3) with 82 noncollinear diffusion directions at *b* = 1000 s/mm2 and six *b* = 0 images.

T1 MRI scans were processed and analysed using the freely available software FreeSurfer (http://surfer.nmr.mgh.harvard.edu/), using automatic

segmentation and parcellation protocols to obtain 68 cortical and 14 subcortical gray matter brain regions (Desikan *et al*, 2006; Fischl *et al*, 2002). A robust tensor fitting method was used to retrieve the preferred diffusion direction from the DWI data in each voxel of a brain mask (Chang *et al*, 2012). Using streamline tractography, eight streamlines were started in each white matter voxel and propagated by following the main diffusion direction from voxel to voxel (Mori *et al*, 1999). Propagation of a streamline was ended when the streamline reached a voxel with fractional anisotropy (FA) < 0.1, when the path angle was > 45°, or when a path exited the brain mask.

Whole-brain connectivity matrices were generated for each of the 54 MZ twins in this study, by combining the mentioned 82 brain regions with the total collection of reconstructed fiber streamlines. Following conventional protocols (van den Heuvel and Sporns, 2011), edge weights were assigned as the count of the number of streamlines (NOS) touching a given pair of regions. A schematic representation of these steps is shown in Figure 1. Complementarily, in recognition that the NOS across each pair of brain of regions may be volume-dependent (van den Heuvel *et al*, 2011), the previous edge weights were mapped to a second connectivity matrix by dividing them by the sum of the volumes of the two connected brain regions. As statistical significance of the results shown below was very similar using either the original or volume-adjusted edge weights, and since this volume adjustment may be overly conservative (van den Heuvel *et al*, 2011; Zalesky and Fornito, 2009), only NOS-based findings are reported in the main text (results using volume-corrected data revealed overlapping findings and are reported as *Supplementary Material*).

---------- Figure 1 ----------

Measures of hippocampal centrality within the brain network. Three different nodal centrality measures were separately computed for both left and right hippocampus: nodal strength, betweenness centrality and eigenvector centrality. These three quantities were included in view that they have widely been studied in the literature (Borgatti and Everett, 2006). Centrality measures were computed using the Brain Connectivity Toolbox (http://www.brain-connectivity-toolbox.net) (Rubinov and Sporns, 2010), run in MATLAB (Mathworks Inc., USA). Detailed mathematical descriptions of these metrics can be found elsewhere (Borgatti *et al*, 2006; Bounova and de Weck, 2012).

In the present context, these quantities represent: the connectivity between the hippocampus and the rest of the brain (*i*, strength), how often the hippocampus bridges through the shortest path between any two other nodes (*ii*, betweenness centrality), and how strong the connections are between the hippocampus and the brain regions with the highest connectivity (*iii*, eigenvector centrality).

Statistical analysis. All inter-subject analyses were conducted using logistic regression models using R's software packages *rms* and *mztwinreg* (Córdova-Palomera, 2015; Harrel, 2013; R Development Core Team, 2011). Separate logistic regression models assessed left and right hippocampal centrality measures.

First, to assess the extent to which the *FKBP5* genotype and the hippocampal centrality measures relate to the outcome of depression –considering each individual as a separate observation–, the model

$$\text{logit}(\pi) = \beta_0 + \beta_1(gender) + \beta_2(age) + \beta_3(rs1360780) + \beta_4(strength) + \beta_5(bet.) + \beta_5(eigenv.)$$

was fitted. Here, π stands for the probability of an individual being depressed, and the left or right hippocampal metrics are introduces in the terms $strength$ (nodal strength), $bet.$ (betweenness centrality) and $eigenv.$ (eigenvector centrality); $rs1360780$ is a three-level numeric variable representing the number of minor alleles ("T" allele) in a given individual. The latter convention was adopted in recognition of the well-established quantitative gene expression changes caused by the rs1360780 SNP (Binder *et al*, 2004; Fujii *et al*, 2014a); its use as a categorical variable did not alter the main results and conclusions derived from the analyses. As noticed from the equation, gender and age were included as covariates to control for potential confounding. Additionally, the Huber-White method was used to adjust the variance-covariance matrix of these regression fits, in order to account for the non-independence of twin data (i.e., heteroskedasticity) (DeMaris, 1995; Harrel, 2013).

Secondly, since previous research has shown that both depression and the brain network metrics are influenced by genes and environment (Bohlken *et al*, 2014; Domschke and Reif, 2012; Leonardo and Hen, 2006), an additional regression procedure (Begg and Parides, 2003; Córdova-Palomera, 2015) was implemented to determine whether the above mentioned results were driven by the familial or non-genetic factors. Specifically, the previous regression model was adjusted by using

$$\text{logit}(\pi_{ij}) = \beta_0 + \beta_B\mu_{i.} + \beta_W\left(X_{ij} - \mu_{i.}\right)$$

in order to obtain estimates of both *a*) familial factors (genetic plus shared environment, β_B) and *b*) unique environmental influences (from non-shared events within a pair, β_W) on every graph-theoretical nodal centrality measure (i.e., strength, betweenness centrality and eigenvector centrality). Subindex $i \in \{1, \dots, n\}$ stands for pair number (here, *n* = 27 MZ pairs) and $j \in \{1, 2\}$ refers to co-twin number (randomly assigned). π_{ij} stands for the probability that co-twin j from the i-th pair has of being affected by depression. β_0 represents the intercept; $\mu_{i.} = (X_{i1} + X_{i2})/2$ is the mean nodal centrality measure of the i-th pair, and $X_{ij} - \mu_{i.}$ denotes the deviation of co-twin *j* from the pair's mean nodal centrality measure. In this set of analyses, each of these three nodal centrality measures is considered in the same regression model; left and right hippocampal measures (parsed out as familial and unique environmental estimates) are analyzed separately. As in the first model, these analyses were adjusted for gender and age, and the Huber-White estimator was incorporated.

Additionally, interaction effects between *FKBP5*'s rs1360780 and the hippocampal metrics were tested. Additive models were chosen in view of four research evidences: *i*) interaction effects in the psychiatric literature are more robust when measured on additive scales than on multiplicative ones (Clarke *et al*, 2011; Kendler and Gardner, 2010), *ii*) additive interactions are closer to true biological effects (Han *et al*, 2012), *iii*) small sample sizes allow a better detection of additive than multiplicative effects (VanderWeele, 2012) and *iv*) testing multiplicative interactions with the above equations would require largely saturated regression models, with high probability of collinearity. In short, the interaction between rs1360780 and the hippocampal centrality metrics was tested using a variant of the likelihood ratio test (Han *et al*, 2012; Harrel, 2013) to compare the full-regression results (SNP + hippocampal metrics) against each of the separate models

(i.e., the SNP and the hippocampal metrics analyzed in independent models).

Logistic regression plots were generated with *ggplot2* (Wickham, 2009) using the univariate version of the above models (residual regression fitting). Following previous indications on interaction effect analysis in the behavioral sciences (Aguinis and Stone-Romero, 1997), 90% confidence intervals are depicted.

When appropriate, multiple testing adjustments of the regression coefficients from the different (independent) regression models were implemented using the false discovery rate (FDR) approach. The adoption of this Type-I error rate correction is based on previous literature of statistical analysis for biological and behavioral data (Benjamini and Hochberg, 1995; Glickman *et al*, 2014; Liu *et al*, 2004; Nakagawa, 2004).

Results

A first set of analyses evaluated the association between the common *FKBP5* functional variant rs1360780 and depression, adjusting for gender and age and correcting for potential heteroskedasticity (due to the correlated nature of data from twins). No statistically significant association was found (β = -0.61, S.E. = 0.4, p = 0.128). Likewise, the association between depression and each of the three hippocampal centrality measures was evaluated, using two regression models (one per hemisphere). None of the left-hippocampal metrics was associated with depression; nevertheless, the right hippocampus did show a statistically significant association with depression (nodal strength: β = -0.99, S.E. = 0.39, p = 0.011; eigenvector centrality: β = 0.92, S.E. = 0.37, p = 0.013) (Table 2). These results are indicative of a right hippocampal nodal strength decrease in depression (i.e., depressed individuals would have less total NOS count from their right hippocampus) (Figure 2: B). Likewise, they would suggest an eigenvector centrality increase of the right hippocampus in affected individuals: depressed individuals of this sample had relatively strong connections from the hippocampus to the regions with the highest connectivity in the brain (Figure 2: D).

---------- Table 2 ----------

---------- Figure 2 ----------

Then, the additive interaction between rs1360780 and hippocampal centrality was tested to determine whether their combined effect was related to depressive psychopathology. The inclusion of both the *FKBP5* genotype and the right hippocampal metrics in the same regression model suggested an additive interaction effect (likelihood ratio tests: X^2 = 10.84, d.f. = 3, p = 0.013 full model vs. only genotype; X^2 = 5.97, d.f. = 1, p = 0.015 full model vs. only the hippocampal metrics) (Table 2). Namely, the inclusion of both rs1360780 and the hippocampal centrality metrics in a same logistic regression provided better model fitting parameters than their separate use. Accordingly, CC genotype carriers of the rs1360780 who have low nodal strength in the right hippocampus show higher depression risk than their T-carrier counterparts (Figure 2: C). Likewise, the results in Table 2 suggest that C homozygotes with high (right) hippocampal eigenvector centrality show an increased probability of depression as compared to CT and TT genotype individuals (Figure 2: E).

Additionally, Table 2 shows no evidence of interaction effects between the left hippocampal metrics and the *FKBP5* genotype (likelihood ratio tests: X^2 = 6.05, d.f. =

3, $p = 0.109$ full model vs. only genotype; $X^2 = 3.84$, d.f. = 1, $p = 0.05$ full model vs. only hippocampal metrics).

Additional analyses were performed to determine whether the previously mentioned associations were caused by either unique environmental or familial factors (genes and shared environment) (Table 3). The results indicate that the associations between both nodal strength and eigenvector centrality of the right hippocampus are mostly driven by familial factors. Namely, some familial influences (genes plus shared twin environment) would alter the hippocampal connectivity to modify the risk for depression. The analyses also suggested that the unique environmental factors altering right hippocampal eigenvector centrality may play a role in the etiology of depression. Nevertheless, none of these three associations remained statistically after FDR adjustments (Table 3).

Finally, the potential environmental or familial roots of the associations between hippocampal centrality and the *FKBP5* genotype were assessed. The results indicate that the additive interaction effects between *FKBP5* and both (right hippocampal) nodal strength and eigenvector centrality were mostly driven by familial factors (Table 3 and Figure 3); the statistical significance of these two associations survived FDR correction at $p < 0.05$.

---------- Table 3 ----------

---------- Figure 3 ----------

Discussion

In this work, a genetically-informative design was implemented to evaluate putative relationships between graph theoretical measures of hippocampal centrality, the common functional *FKBP5* variant rs1360780 and depression risk. To separately analyze the influence of familial and unique environmental factors altering the relationship between hippocampal structural connectivity and depression, a MZ twin-based model was performed. The overall results indicate that the additive effect of right hippocampal connectivity alterations and the *FKBP5* genotype influence depression risk. They also indicate that these associations may be mainly driven by familial factors altering the connections between the hippocampus and the rest of the brain.

Right hippocampal centrality alterations in the depressed brain. The first result of this work is the association between the graph theoretical measures of right hippocampal centrality and depression risk. When considering all 54 twins as independent individuals –adjusting for the correlated nature of twin data–, there were associations between depression risk and both nodal strength and eigenvector centrality of the hippocampus (Table 2). These data indicate lower hippocampal nodal strength in depression, which could be understood as a reduction in the number of connections (NOS) linking the hippocampus and all other brain regions in depressed individuals (Figure 2: B). In line with previous clinical findings (Liao *et al*, 2013; Long *et al*, 2015), this result may be indicative of a communicational deficit in the depressed hippocampus.

Similarly, there was evidence of disrupted right hippocampal (eigenvector) centrality in depression. This result is particularly relevant in view of recent findings of the hippocampus as a key hub for communicational dynamics in the brain (Misic *et al*, 2014). The current data show that the right hippocampus has a more central position in the

depressed brain network than in its healthy counterpart, which may lead to a disruption of information transfer mechanisms. Analogous eigenvector centrality alterations of some limbic brain regions have been found in other DWI studies of depression and related conditions (Qin *et al*, 2014; Teicher *et al*, 2014).

Despite the overall NOS reductions (decreased strength), the hippocampus of depressed individuals had a prominent position in the brain network: it is well connected to the hub regions. This combination of network parameters (decreased strength and increased centrality) may be related to an excessive –and perhaps abnormal– information flow traversing the hippocampus in depression (Table 2 and Figure 2). It is also important mentioning that the associations between right hippocampal centrality and depression were driven by familial factors in the case of nodal strength, and by both genetic and environmental influences on eigenvector centrality (Table 3).

The rs1360780 SNP (*FKBP5*) interacts with hippocampal centrality to increase depression risk. The present findings also indicate that depression risk is partly explained by an additive interaction effect between right hippocampal connectivity and the common functional *FKBP5* variant rs1360780 (Table 2, Table 3). Specifically, they suggest that individuals having altered hippocampal connectivity who also carry the CC genotype of rs1360780 have an additional percentage of risk for depression (Figure 2: C and F, and Figure 3: B and D).

As mentioned earlier, recent reports are consistently showing that *FKBP5*'s rs1360780 is linked to a system-wide (i.e., not only cerebral) biological disruption in both health and depression (Fujii *et al*, 2014a; Fujii *et al*, 2014b; Menke *et al*, 2013), and that there are interaction effects between rs1360780 and early/childhood adverse events that partly predict the risk for depression-related psychopathology (Appel *et al*, 2011; Klengel *et al*, 2013; Roy *et al*, 2010). Likewise, there is some –still not definite– evidence of an association between the rs1360780 and the clinical response to antidepressant drug treatment (Binder *et al*, 2004; Kirchheiner *et al*, 2008; Zou *et al*, 2010). However, it is worth recalling that previous reports have likewise failed to find direct associations between this SNP and depression status (Binder *et al*, 2004), or have also found only moderate ethnicity- or gender-specific effects (Lavebratt *et al*, 2010; Lekman *et al*, 2008).

The present results for the *FKBP5* can be better understood in light of previous literature reports. First, there was no statistically significant association between the rs1360780 SNP and depressive psychopathology (Table 2 and Table 3), consistent with previous evidence (Binder *et al*, 2004; Zou *et al*, 2010). However, the current findings indicate that the familial liability for hippocampal changes that cause depression is dampened by rs1360780's T allele (Table 3 and Figure 3). Since the T allele of this SNP has been found to moderately predict antidepressant treatment response (Binder *et al*, 2004; Kirchheiner *et al*, 2008), one could hypothesize that this allele regulates the connectivity/communication deficits of the hippocampus observed in depression (Figure 2: B and E), making this brain region more responsive to therapeutic factors (Figure 2: C and F, and Figure 3).

Additional considerations and limitations of the study. Notably, all the statistically significant associations found here were driven by right –but not left– hippocampal alterations. The right hippocampus has widely been

recognized as a central brain structure involved in spatial memory information processing (Maguire *et al*, 1997; Piekema *et al*, 2006), a cognitive process showing some alterations in depression and related stress phenotypes (Marazziti *et al*, 2010; Wong *et al*, 2007). Related findings on a role for *FKBP5*'s rs1360780 in cognition (Fujii *et al*, 2014b) may suggest that the current results are linked to cognitive impairments in depressive psychopathology.

Some methodological limitations of this study should be noted. First, the sample size was modest; however, the associations found here (Table 2 and Table 3) may support the presence of relatively strong effects. Secondly, the brain atlas used to obtain all connectivity matrices was contains only 82 ROIs across the whole brain. Though it implies that the present results are not directly comparable with other studies using different parcellation schemes, this is not a problem only within the current study. Choice of parcellation schemes is an important matter with large implications for brain connectomics research (de Reus and van den Heuvel, 2013). In order to tackle this issue, future studies may combine higher-resolution brain scans with finer-grained anatomical atlases.

Funding and disclosures

Supported by the Spanish SAF2008-05674-C03-01, European Twins Study Network on Schizophrenia Research Training Network (grant number EUTwinsS, MRTN-CT-2006-035987), the Catalan 2014SGR1636 and the Ministry of Science and Innovation (PIM2010ERN-00642) in frame of ERA-NET NEURON. MPvdH was supported by a VENI grant of the Dutch Council for Research (VENI: 451-12-001 NWO) and a Fellowship of the Brain Center Rudolf Magnus.

All authors report no biomedical financial interests or potential conflicts of interest.

Acknowledgements

Ximena Goldberg and Silvia Alemany contributed to sample collection. MRI technicians César Garrido and Santi Sotés also contributed to this work. Anna Valldeperas contributed to genotyping. We are indebted to the Medical Image core facility of the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) for the technical help.

References

Aguinis H, Stone-Romero EF (1997). Methodological artifacts in moderated multiple regression and their effects on statistical power. *Journal of Applied Psychology* **82**(1): 192.

Alemany S, Mas A, Goldberg X, Falcon C, Fatjo-Vilas M, Arias B*, et al* (2013). Regional gray matter reductions are associated with genetic liability for anxiety and depression: an MRI twin study. *Journal of affective disorders* **149**(1-3): 175-181.

Appel K, Schwahn C, Mahler J, Schulz A, Spitzer C, Fenske K*, et al* (2011). Moderation of adult depression by a polymorphism in the FKBP5 gene and childhood physical abuse in the general population. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **36**(10): 1982-1991.

Begg MD, Parides MK (2003). Separation of individual-level and cluster-level covariate effects in regression analysis of correlated data. *Statistics in medicine* **22**(16): 2591-2602.

Benjamini Y, Hochberg Y (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B (Methodological)*: 289-300.

Binder EB, Bradley RG, Liu W, Epstein MP, Deveau TC, Mercer KB*, et al* (2008). Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA : the journal of the American Medical Association* **299**(11): 1291-1305.

Binder EB, Salyakina D, Lichtner P, Wochnik GM, Ising M, Putz B*, et al* (2004). Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nature genetics* **36**(12): 1319-1325.

Bohlken MM, Mandl RC, Brouwer RM, van den Heuvel MP, Hedman AM, Kahn RS*, et al* (2014). Heritability of structural brain network topology: a DTI study of 156 twins. *Human brain mapping* **35**(10): 5295-5305.

Borgatti SP, Everett MG (2006). A Graph-theoretic perspective on centrality. *Social Networks* **28**(4): 466-484.

Bounova G, de Weck O (2012). Overview of metrics and their correlation patterns for multiple-metric topology analysis on heterogeneous graph ensembles. *Physical Review E* **85**(1): 016117.

Bullmore E, Sporns O (2009). Complex brain networks: graph theoretical analysis of structural and functional systems. *Nature reviews Neuroscience* **10**(3): 186-198.

Campbell S, Marriott M, Nahmias C, MacQueen GM (2004). Lower hippocampal volume in patients suffering from depression: a meta-analysis. *The American journal of psychiatry* **161**(4): 598-607.

Clarke MC, Tanskanen A, Huttunen M, Leon DA, Murray RM, Jones PB*, et al* (2011). Increased risk of schizophrenia from additive interaction between infant motor developmental delay and obstetric complications: evidence from a population-based longitudinal study. *The American journal of psychiatry* **168**(12): 1295-1302.

Córdova-Palomera A (2015). mztwinreg: Regression Models for Monozygotic Twin Data.

Chang LC, Walker L, Pierpaoli C (2012). Informed RESTORE: A method for robust estimation of diffusion tensor from low redundancy datasets in the presence of physiological noise artifacts. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine* **68**(5): 1654-1663.

Chen Y, Andres AL, Frotscher M, Baram TZ (2012). Tuning synaptic transmission in the hippocampus by stress: the CRH system. *Frontiers in cellular neuroscience* **6**: 13.

de Reus MA, van den Heuvel MP (2013). The parcellation-based connectome: limitations and extensions. *NeuroImage* **80**: 397-404.

DeMaris A (1995). A tutorial in logistic regression. *Journal of Marriage and the Family*: 956-968.

Derogatis LR, Melisaratos N (1983). The Brief Symptom Inventory: an introductory report. *Psychological medicine* **13**(3): 595-605.

Desikan RS, Segonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D*, et al* (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage* **31**(3): 968-980.

Dick DM, Agrawal A, Keller MC, Adkins A, Aliev F, Monroe S*, et al* (2015). Candidate Gene-Environment Interaction Research: Reflections and Recommendations. *Perspectives on psychological science : a journal of the Association for Psychological Science* **10**(1): 37-59.

Domschke K, Reif A (2012). Behavioral genetics of affective and anxiety disorders. *Current topics in behavioral neurosciences* **12**: 463-502.

Eisch AJ, Petrik D (2012). Depression and hippocampal neurogenesis: a road to remission? *Science* **338**(6103): 72-75.

Fani N, Gutman D, Tone EB, Almli L, Mercer KB, Davis J*, et al* (2013). FKBP5 and attention bias for threat: associations with hippocampal function and shape. *JAMA psychiatry* **70**(4): 392-400.

Fani N, King TZ, Reiser E, Binder EB, Jovanovic T, Bradley B*, et al* (2014). FKBP5 genotype and structural integrity of the posterior cingulum. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **39**(5): 1206-1213.

First MB (1997). *Structured clinical interview for DSM-IV axis I disorders : SCID - I : clinician version : administration booklet* American Psychiatric Press: Washington, D.C.

Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C*, et al* (2002). Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* **33**(3): 341-355.

Fujii T, Hori H, Ota M, Hattori K, Teraishi T, Sasayama D*, et al* (2014a). Effect of the common functional FKBP5 variant (rs1360780) on the hypothalamic-pituitary-adrenal axis and peripheral blood gene expression. *Psychoneuroendocrinology* **42**: 89-97.

Fujii T, Ota M, Hori H, Hattori K, Teraishi T, Matsuo J*, et al* (2014b). The common functional FKBP5 variant rs1360780 is associated with altered cognitive function in aged individuals. *Scientific reports* **4**: 6696.

Glickman ME, Rao SR, Schultz MR (2014). False discovery rate control is a recommended alternative to Bonferroni-type adjustments in health studies. *Journal of clinical epidemiology* **67**(8): 850-857.

Graffelman J, Moreno V (2013). The mid p-value in exact tests for Hardy-Weinberg equilibrium. *Statistical applications in genetics and molecular biology* **12**(4): 433-448.

Graham J, Salimi-Khorshidi G, Hagan C, Walsh N, Goodyer I, Lennox B*, et al* (2013). Meta-analytic evidence for neuroimaging models of depression: state or trait? *Journal of affective disorders* **151**(2): 423-431.

Guidotti G, Calabrese F, Anacker C, Racagni G, Pariante CM, Riva MA (2013). Glucocorticoid receptor and FKBP5 expression is altered following exposure to chronic stress: modulation by antidepressant treatment. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **38**(4): 616-627.

Guilherme R, Drunat S, Delezoide AL, Oury JF, Luton D (2009). Zygosity and chorionicity in triplet pregnancies: new data. *Human reproduction* **24**(1): 100-105.

Han SS, Rosenberg PS, Garcia-Closas M, Figueroa JD, Silverman D, Chanock SJ*, et al* (2012). Likelihood ratio test for detecting gene (G)-environment (E) interactions under an additive risk model exploiting G-E independence for case-control data. *American journal of epidemiology* **176**(11): 1060-1067.

Harrel F (2013). rms: Regression Modeling Strategies.

Kendler KS, Gardner CO (2010). Interpretation of interactions: guide for the perplexed. *The British journal of psychiatry : the journal of mental science* **197**(3): 170-171.

Kirchheiner J, Lorch R, Lebedeva E, Seeringer A, Roots I, Sasse J*, et al* (2008). Genetic variants in FKBP5 affecting response to antidepressant drug treatment. *Pharmacogenomics* **9**(7): 841-846.

Klengel T, Mehta D, Anacker C, Rex-Haffner M, Pruessner JC, Pariante CM*, et al* (2013). Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nature neuroscience* **16**(1): 33-41.

Korgaonkar MS, Fornito A, Williams LM, Grieve SM (2014). Abnormal structural networks characterize major depressive disorder: a connectome analysis. *Biological psychiatry* **76**(7): 567-574.

Lavebratt C, Aberg E, Sjoholm LK, Forsell Y (2010). Variations in FKBP5 and BDNF genes are suggestively associated with depression in a Swedish population-based cohort. *Journal of affective disorders* **125**(1-3): 249-255.

Lekman M, Laje G, Charney D, Rush AJ, Wilson AF, Sorant AJ*, et al* (2008). The FKBP5-gene in depression and treatment response--an association study in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) Cohort. *Biological psychiatry* **63**(12): 1103-1110.

Leonardo ED, Hen R (2006). Genetics of affective and anxiety disorders. *Annual review of psychology* **57**: 117-137.

Leow A, Ajilore O, Zhan L, Arienzo D, GadElkarim J, Zhang A*, et al* (2013). Impaired inter-hemispheric integration in bipolar disorder revealed with brain network analyses. *Biological psychiatry* **73**(2): 183-193.

Liao Y, Huang X, Wu Q, Yang C, Kuang W, Du M*, et al* (2013). Is depression a disconnection syndrome? Meta-analysis of diffusion tensor imaging studies in patients with MDD. *Journal of psychiatry & neuroscience : JPN* **38**(1): 49-56.

Liu W, Jamshidian M, Zhang Y (2004). Multiple comparison of several linear regression models. *Journal of the American Statistical Association* **99**(466): 395-403.

Long Z, Duan X, Wang Y, Liu F, Zeng L, Zhao JP*, et al* (2015). Disrupted structural connectivity network in treatment-naive depression. *Progress in neuro-psychopharmacology & biological psychiatry* **56**: 18-26.

MacQueen GM, Campbell S, McEwen BS, Macdonald K, Amano S, Joffe RT*, et al* (2003). Course of illness, hippocampal function, and hippocampal volume in major depression. *Proceedings of the National Academy of Sciences of the United States of America* **100**(3): 1387-1392.

Maguire EA, Frackowiak RS, Frith CD (1997). Recalling routes around london: activation of the right hippocampus in taxi drivers. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **17**(18): 7103-7110.

Mandelli L, Serretti A (2013). Gene environment interaction studies in depression and suicidal behavior: An update.

Neuroscience and biobehavioral reviews **37**(10 Pt 1): 2375-2397.

Marazziti D, Consoli G, Picchetti M, Carlini M, Faravelli L (2010). Cognitive impairment in major depression. *European journal of pharmacology* **626**(1): 83-86.

Menke A, Klengel T, Rubel J, Bruckl T, Pfister H, Lucae S*, et al* (2013). Genetic variation in FKBP5 associated with the extent of stress hormone dysregulation in major depression. *Genes, brain, and behavior* **12**(3): 289-296.

Miller BR, Hen R (2015). The current state of the neurogenic theory of depression and anxiety. *Current opinion in neurobiology* **30**: 51-58.

Misic B, Goni J, Betzel RF, Sporns O, McIntosh AR (2014). A network convergence zone in the hippocampus. *PLoS computational biology* **10**(12): e1003982.

Mori S, Crain BJ, Chacko VP, van Zijl PC (1999). Three-dimensional tracking of axonal projections in the brain by magnetic resonance imaging. *Annals of neurology* **45**(2): 265-269.

Mosing MA, Gordon SD, Medland SE, Statham DJ, Nelson EC, Heath AC*, et al* (2009). Genetic and environmental influences on the co-morbidity between depression, panic disorder, agoraphobia, and social phobia: a twin study. *Depression and anxiety* **26**(11): 1004-1011.

Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C*, et al* (2012). Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380**(9859): 2197-2223.

Nakagawa S (2004). A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behavioral Ecology* **15**(6): 1044-1045.

Northoff G (2013). Gene, brains, and environment-genetic neuroimaging of depression. *Current opinion in neurobiology* **23**(1): 133-142.

Pagliaccio D, Luby JL, Bogdan R, Agrawal A, Gaffrey MS, Belden AC*, et al* (2014). Stress-system genes and life stress predict cortisol levels and amygdala and hippocampal volumes in children. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **39**(5): 1245-1253.

Parasuraman R, Jiang Y (2012). Individual differences in cognition, affect, and performance: behavioral, neuroimaging, and molecular genetic approaches. *NeuroImage* **59**(1): 70-82.

Piekema C, Kessels RP, Mars RB, Petersson KM, Fernandez G (2006). The right hippocampus participates in short-term memory maintenance of object-location associations. *NeuroImage* **33**(1): 374-382.

Qin J, Wei M, Liu H, Yan R, Luo G, Yao Z*, et al* (2014). Abnormal brain anatomical topological organization of the cognitive-emotional and the frontoparietal circuitry in major depressive disorder. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine* **72**(5): 1397-1407.

R Development Core Team (2011). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing: Vienna, Austria.

Ressler KJ, Mayberg HS (2007). Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. *Nature neuroscience* **10**(9): 1116-1124.

Roy A, Gorodetsky E, Yuan Q, Goldman D, Enoch MA (2010). Interaction of FKBP5, a stress-related gene, with childhood trauma increases the risk for attempting suicide. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **35**(8): 1674-1683.

Rubinov M, Sporns O (2010). Complex network measures of brain connectivity: uses and interpretations. *NeuroImage* **52**(3): 1059-1069.

Ruiperez M, Ibáñez MI, Lorente E, Moro M, Ortet G (2001). Psychometric properties of the Spanish version of the BSI: Contributions to the relationship between personality and psychopathology. *Eur J Psychol Assess* **17**(3): 241.

Saveanu RV, Nemeroff CB (2012). Etiology of depression: genetic and environmental factors. *The Psychiatric clinics of North America* **35**(1): 51-71.

Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA (2011). Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. *Nature* **476**(7361): 458-461.

Teicher MH, Anderson CM, Ohashi K, Polcari A (2014). Childhood maltreatment: altered network centrality of cingulate, precuneus, temporal pole and insula. *Biological psychiatry* **76**(4): 297-305.

van den Heuvel MP, Sporns O (2011). Rich-club organization of the human connectome. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **31**(44): 15775-15786.

VanderWeele TJ (2012). Sample Size and Power Calculations for Additive Interactions. *Epidemiologic methods* **1**(1): 159-188.

Wickham H (2009). *ggplot2: elegant graphics for data analysis* Springer Science & Business Media.

Wittchen HU, Kessler RC, Beesdo K, Krause P, Hofler M, Hoyer J (2002). Generalized anxiety and depression in primary care: prevalence, recognition, and management. *The Journal of clinical psychiatry* **63 Suppl 8**: 24-34.

Wong TP, Howland JG, Robillard JM, Ge Y, Yu W, Titterness AK*, et al* (2007). Hippocampal long-term depression mediates acute stress-induced spatial memory retrieval impairment. *Proceedings of the National Academy of Sciences of the United States of America* **104**(27): 11471-11476.

Zalesky A, Fornito A (2009). A DTI-derived measure of cortico-cortical connectivity. *IEEE transactions on medical imaging* **28**(7): 1023-1036.

Zbozinek TD, Rose RD, Wolitzky-Taylor KB, Sherbourne C, Sullivan G, Stein MB*, et al* (2012). Diagnostic overlap of generalized anxiety disorder and major depressive disorder in a primary care sample. *Depression and anxiety* **29**(12): 1065-1071.

Zou YF, Wang F, Feng XL, Li WF, Tao JH, Pan FM*, et al* (2010). Meta-analysis of FKBP5 gene polymorphisms association with treatment response in patients with mood disorders. *Neuroscience letters* **484**(1): 56-61.

Tables

	CONCORDANT (12 subjects, 10 female)		DISCORDANT (20 subjects, 14 female)		HEALTHY (22 subjects, 10 female)		Group comparison
rs1360780 genotype	**Frequency**	**%**	**Frequency**	**%**	**Frequency**	**%**	**Fisher's *p*[a]**
CC	8	66.7	12	60	10	45.5	0.843
CT	2	16.7	4	20	6	27.3	
TT	2	16.7	4	20	6	27.3	
	Mean (SD)	**Range**	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**	**X-squared[b]; *p***
Age	37 (12.1)	23-51	33.8 (10.9)	21-53	34.5 (8.1)	22-48	0.8; 0.684
Total BSI	37.3 (26.7)	6-108	21.7 (13.5)	4-45	9.4 (8.1)	1-33	19.4; $6x10^{-5}$*
Depressive symptoms (BSI subscale)	8.7 (7.5)	1-24	4.4 (3.3)	0-12	1.5 (1.6)	0-6	14.6; $7x10^{-4}$*

Table 1. Demographic, psychopathological and genotypic data for DSM-IV diagnostic concordant, discordant and healthy MZ twin pairs. *Notes:* SD, standard deviation; BSI, Brief Symptom Inventory; [a]; Fisher's exact test for count data; [b], Kruskal-Wallis X-squared, as these variables were continuous; *, statistically significant *p*-value.

		Independent model				Additive model				Likelihood ratio test		
		β	**S.E.**	***p* (>|Z|)**	**C-index**	***β***	**S.E.**	***p* (>|Z|)**	**C-index**	X^2	**d.f.**	***p***
LEFT	**Strength**	-0.57	0.48	0.238	0.756	-0.75	0.7	0.286	0.797	*Additive model vs. Hippocampal metrics*		
	Betweenness c.	0.57	0.38	0.14		0.64	0.52	0.222		3.84	1	0.05
	Eigenvenctor c.	0.43	0.48	0.372		0.62	0.48	0.201		*Additive model vs.* FKBP5		
	FKBP5	-0.61	0.4	0.128	0.727	-0.83	0.56	0.141		6.05	3	0.109
RIGHT	**Strength**	-0.99	0.39	0.011**	0.756	-1.29	0.5	0.009**	0.806	*Additive model vs. Hippocampal metrics*		
	Betweenness c.	0.18	0.39	0.655		0.26	0.44	0.556		5.97	1	0.015
	Eigenvenctor c.	0.92	0.37	0.013**		1.42	0.47	0.002**		*Additive model vs.* FKBP5		
	FKBP5	-0.61	0.4	0.128	0.727	-1.16	0.48	0.016**		10.84	3	0.013

Table 2. Logistic regression test results for the association between depression and both hippocampal centrality and *FKBP5* genotype. The statistical analyses were performed independently for the left and right hippocampal centrality metrics. S.E., standard error; d.f., degrees of freedom; c., centrality; **, statistically significant *p*-value after FDR multiple testing adjustment.

		Independent model				Additive model				Likelihood ratio test		
		β	**S.E.**	***p* (>|Z|)**	**C-index**	***β***	**S.E.**	***p* (>|Z|)**	**C-index**	X^2	**d.f.**	***p***
RIGHT	**Strength (fam.)**	-0.84	0.41	0.039*	0.776	-1.14	0.5	0.021**	0.82	*Additive model vs. Hippocampal metrics*		
	Strength (env.)	-0.73	0.47	0.121		-0.83	0.54	0.121				
	Betweenness c. (fam.)	0.35	0.46	0.448		0.44	0.48	0.364		6.18	1	0.013
	Betweenness c. (env.)	-0.01	0.38	0.987		0.09	0.47	0.853		*Additive model vs.* FKBP5		
	Eigenvenctor c. (fam.)	0.75	0.41	0.069		1.26	0.45	0.005**				
	Eigenvenctor c. (env.)	0.73	0.37	0.049*		0.97	0.5	0.054		12.21	6	0.057
	FKBP5	-0.61	0.4	0.128	0.727	-1.22	0.49	0.012**				

Table 3. Logistic regression test results for the association between depression, the *FKBP5* genotype and both familial and environmental factors altering right hippocampal centrality. S.E., standard error; d.f., degrees of freedom; c., centrality; *, *p*-value < 0.05; **, statistically significant *p*-value after FDR multiple testing adjustment.

Figure legends

Figure 1. Schematic representation of the pre-processing steps and hippocampal network metrics considered. A: The diffusion weighted image is co-registered to the anatomical T1 3D volume, and the brain is subdivided into 82 ROIs (41 per hemisphere), to retrieve the NOS between brain regions as shown in B; C: the NOS between each pair of regions is mapped to an edge weight in a connectivity matrix; D: nodal centrality metrics are computed for both the left and right hippocampus (larger nodes in the brain networks). The brain network shown here represents the average connectivity values of 5 twins randomly chosen from different healthy pairs.

Figure 2. Right hippocampal centrality alterations associate with increased risk of depression, and the *FKBP5* gene moderates this association. For simplicity, *FKBP5* genotype effects are represented with two levels: C homozygotes and T (minor frequency allele) carriers. A: the right hippocampus (larger orange node) typically exhibits moderate nodal strength in healthy individuals (the network represents the mean nodal strength values of 5 twins randomly chosen from 5 different healthy pairs); B: a decrease in nodal strength of the right hippocampus associates with increased risk for depression; C: homozygotes for the C allele of *FKBP5*'s rs1360780 with low nodal strength of the right hippocampus show higher depression risk than T allele carriers; D: the right hippocampus (larger blue node) typically exhibits moderately high eigenvector centrality in healthy individuals (the network represents the mean nodal strength values of 5 twins randomly chosen from 5 different healthy pairs); E: increased eigenvector centrality of the right hippocampus associates with higher risk of depression; F: rs1360780 T allele carriers with high right hippocampal eigenvector centrality exhibit larger risk for depression than C homozygotes.

Figure 3. Familial factors altering right hippocampal centrality associate with increased risk of depression, and the *FKBP5* gene moderates this association. For simplicity, *FKBP5* genotype effects are represented with two levels: C homozygotes and T (minor frequency allele) carriers. A: the right hippocampus (larger orange node) typically exhibits moderate nodal strength in healthy individuals (the network represents the mean nodal strength values of 5 twins randomly chosen from 5 different healthy pairs); B: the *FKBP5* rs1360780 genotype interacts with the familial factors altering right hippocampal nodal strength to increase depression risk; C: the right hippocampus (larger blue node) typically exhibits moderately high eigenvector centrality in healthy individuals (the network represents the mean nodal strength values of 5 twins randomly chosen from 5 different healthy pairs); D: the *FKBP5* rs1360780 genotype interacts with the familial factors altering right hippocampal eigenvector centrality to increase depression risk.

Figures

Figure 1.

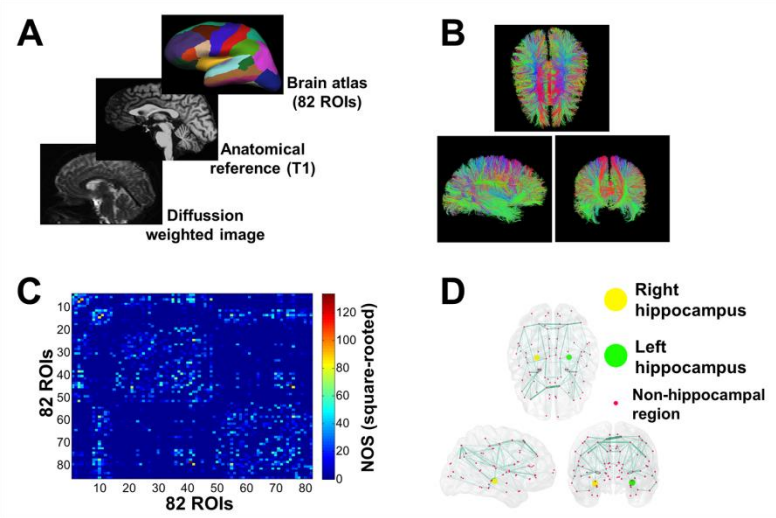


Figure 2.

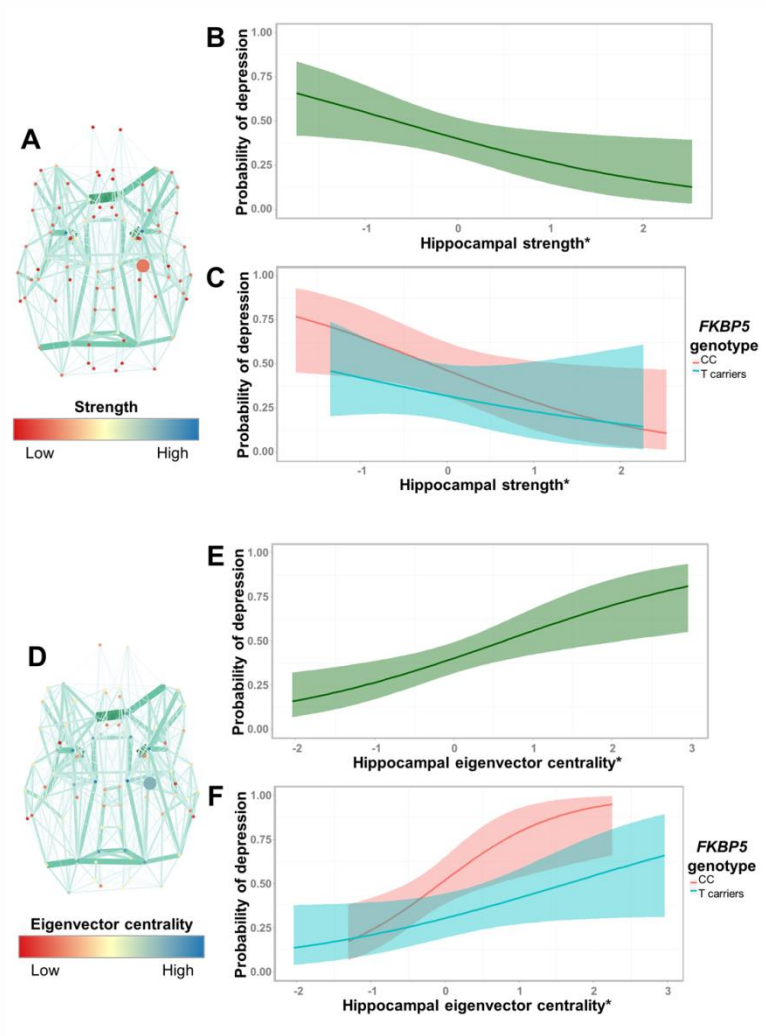
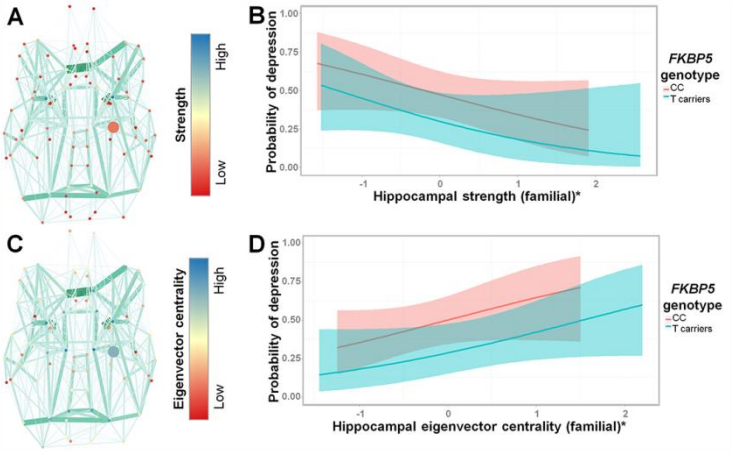


Figure 3.



Advisor's report on the contribution of the Ph.D. candidate to the article

Prof. Dr. Lourdes Fañanás Saura, associate professor at the Faculty of Biology, University of Barcelona, and supervisor of the present doctoral thesis by Aldo Córdova Palomera, hereby certifies that the participation of the Ph.D. candidate in the article "Polymorphic variation in *FKBP5* interacts with hippocampal connectivity to influence the risk for depression: a study in twins" included the following tasks:

- DTI data post-processing.
- Participation in study design.
- Statistical analyses.
- Writing of the first manuscript draft.
- Critical revision of the article.

Prof. Dr. Lourdes Fañanás Saura

Barcelona, June 30th 2015.

Altered amygdalar resting-state connectivity in depression is explained by both genes and environment

Córdova-Palomera A, Tornador C, Falcón C, Bargalló N, Nenadic I, Deco G, Fañanás L

Human Brain Mapping (2015) *In press*

DOI: http://dx.doi.org/10.1002/hbm.22876

Altered Amygdalar Resting-State Connectivity in Depression is Explained by Both Genes and Environment

Aldo Córdova-Palomera,[1,2] Cristian Tornador,[3] Carles Falcón,[4,5] Nuria Bargalló,[2,4,6] Igor Nenadic,[7] Gustavo Deco,[3,8] and Lourdes Fañanás[1,2]*

[1]*Unidad de Antropología, Departamento de Biología Animal, Facultad de Biología and Instituto de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain*
[2]*Centro de Investigaciones Biomédicas en Red de Salud Mental (CIBERSAM), Madrid, Spain*
[3]*Center for Brain and Cognition, Computational Neuroscience Group, Department of Information and Communication Technologies, Universitat Pompeu Fabra, Barcelona, Spain*
[4]*Medical Image Core facility, the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain*
[5]*Centro de Investigación Biomédica en Red en Bioingeniería, Biomedicina y Nanomedicina (CIBER-BBN), Zaragoza, Spain*
[6]*Centro de Diagnóstico por Imagen, Hospital Clínico, Barcelona, Spain*
[7]*Department of Psychiatry and Psychotherapy, Jena University Hospital, Friedrich Schiller University Jena, Jena, Germany*
[8]*Institució Catalana de la Recerca i Estudis Avançats (ICREA), Universitat Pompeu Fabra, Barcelona, Spain*

Abstract: Recent findings indicate that alterations of the amygdalar resting-state fMRI connectivity play an important role in the etiology of depression. While both depression and resting-state brain activity are shaped by genes and environment, the relative contribution of genetic and environmental factors mediating the relationship between amygdalar resting-state connectivity and depression remain largely unexplored. Likewise, novel neuroimaging research indicates that different mathematical representations of resting-state fMRI activity patterns are able to embed distinct information relevant to brain health and disease. The present study analyzed the influence of genes and environment on

Additional Supporting Information may be found in the online version of this article.

Aldo Córdova-Palomera and Cristian Tornador contributed equally to this work.

Contract grant sponsor: Spanish; Contract grant number: SAF2008-05674-C03-01; Contract grant sponsor: European Twins Study Network on Schizophrenia Research Training Network; Contract grant number: EUTwinsS, MRTN-CT-2006-035987; Contract grant sponsor: Catalan; Contract grant number: 2014SGR1636; Contract grant sponsor: Ministry of Science and Innovation in frame of ERA-NET NEURON; Contract grant number: PIM2010ERN-00642; Contract grant sponsor: ERC Advanced Grant DYSTRUCTURE; Contract grant number: 295129; Contract grant sponsor: FET Flagship Human Brain Project; Contract grant number: 604102; Contract grant sponsor: Spanish Government; Contract grant number: PSI2013-42091; Contract grant sponsor: FP7-ICT BrainScaleS; Contract grant number: 269921; Contract grant sponsor: CORONET; Contract grant number: 269459; Contract grant sponsor: EraNet Neuron SEMAINE; Contract grant number: PCIN-2013-026.

*Correspondence to: Prof. Dr. Lourdes Fañanás Saura, Unitat d'Antropologia, Departamento de Biologia Animal, Facultat Biologia, Universitat de Barcelona, Av. Diagonal, 645. Barcelona 08028, Spain. E-mail: lfananas@ub.edu

Received for publication 7 February 2015; Revised 5 May 2015; Accepted 2 June 2015.

DOI: 10.1002/hbm.22876

Published online 00 Month 2015 in Wiley Online Library (wileyonlinelibrary.com).

amygdalar resting-state fMRI connectivity, in relation to depression risk. High-resolution resting-state fMRI scans were analyzed to estimate functional connectivity patterns in a sample of 48 twins (24 monozygotic pairs) informative for depressive psychopathology (6 concordant, 8 discordant and 10 healthy control pairs). A graph-theoretical framework was employed to construct brain networks using two methods: (i) the conventional approach of filtered BOLD fMRI time-series and (ii) analytic components of this fMRI activity. Results using both methods indicate that depression risk is increased by environmental factors altering amygdalar connectivity. When analyzing the analytic components of the BOLD fMRI time-series, genetic factors altering the amygdala neural activity at rest show an important contribution to depression risk. Overall, these findings show that both genes and environment modify different patterns the amygdala resting-state connectivity to increase depression risk. The genetic relationship between amygdalar connectivity and depression may be better elicited by examining analytic components of the brain resting-state BOLD fMRI signals. *Hum Brain Mapp 00:000–000, 2015.*

Key words: amygdala; resting-state fMRI; environment; depression; signal processing; Hilbert transform; MZ twins

INTRODUCTION

Depressive disorders are becoming one of the leading causes of economic burden globally [Murray et al., 2012], with lifetime prevalence estimates reaching up to 20% in some cases [Kessler et al., 2007]. It is generally accepted that depression can partly be traced back to environmental factors such as adverse childhood familial environment, personality traits and stressful adult life events, among others [Kendler et al., 2003; Moffitt et al., 2007]. Likewise, research has demonstrated that an important extent of the risk for this psychopathological disorder can be explained by genetic influences and by the synergic effect of genes and environment [Domschke and Reif, 2012; Leonardo and Hen, 2006; Sullivan et al., 2000].

In this sense, parsing out genes and environment has enormous importance in the search for the etiological origins of mental disorders, as (i) it has been suggested that some alternative phenotypes (i.e., endophenotypes) may have a stronger link to the genetic basis of psychopathology than phenomenologically-derived clinical diagnoses [Glahn et al., 2014; Gottesman and Gould, 2003] and (ii) the identification of non-genetic influences on psychiatric conditions has several epidemiological and public health implications [Duncan and Keller, 2011; Freeman and Stansfeld, 2008; Lundberg, 1998].

Importantly, neuroimaging studies may provide an important reference framework to understand such complex multifactorial basis of disease [Blokland et al., 2012; Hyde et al., 2011; Paus, 2013], and research has shown that resting-state functional magnetic resonance imaging (fMRI) brain network alterations may serve as endophenotypic markers of neuropsychiatric disorders [Glahn et al., 2010].

Novel findings on the genetics of the connectome have pointed out that resting-state functional brain connectivity, as measured by blood-oxygen-level dependent (BOLD) fMRI signals, is influenced by both genes and environment. Specifically, three quantitative genetic studies have reported on the heritability of important resting-state fMRI network features. Glahn et al. [2010] analyzed data of 333 individuals from 29 families to conclude that several features of the default mode network are partly heritable and may be used as endophenotypic measures for psychiatric disorders. In addition, Fornito et al. [2011] examined a sample of 16 monozygotic (MZ) and 13 dizygotic adult twin pairs, and found strong genetic influences on global network efficiency. Likewise, a more recent report by van den Heuvel et al. [2013] using a sample of 21 MZ and 22 dizygotic twin pairs; their main findings indicate an important role for genetic factors on global network metrics of the resting brain. While these three reports constitute sound evidence of large genetic influences–as well as unique environmental factors–underlying BOLD fMRI connectivity patterns during rest, they have mainly focused on global network measures at either the whole-brain or the default mode network in healthy individuals. Complementarily, studies of candidate genes in samples of genetically unrelated individuals have suggested a role for genes such as *ZNF804A*, *APOE*, *COMT*, and *MET* as modulators of different resting-state fMRI network parameters in neuropsychiatric phenotypes such as Alzheimer's disease, schizophrenia and autism [Esslinger et al., 2011; Filippini et al., 2009; Liu et al., 2009; Martin et al., 2014; Rudie et al., 2012; Trachtenberg et al., 2012; Tunbridge et al., 2006].

Overall, these ample evidences indicate that resting-state networks extracted from BOLD fMRI measurements have separate genetic and environmental influences. Likewise, they suggest that the association between some specific genetic or environmental factors and several psychopathological outcomes may be mediated by the disruption of the resting-state networks. Despite this, to the best of our knowledge, no previous study has evaluated the potential genetic or environmental etiology of the resting-state fMRI network alterations underlying depressive disorders. This is an important issue, as recent studies of have consistently

shown alterations of the resting-state fMRI activity patterns in depressed individuals [Dutta et al., 2014].

Specifically, one of the most consistently replicated finding in resting-state fMRI studies of depression is a disruption of the amygdalar activity [Cullen et al., 2014; Dutta et al., 2014; Wang et al., 2014; Zeng et al., 2014]. Briefly, these recent reports have shown alterations of amygdalar connectivity in adolescent depression [Cullen et al., 2014]; disrupted connectivity strength in major depressive disorder patients who previously suffered childhood neglect [Wang et al., 2014]; and important amygdalar modifications that may serve to discriminate a depressed from a healthy brain [Zeng et al., 2014].

Other alterations of the amygdalar activity in resting-state fMRI networks of depressed individuals have widely been described across the literature [Dutta et al., 2014]. For instance, a number of disruptions in the affective network comprising the amygdala, the hippocampus and related regions have been found in depression [Zeng et al., 2012; Zhang et al., 2014a]. These results are somehow consistent with the findings by Sheline and colleagues, who described alterations in sets of brain regions comprising the amygdala, such as the affective and the default mode networks [Sheline et al., 2009, 2010]. Similarly, network alterations of pathways connecting the amygdala and the prefrontal cortex have been found after selective serotonin reuptake inhibitor antidepressant treatment [McCabe and Mishor, 2011]. Overall, these and other related studies support the idea of a disruption of amygdalar resting-state connectivity as one of the main mechanisms underlying large-scale network disruptions in depression [Kaiser et al., 2015].

These resting state connectivity alterations index modifications in the communication between the amygdala and a wide set of regions across the whole brain. In the context of large-scale networks, these disruptions may be thought of as changes in the information processing mechanisms between the amygdala and other cerebral structures [van den Heuvel and Hulshoff Pol, 2010]. It is worth mentioning that resting-state communication between regions can be understood from several alternative viewpoints, some of which have–at least in principle–their own potential relevance in clinical settings [Lee et al., 2013]. Among numerous methods to study brain activity at rest, one of the most promising approaches is the assessment of the spatio-temporal patterns of coactivation between regions through network modeling [Richiardi et al., 2011; Smith, 2012; van den Heuvel and Hulshoff Pol, 2010]. Conventionally, low-frequency periodic time courses of resting-state activation patterns are extracted from a set of anatomical regions, and strong first-order correlations in the temporal configuration of activity between two anatomically separated regions is abstracted as a functional connection [De Vico Fallani et al., 2014; van den Heuvel and Hulshoff Pol, 2010].

While this method of extracting networks from correlated temporal activity between brain regions has undoubtedly led to outstanding neurobiological and clinical findings [Lee et al., 2013], it is essential recognizing that periodic waves can carry information via several different coding systems, some of which constitute the basis of standard devices in communications theory and related technical disciplines [O'Reilly, 1984]. In effect, there is compelling evidence that higher-order brain function may be tightly related to neural communication emerging from the coherent oscillatory activity of the brain regions at specific frequencies [Fries, 2005, 2009]. Namely, information can efficiently be coded and transmitted within different components of neuronal activity waves, which are not straightforwardly deduced by examining its raw time course.

For instance, a common method to analyze brain signals in magnetoencephalography and electroencephalography is the estimation of their analytical representation, which allows transforming one time function—a magnetic or an electric wave recorded over time—into two time functions with meaningful mathematical properties. Of note, novel neuroimaging research has shown that distinct properties of wave-like temporal patterns of fMRI brain activity are able to embed information of particular biological relevance [Glerean et al., 2012], which may have implications for depression [Liu et al., 2014]. It is important noting that recent findings indicate that analytic properties such as the phase or the amplitude envelope of resting-state fMRI oscillations may explain an important extent of the relationship between brain structure and function [Glerean et al., 2012; Guggisberg et al., 2014; Ponce-Alvarez et al., 2015], suggesting such properties could be feasible endophenotype candidates in depression.

Considering these elements, the current study was aimed at determining the relevance of genetic and environmental factors leading to depression by altering amygdalar resting-state fMRI activity. To do so, whole brain resting-state fMRI time series were extracted from a group of 48 MZ twins (24 pairs) informative for depressive psychopathology. Insofar as members of a monozygotic (MZ) twin pair have almost identical DNA sequences, this work studied their phenotypic similarities and differences to obtain insights on familial and environmental influences. Different centrality measures of amygdalar connectivity were estimated by constructing whole-brain networks from resting-state time series, using two distinct methodologies: (i) the conventional examination of correlations between band-pass filtered time series [Smith et al., 2013] and (ii) a technique for extracting analytical components of fMRI signals, which is able to explain a considerable extent of the relationship between brain morphology and resting-state fMRI activity [Glerean et al., 2012; Ponce-Alvarez et al., 2015].

METHODS

Sample Description

The present sample was gathered from a set of 115 Spanish Caucasian adult twin pairs (230 individuals) from

the general population, who gave permission to be contacted for research purposes. All twins were contacted by telephone and invited to participate in a general study of adult cognitive and psychopathological traits. A battery of psychological and neurocognitive tests was administered to the twins by trained psychologists. Similarly, they were interviewed for medical records. Exclusion criteria applied were age under 18 and over 65 years, current substance misuse or dependence, a medical history of neurological disturbance and presence of sensory or motor alterations. 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 in accordance with the Declaration of Helsinki.

Zygosity of all pairs was assessed by genotyping 16 highly polymorphic microsatellite loci from DNA samples (SSRs; PowerPlex® 16 System Promega Corporation). Identity on all the markers can be used to assign monozygosity with greater than 99% accuracy [Guilherme et al., 2009]. In the whole sample (115 twin pairs), 86 duos were MZ.

From that group of participants, using the previously collected data, a subset of 54 individuals (27 MZ twin pairs) was selected, as they were informative for obstetric and psychopathological traits and gave consent to participate in the MRI part of the present study.

Twins included in this subset of 54 participants met the following criteria: (a) age at scan between 20 and 56 years, (b) both twins right-handed, and (c) none of the twins manifested liability for DSM-IV-R psychiatric diagnoses other than depression and/or anxiety. Pairs where one or both twins manifested either neurological or major medical illnesses were excluded as well (see Measures).

After this point, due to image artifacts or lack of data about six participants, the final sample (i.e., the subset included in all statistical analyses) consisted of 48 individuals (20 males, mean age: 33.6 years).

Psychometric Measures

To evaluate liability for psychopathology in this general population sample, a clinical psychologist applied the Structural Clinical Interview for DSM-IV Axis I Disorders (SCID-I) [First, 1997] in a face-to-face interview to screen for presence of any lifetime psychiatric disorder.

Participants were asked to report if they had received pharmacological or psychological treatment or had consulted a psychiatrist or psychologist as they first participated in the study. Only one individual had life-time exposure to psychopharmacological treatment for depression. However, excluding this individual from the group analyses did not change the significance of the results.

A clinical psychologist applied the Structural Clinical Interview for DSM-IV Axis I Disorders (SCID-I) in a face-to-face interview to screen for the presence of any lifetime depression or related anxiety spectrum disorder. In this sample, six individuals with a history of (mainly) anxious psychopathology were included in the psychopathology-affected group. This apparently broad category of outcomes was used in conjunction with evidence on the comorbidity, shared etiopathology and diagnostic criteria overlap between depressive and anxious disorders [Mosing et al., 2009; Ressler and Mayberg, 2007; Wittchen et al., 2002; Zbozinek et al., 2012], as well as taking into account evidences of amygdalar resting-state alterations across both diagnoses [Oathes et al., 2014]. Remarkably, repeating the statistical analyses removing predominantly anxious individuals did not alter the significance of the results.

Overall, there were ten healthy pairs, six concordant and eight discordant pairs for lifetime DSM-IV diagnoses. Additionally, current depression status and other psychiatric symptoms were evaluated using the Brief Symptom Inventory (BSI) [Derogatis and Melisaratos, 1983; Ruiperez et al., 2001]. The BSI is a self-administered 46-item screening instrument aimed at identifying the experience of psychopathological symptoms during the last 30 days. It is composed by six subscales (depression, phobic anxiety, paranoid ideation, obsession-compulsion, somatization, and hostility) conceived for use in both clinical and nonclinical samples. Items are rated on a five-point scale of distress, according to self-perception of symptoms. Descriptive data from the current sample is summarized in Table I. As shown, all diagnostic concordant pairs were females, and twins with no lifetime history of DSM-IV diagnosis had lower BSI scores—fewer self-reported symptoms—in both the depressive subscale and the whole questionnaire. In addition, neurocognitive data for this sample was collected using the Wechsler Adult Intelligence Scale [Sattler, 2001; Wechsler et al., 1997]. The intelligence quotient (IQ) was estimated from five subtests (block design, digit span, matrix reasoning, information and vocabulary) of this battery. As shown in Table I, IQ scores were similar to those from demographically similar samples [Lynn and Meisenberg, 2010]; of note, there were no intra-group differences in IQ, indicating that neurocognitive influences on resting-state brain signals [Douw et al., 2011; Wang et al., 2011] are not likely to influence subsequent statistical analyses.

MRI Acquisition and Preprocessing

The images were acquired at the MRI Unit of the Image Platform (IDIBAPS, Hospital Clínic de Barcelona), using a TIM TRIO 3 T scanner with an 8-channel head coil (Siemens, Erlangen, Germany). Resting-state fMRI data comprised 210 echo-planar (EPI) BOLD sensitive volumes (TR = 2790 ms, TE = 30 ms, 45 axial slices parallel to anterior-posterior commissure plane acquired in interleaved order, 3.0 mm slice thickness and no gap, FOV = 2075 × 1344 mm^2, voxel size = 2.67 × 2.67 × 3 mm^3).

Additionally, high resolution 3D structural datasets were obtained for anatomical reference, using a T1-weighted magnetization prepared rapid gradient echo, with the following parameters: 3D T1-weighted MPRAGE sequence, TR = 2300

TABLE I. Demographic, psychopathological and neurocognitive data for DSM-IV diagnostic concordant, discordant, and healthy MZ twin pairs

	Concordant (12 subjects, 10 female)		Discordant (16 subjects, 10 female)		Healthy (20 subjects, 8 female)		Group comparison X-squared[a]; *P*
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
Age	42.5 (13)	22–54	37 (10.9)	20–50	30.3 (7.3)	19–39	5.9; 0.052
IQ	105.1 (12.5)	87–127	108.1 (11.8)	87–131	110.5 (5.5)	103–118	1.9; 0.393
Current psychopathology (total BSI)	27.9 (16.5)	6–57	20.9 (13.3)	4–45	10.6 (9.3)	1–33	8.7; 0.013[b]
Current depressive symptoms (BSI subscale)	6.9 (6.5)	1–20	3.5 (2.7)	0–9	1.7 (1.8)	0–6	6.4; 0.04[b]

Notes: SD, standard deviation; IQ, intellectual quotient; BSI, Brief Symptom Inventory
[a]Kruskal–Wallis X-squared, as these variables were continuous
[b]Statistically significant *P*-value

ms, TE = 3.03 ms, TI = 900 ms, Flip angle = 9°, 192 slices in the sagittal plane, matrix size=256 × 256, 1 mm^3 isometric voxel.

Resting-sate time series were obtained by means of standard image processing protocols implemented in the Statistical Parametric Mapping software, version 8 (SPM8) [Friston et al., 1995], running under MATLAB (The Mathworks, Natick, MA). Briefly, after correction of slice-timing differences and head-motion, the fMRI images were coregistered to the 3D (T1) anatomical image and the mean functional image; then, the images were spatially normalized to the standard stereotaxic space MNI [Evans et al., 1993]. Additionally, artifacts related to blood pulsation, head movement and instrumental spikes were removed from the BOLD time series in MNI space, using independent component analysis as implemented in GIFT [Calhoun et al., 2009; Sui et al., 2009]. No global signal regression or spatial smoothing was applied. Mean BOLD time series were extracted from the 90 regions of interest (ROIs) in the standard Automatic Anatomical Labeling (AAL) atlas [Tzourio-Mazoyer et al., 2002]. The atlas was previously masked with the binarized subjective tissue probability maps to isolate the mean value of the regions from the gray matter via a conventional protocol [Power et al., 2014; Villain et al., 2010]. The following mask was used: [Atlas * (GM > WM) * (GM > CSF) * (GM > 0.1)], where GM stands for gray matter, WM is the white matter and CSF stands for cerebrospinal fluid. Afterward, the BOLD time series for each region were band-pass filtered within the resting-state fMRI narrowband going from 0.04 to 0.07 Hz [Achard et al., 2006; Glerean et al., 2012]. A schematic representation of these steps is shown in sections A and B of Figure 1.

Statistical Analyses

Extraction of functional connectivity networks for each individual

Two different approaches were used in this study to estimate functional connectivity from the band-passed time series described above. First, a conventional approach to examine correlations between fMRI BOLD time series [ninety $x(t)$ series per individual: one for each AAL ROI] was used [Smith et al., 2013]. Briefly, the partial correlation matrix was obtained from the 90 ROIs at the 210 slices scanned over time. Partial correlation coefficients give a measure of the extent of association between two variables (i.e., every pair of ROIs) controlling for the effect of the other variables (i.e., the remaining ROIs). This step produced a 90 × 90 matrix representing the functional connectivity (FC) between each pair of brain ROIs, which was then normalized using Fischer's z transform [Fox et al., 2005; Jenkins and Watts, 1968]. Then, following a previous technical report [Schwarz and McGonigle, 2011], a soft threshold procedure was implemented to remove negative edges, as their particular network topology can drastically alter the properties of brain fMRI connectivity networks. The leftmost part of sections D and E in Figure 1 schematizes this procedure, applied to a random individual's data.

Complementarily, in view of recent reports showing a major role for the analytic components of resting-state BOLD time series in shaping the relationship between structure and function of the brain [Ponce-Alvarez et al., 2015], the time series from the 90 ROIs were further processed. Specifically, the analytic representation of the real valued signals built from the band-passed (0.04–0.07 Hz) BOLD time series was computed with the Hilbert transform. Namely, given a BOLD time series $x(t)$ for a particular ROI, its analytic representation is the complex signal

$$x_a(t) = x(t) + i\mathrm{H}[x(t)],$$

where $H[\cdot]$ is the Hilbert transform, and i stands for $\sqrt[2]{-1}$. This new signal $x_a(t)$ has the same Fourier transform as $x(t)$, but is defined only for positive frequencies. Likewise, if $x(t)$ is expressed as an amplitude-modulated signal $a(t)$ with carrier frequency $\varphi(t)$, so that $x(t) = a(t)\cos[\varphi(t)]$. Its Hilbert transform gives

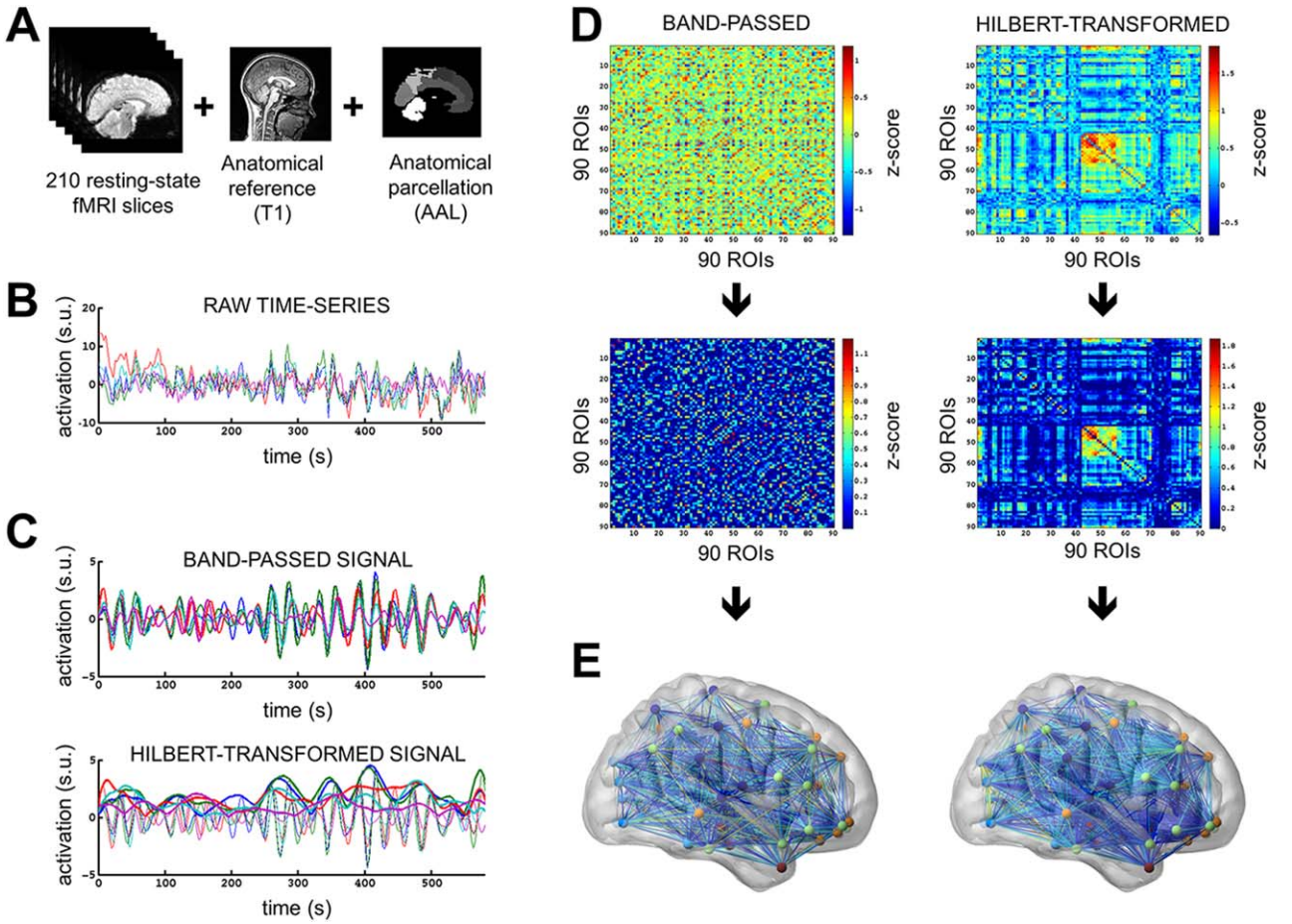


Figure 1.

Schematic representation of the construction of two functional networks for one brain. (**A**) The 210 resting-state fMRI volumes (slices) are co-registered to the anatomical T1 3D reference volume, and each voxel is mapped to one of the 90 ROIs in the AAL atlas. (**B**) After artefact removal, a time-series of the mean (BOLD) activation probability for each of the 90 ROIs is obtained. This is built upon the 210 fMRI slices acquired through 9:56 minutes of scan time. (**C**) (Top): A band-pass filter is applied to obtain the resting-state fMRI narrowband signal (0.04–0.07 Hz). (Bottom): An additional processing step to the above band-passed (0.04–0.07 Hz) time-series: the envelope extraction using the Hilbert transform. (**D**) Two partial correlation matrices are obtained from the previous time-series (band-passed and Hilbert transformed); they are z-transformed to normalize correlation values across individuals. Warm (cold) colors in these matrices represent large (small) correlation values between ROIs. The left tail of these correlation matrices (i.e., edges with negative z-scores) are set to 0 following a soft-thresholding procedure. (**E**) Graph-theoretical measures of nodal centrality are obtained for each brain region. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

$$x_a(t) = a(t)e^{i\phi(t)},$$

where $a(t)$ represents the instantaneous envelope and $\phi(t)$ stands for the instantaneous phase. In the present study, the value of the signal envelope $a(t)$ is used to later estimate a 90 × 90 partial correlation matrix as described above, which is later z-transformed and soft-thresholded. The lowermost part of sections D and E in Figure 1 represent this procedure applied to data from one participant.

Measures of amygdalar centrality within the brain network

The AAL 90 atlas contains two amygdalar ROIs, from the left and right brain hemispheres. Graph-theoretical measures of amygdalar centrality within the brain were computed to later evaluate potential impairments amygdalar resting-stat fMRI activity within the context of the whole brain, and parsing out genetic and environmental factors. It is worth noting that there is previous evidence of differential genetic and environmental influences on

BOLD fMRI-derived graph-theoretical metrics in the brain [van den Heuvel et al., 2013], which justifies the adoption of this perspective.

Four different nodal centrality measures were separately computed for both left and right amygdala ROIs (i.e., eight independent scalars for each individual): (i) degree, (ii) betweenness centrality, (iii) local clustering coefficient, and (iv) eigenvector centrality. These four specific quantities were included in view that they have widely been studied in the literature [Borgatti and Everett, 2006], and as most nodal centrality metrics can be obtained by parameter-tuning from degree to eigenvector centrality, which represent limiting cases [Benzi and Klymko, 2015]. Due to the soft-thresholding procedure [Schwarz and McGonigle, 2011] adopted here, the weighted version of these metrics was estimated, and the centrality measures were computed using the Massachusetts Institute of Technology's *Matlab Tools for Network Analysis* toolbox [Bounova and de Weck, 2012]. Detailed mathematical descriptions of these metrics can be found elsewhere [Borgatti and Everett, 2006; Bounova and de Weck, 2012].

In the present context, these quantities represent: the number of links directly incident upon the amygdala (i, degree), how often the amygdala bridges through the shortest path between any two other nodes (ii, betweenness centrality), the extent to which the amygdala's neighbors are neighbors of each other (iii, local clustering coefficient), and the frequency of connections between the amygdala and highly connected brain regions (iv, eigenvector centrality).

Intersubject analyses: estimation of genetic and environmental influences on amygdalar resting-state activity

To determine the relationship between depression risk and both genetic and environmental factors altering amygdalar functional connectivity at rest, general linear models were executed, using a regression procedure described elsewhere [Begg and Parides, 2003], as implemented using the R's software packages *rms* and *mztwinreg* [Córdova-Palomera, 2015; Harrel, 2014; R Development Core Team, 2011]. Specifically, the logistic model

$$\text{logit}(\pi_{ij}) = \beta_0 + \beta_B \mu_{i.} + \beta_W (X_{ij} - \mu_{i.})$$

is built by first obtaining estimates of both (a) familial factors (genetic plus shared environment, β_B) and (b) unique environmental influences (from nonshared events within a pair, β_W) on a graph-theoretical nodal centrality measure (i.e., degree, betweenness centrality, local clustering coefficient or eigenvector centrality). Subindex $i \in \{1, \ldots, n\}$ stands for pair number (here, $n = 24$ MZ pairs) and $j \in \{1,\ 2\}$ refers to co-twin number (randomly assigned). π_{ij} stands for the probability that co-twin j from the i-th pair has of being affected by depression. β_0 represents the intercept; $\mu_{i.} = (X_{i1} + X_{i2})/2$ is the mean nodal centrality measure of the i-th pair, and $X_{ij} - \mu_{i.}$ denotes the deviation of cotwin j from the pair's mean nodal centrality measure. In the next set of analyses, each of the four nodal centrality measures is considered in a regression model; left and right amygdalar measures (parsed out as familial and unique environmental estimates) are included in it. To control for potential confounding demographics (Table I), all analyses were adjusted for gender and age. Besides, the Huber–White method was used to adjust the variance-covariance matrix of these regression fits, to account for the non-independence of twin data (i.e., heteroskedasticity) [DeMaris, 1995; Harrel, 2014; White, 1982]. Previous reports have shown the usefulness of this between-within model to parse out familial and unique environmental factors underlying phenotypic relationships [Carlin et al., 2005; Frisell et al., 2012].

Power analysis estimations for these multiple regression models were conducted following standard protocols [Cohen, 1988; Champely, 2012]. After including all covariates, each of the above mentioned models has 4 and 71 numerator and denominator degrees of freedom. Using the conventional significance level of 0.05, the present sample has a power of 80.6% to detect moderately large effects (Cohen's $f^2 \sim 0.35$), which are expected for neuroimaging endophenotypes of brain disorders [Glahn et al., 2007; Rose and Donohoe, 2013]. However, to examine all 90 ROIs, lowering the significance level to 0.05/90—to adjust for multiple testing—would have decreased the power to 20.9%. Instead of analyzing all 90 ROIs, and given the scope and the aims of the present study, five different types of amygdalar communication mechanisms were studied in detail. This choice of biologically feasible mechanisms in hypothesis-driven research to avoid overly conservative multiple testing adjustments has previously been proposed as an adequate paradigm in epidemiological and medical statistics [Cook and Farewell, 1996; Perneger, 1998]. Exploratory *post-hoc* tests compared the number of statistical associations found for the amygdala with the results that would have been found for the other 89 ROIs (see Results and Supporting Information Figure); they suggested that the amygdala could be the most relevant ROI in this fMRI design.

Although part of the phenotypical variance of depression may be explained by gene-environment interaction effects, the current data may have limited statistical power to detect such associations [Jaccard and Wan, 1995; Jaccard et al., 1990; Mathieu et al., 2012]. Accordingly, the results presented here focus mainly on the separate influence of familial and environmental factors.

Finally, when appropriate, multiple testing adjustments of the regression coefficients from the different (independent) regression models were implemented using the false discovery rate (FDR) approach. The adoption of this Type-I error rate correction is based on previous literature of statistical analysis for biological and behavioral data [Benjamini and Hochberg, 1995; Cook and Farewell, 1996; Glickman et al., 2014; Liu et al., 2004; Nakagawa, 2004; Perneger, 1998].

TABLE II. Descriptive data of the four centrality measures analyzed for both left and right amygdalar ROIs

		Individual level (n = 48 subjects)				Intrapair differences (n = 24 MZ pairs)			
		Amplitude correlation[a]		Amplitude envelope correlation (Hilbert-transformed)[b]		Amplitude correlation[a]		Amplitude envelope correlation (Hilbert- transformed)[b]	
Nodal centrality measure	Brain hemisphere	Mean (S.D.)	Range	Mean (S.D.)	Range	Spear-man's Rho[c]	P-value	Spear-man's Rho[c]	P-value
Degree	Left	12.9 (2.3)	9.1 to 19.1	36.7 (21.9)	6.3 to 110	−0.05	0.827	0	0.991
	Right	12.5 (2)	8.7 to 15.9	32.2 (19.5)	4.4 to 88.9	−0.2	0.357	0.11	0.617
Betweenness centrality	Left	0.2 (0.2)	0 to 1.1	0.3 (0.3)	0 to 1.2	0.3	0.159	−0.22	0.308
	Right	0.3 (0.4)	0 to 1.3	0.4 (0.5)	0 to 2.3	−0.51	0.014[c]	−0.22	0.307
Local clustering coefficient	Left	0.3 (0.1)	0.2 to 0.4	0.4 (0)	0.3 to 0.5	−0.43	0.043[c]	0	0.977
	Right	0.3 (0.1)	0.2 to 0.4	0.4 (0)	0.3 to 0.5	−0.21	0.331	0.12	0.589
Eigenvector centrality	Left	0 (0.1)	−0.2 to 0.1	0 (0.1)	−0.1 to 0.2	−0.07	0.739	0.1	0.663
	Right	0 (0.1)	−0.1 to 0.1	0 (0.1)	−0.1 to 0.1	−0.09	0.682	0.05	0.834

As mentioned above (Section Extraction of functional connectivity networks for each individual), two different functional connectivity network construction procedures were employed. Namely,
[a]The conventional soft-thresholding method of band-passed low-frequency oscillations [Smith et al., 2013].
[b]The amplitude envelope extraction from the previous band-passed time-series [Glerean et al., 2012].
[c]Statistically-significant P-value. S.D., standard deviation; MZ, monozygotic.

RESULTS

As mentioned before, though scarce, there is some evidence of dissimilarities within MZ pairs for graph-theoretical measures of brain functional connectivity at rest [van den Heuvel et al., 2013]. Hence, to verify that these parameters are driven not only by genetic but also by environmental factors, a preliminary step consisted in the estimation of intrapair correlations in graph-theoretical-based connectivity measures. Table II shows these descriptive parameters.

As presented in Table II, there was an important extent of MZ intrapair differences across all these metrics, as indicated by their low and mostly nonsignificant correlation coefficients. Of note, even when there were statistically significant intrapair correlations in nodal centralities (left local clustering coefficient and right betweenness centrality in the conventional processing protocol), the correlation coefficients were moderate (Spearman's rho equaling −0.41 and −0.51, respectively). Remarkably, no statistically significant intrapair correlations are observed when the resting-state time series are Hilbert-transformed. These observations justify the ensuing procedure to parse out genetic and environmental factors underlying amygdalar resting-state activity centrality.

It is also worth noting that the nodal centrality measures computed from the amplitude envelope of the low-frequency envelope (i.e., using the Hilbert-transform of the 0.04–0.07 Hz signal) consistently showed less intrapair correlations than their non-transformed counterparts (typically smaller absolute rho and larger P-values, as displayed in Table II). This fact probably indicates that some environmental factors are cannot be straightforwardly deduced from the raw fMRI time-series but may probably be disclosed by performing different signal processing techniques such as amplitude envelope extraction.

The last set of analyses conducted here allowed the estimation of both genetic and environmental influences on amygdalar resting-state fMRI activity that may influence risk on depressive psychopathology. As indicated in Table III, the conventional brain network construction by examining the (band-pass filtered) low-frequency oscillations during rest indicated that nongenetic factors that alter amygdalar communication with the whole brain can increase depression risk. More explicitly, nongenetic factors alter left amygdalar connectivity to increase depression risk in two ways: by increasing its degree centrality and by decreasing its local clustering coefficient (i.e., left-amygdalar hypersynchronization with the rest of the brain, and less synchronization between its functional neighbors). Likewise, environmental factors may induce reductions in right-amygdalar betweenness centrality (i.e., its intermediate role in the synchrony between any two brain regions) to rise depression risk. It is important noting that these three associations between environmental factors altering amygdalar activity and depression risk should be taken with caution, as two of them were significant only at a trend level when adjusting for multiple comparisons (FDR-adjusted P-values: left degree = 0.08, left clustering coefficient = 0.02, right betweenness centrality = 0.075).

The above mentioned environmental influences on resting-state amygdalar connectivity—for both band-passed amplitude correlations and Hilbert-transformed amplitude envelope correlations—, as well as how they may influence depression risk, are depicted in Figure 2. Moreover, it is interesting noticing that the influence of familial factors on amygdalar connectivity was detected

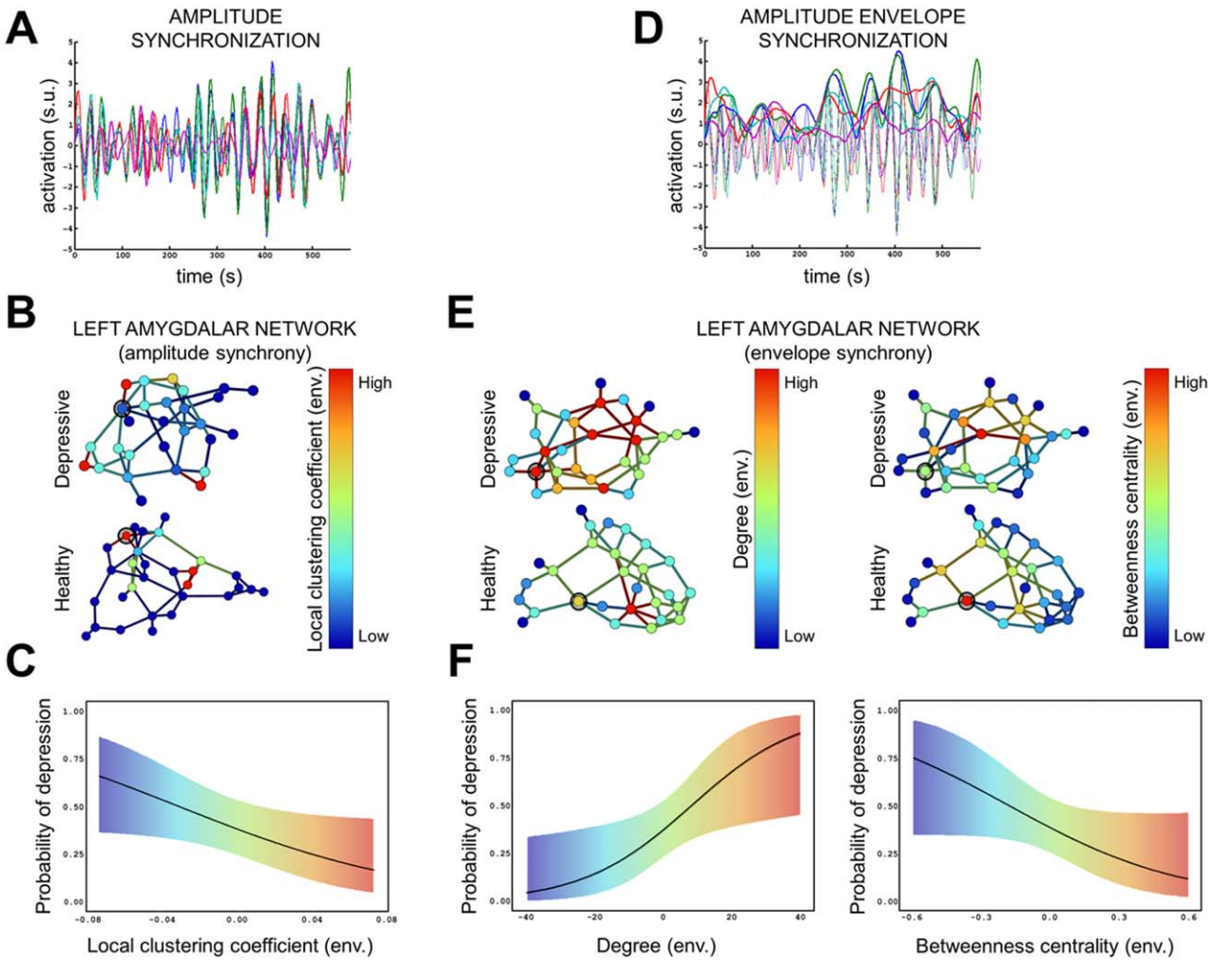


Figure 2.

Environmental factors altering resting-state amygdalar connectivity relate to depression. Only results that survived FDR multiple testing adjustments are shown. (**A**) The conventional approach to resting-state connectivity analysis, based on the estimation of a whole-brain partial correlation matrix allowed detecting environmentally-induced amygdalar connectivity alterations potentially linked to depression (as shown in sections B and C). (**B**, **C**) The environmental influences on the left amygdala (highlighted node) may decrease its local clustering coefficient to induce depression. (**D**) The amplitude envelope obtained from the Hilbert-transformed resting-state signal allowed identifying more environmentally-induced modifications of the amygdalar connectivity that could be related to depression (as shown in sections E and F). (**E**, **F**) Some environmental factors may alter the left amygdala (highlighted node) to induce depression, mainly by increasing its nodal degree (leftmost panels in E, F) and decreasing its betweenness centrality (rightmost panels in E, F). For simplicity, the logistic regression curves shown in C and F were estimated from univariate models. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

only when computing the Hilbert-transformed amplitude envelope correlations (Fig. 3).

Notably, the additional processing step of amplitude envelope estimation using the Hilbert transform showed complementary results, with logistic regression models outperforming their conventional processing counterparts. As indicated by the better discrimination indexes obtained using amplitude envelopes (overall R^{2}'s in Table III), fMRI resting-state amygdalar centrality measures typically provide better indications of depression risk when they are derived from the Hilbert-transformed signal. This was the case when analyzing left and right amygdalar degree, betweenness centrality and local clustering coefficient. It is important mentioning that amygdalar eigenvector centrality did not seem related to depression risk in none of the models considered here.

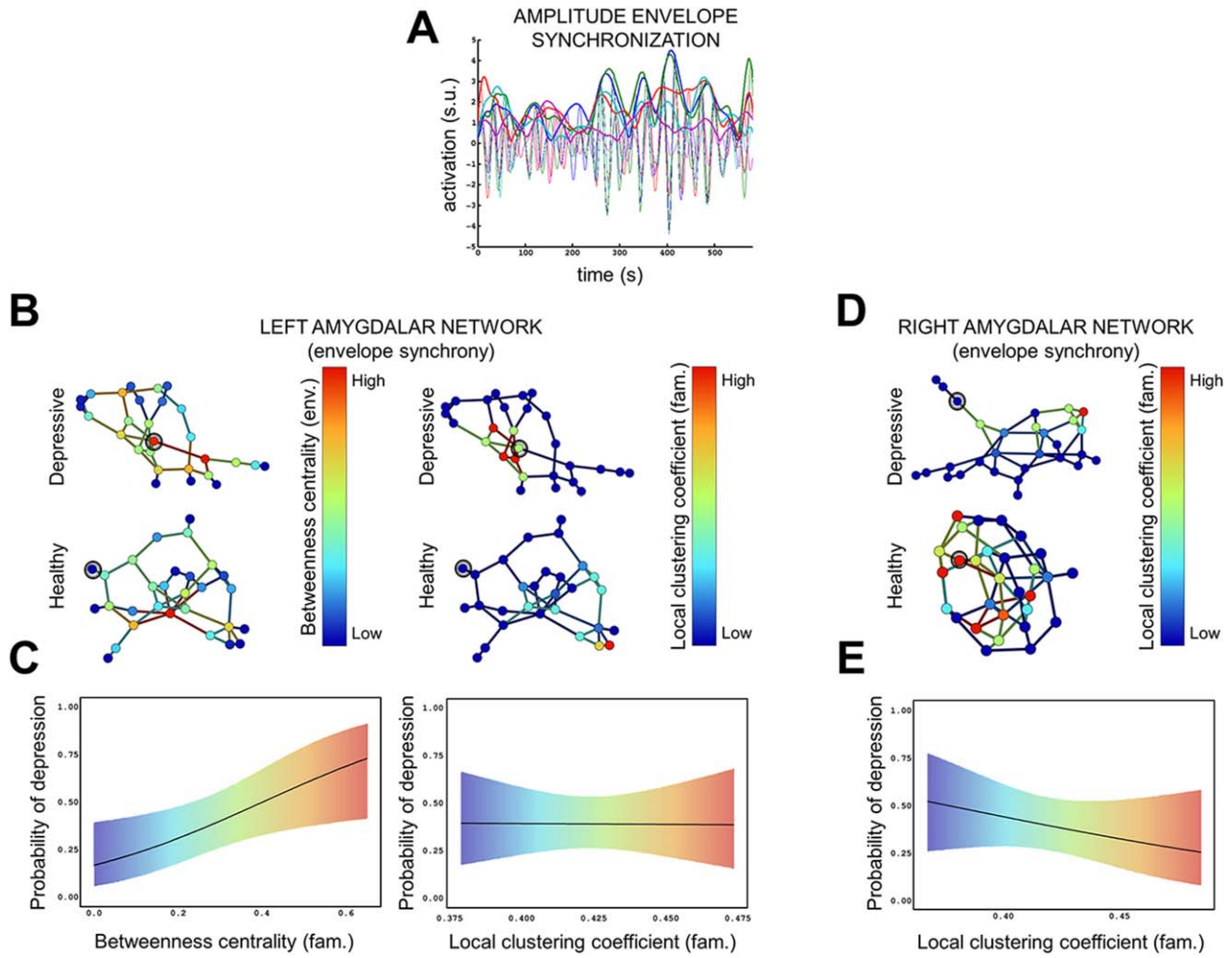


Figure 3.

Familial factors altering resting-state amygdalar connectivity relate to depression. Only results that survived FDR multiple testing adjustments are shown. (**A**) The amplitude envelope, derived from the analytical representation of resting-state fMRI signals, allowed identifying amygdalar connectivity alterations induced by familial factors (genes and shared environment) potentially linked to depression (as shown in sections B–E). (**B**, **C**) The familial influences on the left amygdala (highlighted node) may increase its betweenness centrality (leftmost panels in B, C) and decrease its local clustering coefficient (rightmost panels in B, C) to induce depression. (**D**, **E**) Some familial factors may alter the right amygdala (highlighted node) to induce depression, mainly by decreasing its local clustering coefficient. For simplicity, the logistic regression curves shown in C and E were estimated from univariate models. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Remarkably, the amplitude envelope of the whole-brain resting state fMRI signal confirmed the previous finding of an environmentally induced amygdalar hypersynchronization in depression (FDR-adjusted *P*-value for degree centrality = 0.007), and also suggested a role for left amygdalar betweenness centrality (FDR-adjusted *P*-value = 0.007). While the above mentioned findings mainly support the role of amygdalar connectivity alterations in mediating associations between exclusively environmental factors and depression, the results of the novel Hilbert-transform approach showed a different and very interesting property: they permitted recognizing that some familial factors (i.e., genes plus shared environment) that determine amygdalar resting-state fMRI activity are significantly contributing to the depression risk. Specifically, some familial factors seemed to alter both left and right amygdalar clustering coefficients (a measure of the synchrony among brain regions partly synchronized with the amygdala) to induce depression (FDR-adjusted *P*-values: left = 0.011, right- = 0.021). Likewise, familial factors increased both left and right amygdala's betweenness centrality in depression (FDR-adjusted *P*-values: left = 0.007, right = 0.064).

TABLE III. Estimation of the genetic and environmental influences on amygdalar resting-state activity that lead to depressive psychopathology

		Amplitude correlation[a]					Amplitude envelope correlation (Hilbert-transformed)[b]				
		Familial factors		Unique environment			Familial factors		Unique environment		
Nodal centrality measure	Brain hemisphere	β_B	*P*-value	β_W	*P*-value	Overall R^2	β_B	*P*-value	β_W	*P*-value	Overall R^2
Degree	Left	0.12	0.609	0.39	0.04[c]	0.224	0.01	0.817	0.07	0.002[d]	0.316
	Right	0.06	0.771	−0.17	0.41		0	0.985	0	0.853	
Betweenness centrality	Left	1.69	0.489	0.2	0.862	0.257	7.01	0.002[d]	−4.65	0.003[d]	0.508
	Right	−1.47	0.415	2.4	0.019[c]		2.44	0.032[c]	−0.41	0.739	
Local clustering coefficient	Left	−0.62	0.956	−18.4	0.005[d]	0.263	82.18	0.006[d]	−13.87	0.316	0.317
	Right	−2.34	0.833	4.86	0.474		−70.55	0.005[d]	12.98	0.366	
Eigenvector Centrality	Left	5.6	0.771	10.95	0.454	0.219	−7.38	0.471	2.9	0.73	0.18
	Right	−7.56	0.723	−18.73	0.222		0.13	0.992	0.5	0.937	

[a]The conventional soft-thresholding method for band-passed low-frequency oscillations [Smith et al., 2013].
[b]The amplitude envelope extraction from the previous band-passed time-series [Glerean et al., 2012].
[c]Statistically significant at unadjusted $P \leq 0.05$, but showing only a trend towards association ($P \leq 0.1$) after FDR adjustment
[d]Statistically significant before and after FDR adjustment at $P \leq 0.05$.

DISCUSSION

This study implemented a genetically-informative design to test the potential relationship between amygdalar resting-state fMRI activity and depression risk. The separate influence of familial and unique environmental factors altering the relationship between amygdalar activity and depression was analyzed using two different approaches to functional connectomics from resting-state fMRI. First, the conventional procedure to estimate temporal correlations between BOLD activity of paired brain ROIs was used to construct brain networks. Results using this method suggested that unique environmental factors modify the amygdalar resting-state activity to increase depression risk. Afterward, the amplitude envelope of the whole-brain resting-state activity patterns was computed to search for other informative patterns potentially embedded within the BOLD signal. This approach confirmed that the environment may modify the amygdalar functionality to lead to depression; it also set forth that familial factors (genes plus shared twin environment) affecting amygdalar resting-state patterns could play a role in depression risk.

The Environment and Amygdalar Centrality in the Depressed Brain

A first noteworthy result is the indication that the amygdalar degree—which here represents the extent of amygdala-whole-brain synchronization—is increased in depressed individuals. This communicational impairment somehow parallels previous findings of hypersynchronized oscillations in other pathological states. For instance, there is evidence of a decreased resting-state communicational complexity (i.e., a synchronization increase) in schizophrenia and autism [Andreou et al., 2014; Billeci et al., 2013; Sokunbi et al., 2013]; these hypersynchronized patterns have their limit expression in the neural activity of epileptic individuals [Stamoulis et al., 2010; Zhang et al., 2014b]. Of note, reduced communicational complexity, as indexed by redundant information across distinct sources, has largely been studied in other mathematical disciplines [Shannon, 1997]. The present findings are in this direction by suggesting a disease-associated overlap in the information carried by oscillations in the amygdala and in the rest of the brain. They also point out that environmental factors prompt such increased connectivity; this result was detected when analyzing the conventional resting-state time-series, and was clearer when examining their amplitude envelope synchronization (Fig. 2 and Table III).

Likewise, unique environmental factors altering left amygdalar betweenness centrality and local clustering coefficient seemed to predispose to depressive psychopathology (Fig. 2 and Table III). The potential biological meaning of these functional alterations can be interpreted as follows: first, the nodal clustering changes observed here would indicate a functional decoupling between brain regions with BOLD oscillatory patterns similar to (i.e., synchronized with) those of the amygdala. Similar local clustering coefficient alterations have been shown in the structural connectivity networks of MDD patients, across a number of limbic-emotional regions such as the left hippocampus [Qin et al., 2014]. Second, resting-state network alterations such as betweenness centrality of a number of brain regions have been found to predict depression status [Lord et al., 2012]. In the present context, these centrality disruptions may implicate a failure of the amygdala to bridge the shortest paths between pairs of synchronized nodes.

Familial Factors Altering Amygdalar Centrality in the Depressed Brain

When observing the model-fitting statistics across the distinct models considered here, the analysis of amygdalar betweenness centrality gave the best discrimination indexes ($R^2 = 0.508$; Table III). Of note, both genetic and environmental influences on left amygdalar betweenness centrality were significantly associated with depressive psychopathology, even after multiple testing adjustments.

Similarly, depression risk was associated with the familial factors altering both left and right amygdalar betweenness centrality of the Hilbert-transformed data (Fig. 3 and Table III).

The results of this study advocate for the use of analytic components of BOLD fMRI signals—such as the amplitude envelope—, particularly when studying the genetic influences leading to functional alterations of the amygdala in depression. This may have important implications considering the relevance of genetic factors such as the serotonin transporter genotype (5-HTTLPR) in modulating the amygdala during both resting-state and task-related fMRI paradigms [El-Hage et al., 2013; Li et al., 2012; Munafo et al., 2008]. The present findings suggest that the genetic bases of amygdalar activity may probably be better elicited by examining specific analytical properties of fMRI signals.

Additional Considerations

It is important mentioning that the left amygdala showed more robust statistical associations with depression than its right counterpart; most of its associations remained multiple testing adjustments. This is consistent with previous reports of partially lateralized amygdalar activity patterns at rest [Roy et al., 2009].

None of the analyses conducted here suggested a role for eigenvector centrality alterations of the amygdala in depression. As mentioned above, Benzi and Klymko [2015] have previously shown that degree and eigenvector centrality constitute limiting cases across a wide range of different nodal centrality measures, including the clustering coefficient. Though graph metrics derived from spectral graph theory (such as eigenvector-related measures) may be a landmark of brain anatomy across different species [de Lange et al., 2014], the current results suggest that such measures are not disrupted in the resting-state functional activity of the amygdala in depression. As likewise noticed, eigenvector centrality represents, in this context, the extent of synchrony between the amygdala to other highly synchronized brain regions.

Finally, some methodological limitations of this study should be noted. First, the sample size was modest; nevertheless, the associations found here (and their corresponding model fitting statistics shown in Table III) would support the presence of relatively strong effects. Likewise, the parcellation scheme adopted to construct the brain connectivity matrix was built upon the AAL atlas, which contains 90 ROIs across the whole brain. Hence, the present results are not directly comparable with other studies using different parcellation schemes. While this is certainly important, it is worth noting that it is not a problem only within the current report; choice of parcellation schemes is an important subject with large implications for brain connectomics research [de Reus and van den Heuvel, 2013]. To address this issue, future studies may combine higher-resolution neuroimaging scans with finer-grained anatomical atlases.

ACKNOWLEDGMENTS

Ximena Goldberg and Silvia Alemany contributed to sample collection. MRI technicians César Garrido and Santi Sotés also contributed to this work. The authors are indebted to the Medical Image core facility of the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) for the technical help.

REFERENCES

Achard S, Salvador R, Whitcher B, Suckling J, Bullmore E (2006): A resilient, low-frequency, small-world human brain functional network with highly connected association cortical hubs. J Neurosci 26:63–72.

Andreou C, Nolte G, Leicht G, Polomac N, Hanganu-Opatz IL, Lambert M, Engel AK, Mulert C (2014): Increased resting-state gamma-band connectivity in first-episode schizophrenia. Schizophr Bull: sbu121.

Begg MD, Parides MK (2003): Separation of individual-level and cluster-level covariate effects in regression analysis of correlated data. Stat Med 22:2591–602.

Benjamini Y, Hochberg Y (1995): Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Stat Soc Ser B (Methodological) 57:289–300.

Benzi M, Klymko C (2015): On the Limiting Behavior of Parameter-Dependent Network Centrality Measures. SIAM J Matrix Anal & Appl, 36:686–706.

Billeci L, Sicca F, Maharatna K, Apicella F, Narzisi A, Campatelli G, Calderoni S, Pioggia G, Muratori F (2013): On the application of quantitative EEG for characterizing autistic brain: A systematic review. Front Hum Neurosci 7:442

Blokland GA, de Zubicaray GI, McMahon KL, Wright MJ (2012): Genetic and environmental influences on neuroimaging phenotypes: A meta-analytical perspective on twin imaging studies. Twin Res Hum Genet 15:351–371.

Borgatti SP, Everett MG (2006): A graph-theoretic perspective on centrality. Soc Netw 28:466–484.

Bounova G, de Weck O (2012): Overview of metrics and their correlation patterns for multiple-metric topology analysis on heterogeneous graph ensembles. Phys Rev E 85:016117

Calhoun VD, Liu J, Adali T (2009): A review of group ICA for fMRI data and ICA for joint inference of imaging, genetic, and ERP data. NeuroImage 45:S163–S172.

Carlin JB, Gurrin LC, Sterne JA, Morley R, Dwyer T (2005): Regression models for twin studies: A critical review. Int J Epidemiol 34:1089–1099.

Cohen J (1988): Statistical Power Analysis for the Behavioral Sciences, Vol. xxi. Hillsdale, N.J.: L. Erlbaum Associates, 567 p.

Cook RJ, Farewell VT (1996): Multiplicity considerations in the design and analysis of clinical trials. J R Stat Soc Ser A (Stat Soc) 159:93–110.

Córdova-Palomera A (2015): mztwinreg: Regression Models for Monozygotic Twin Data. R package version 1.0–1. Retrieved from: http://CRAN.R-project.org/package=mztwinreg.

Cullen KR, Westlund MK, Klimes-Dougan B, Mueller BA, Houri A, Eberly LE, Lim KO (2014): Abnormal amygdala resting-state functional connectivity in adolescent depression. JAMA Psychiatry 71:1138–1147.

Champely S (2012): pwr: Basic functions for power analysis. R package version 1.1.1. Retrieved from: http://CRAN.R-project.org/package=pwr.

de Lange SC, de Reus MA, van den Heuvel MP (2014): The Laplacian spectrum of neural networks. Front Comput Neurosci 7:189

de Reus MA, van den Heuvel MP (2013): The parcellation-based connectome: Limitations and extensions. NeuroImage 80:397–404.

De Vico Fallani F, Richiardi J, Chavez M, Achard S (2014): Graph analysis of functional brain networks: Practical issues in translational neuroscience. Philos Trans R Soc London Ser B, Biol Sci 369: 20130520

DeMaris A (1995): A tutorial in logistic regression. J Marriage Family 57:956–968.

Derogatis LR, Melisaratos N (1983): The Brief Symptom Inventory: An introductory report. Psychol Med 13:595–605.

Domschke K, Reif A (2012): Behavioral genetics of affective and anxiety disorders. Curr Top Behav Neurosci 12:463–502.

Douw L, Schoonheim M, Landi D, Van der Meer M, Geurts J, Reijneveld J, Klein M, Stam C (2011): Cognition is related to resting-state small-world network topology: An magnetoencephalographic study. Neuroscience, 175:169–177.

Duncan LE, Keller MC (2011): A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. Am J Psychiatry 168:1041–1049.

Dutta A, McKie S, Deakin JF (2014): Resting state networks in major depressive disorder. Psychiatry Res 224:139–151.

El-Hage W, Zelaya F, Radua J, Gohier B, Alsop DC, Phillips ML, Surguladze SA (2013): Resting-state cerebral blood flow in amygdala is modulated by sex and serotonin transporter genotype. NeuroImage 76:90–97.

Esslinger C, Kirsch P, Haddad L, Mier D, Sauer C, Erk S, Schnell K, Arnold C, Witt SH, Rietschel M, Cichon S, Walter H, Meyer-Lindenberg A (2011): Cognitive state and connectivity effects of the genome-wide significant psychosis variant in ZNF804A. NeuroImage 54:2514–2523.

Evans AC, Collins DL, Mills S, Brown E, Kelly R, Peters TM (1993): 3D statistical neuroanatomical models from 305 MRI volumes. In IEEE Conference Record Nuclear Science Symposium and Medical Imaging Conference, San Francisco, CA, pp. 1813–1817.

Filippini N, MacIntosh BJ, Hough MG, Goodwin GM, Frisoni GB, Smith SM, Matthews PM, Beckmann CF, Mackay CE (2009): Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. Proc Natl Acad Sci USA 106:7209–7214.

First MB (1997): Structured Clinical Interview for DSM-IV Axis I Disorders: SCID - I: Clinician Version: Administration Booklet. Washington, D.C.: American Psychiatric Press.

Fornito A, Zalesky A, Bassett DS, Meunier D, Ellison-Wright I, Yucel M, Wood SJ, Shaw K, O'Connor J, Nertney D, Mowry BJ, Pantelis C, Bullmore ET (2011): Genetic influences on cost-efficient organization of human cortical functional networks. J Neurosci 31:3261–3270.

Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle, ME (2005): The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proceedings of the Natl Acad Sci USA 102:9673–9678.

Freeman HL, Stansfeld SA (2008): The Impact of the Environment on Psychiatric Disorder. London, New York: Routledge, 330 p.

Fries P (2005): A mechanism for cognitive dynamics: Neuronal communication through neuronal coherence. Trends Cogn Sci 9:474–480.

Fries P (2009): Neuronal gamma-band synchronization as a fundamental process in cortical computation. Annu Rev Neurosci 32:209–224.

Frisell T, Oberg S, Kuja-Halkola R, Sjolander A (2012): Sibling comparison designs: Bias from non-shared confounders and measurement error. Epidemiology 23:713–720.

Friston KJ, Holmes AP, Poline JB, Grasby PJ, Williams SC, Frackowiak RS, Turner R (1995): Analysis of fMRI time-series revisited. NeuroImage 2:45–53.

Glahn DC, Knowles EE, McKay DR, Sprooten E, Raventos H, Blangero J, Gottesman II, Almasy L (2014): Arguments for the sake of endophenotypes: Examining common misconceptions about the use of endophenotypes in psychiatric genetics. Am J Med Genet Part B, Neuropsychiatric Genet 165B:122–130.

Glahn DC, Thompson PM, Blangero J (2007): Neuroimaging endophenotypes: Strategies for finding genes influencing brain structure and function. Hum Brain Mapp 28:488–501.

Glahn DC, Winkler AM, Kochunov P, Almasy L, Duggirala R, Carless MA, Curran JC, Olvera RL, Laird AR, Smith SM, Beckmann CF, Fox PT, Blangero J (2010): Genetic control over the resting brain. Proc Natl Acad Sci USA 107:1223–1228.

Glerean E, Salmi J, Lahnakoski JM, Jaaskelainen IP, Sams M (2012): Functional magnetic resonance imaging phase synchronization as a measure of dynamic functional connectivity. Brain Connect 2:91–101.

Glickman ME, Rao SR, Schultz MR (2014): False discovery rate control is a recommended alternative to Bonferroni-type adjustments in health studies. J Clin Epidemiol 67:850–857.

Gottesman II, Gould TD (2003): The endophenotype concept in psychiatry: Etymology and strategic intentions. Am J Psychiatry 160:636–645.

Guggisberg AG, Rizk S, Ptak R, Di Pietro M, Saj A, Lazeyras F, Lovblad KO, Schnider A, Pignat JM (2014): Two intrinsic coupling types for resting-state integration in the human brain. Brain Topogr 28:318–329.

Guilherme R, Drunat S, Delezoide AL, Oury JF, Luton D (2009): Zygosity and chorionicity in triplet pregnancies: New data. Hum Reprod 24:100–105.

Harrel F (2014): rms: Regression Modeling Strategies. R package version 4.2-1. Retrieved from: http://CRAN.R-project.org/package=rms.

Hyde LW, Bogdan R, Hariri AR (2011): Understanding risk for psychopathology through imaging gene-environment interactions. Trends Cogn Sci 15:417–427.

Jaccard J, Wan CK (1995): Measurement error in the analysis of interaction effects between continuous predictors using multiple regression: Multiple indicator and structural equation approaches. Psychol Bull 117:348

Jaccard J, Wan CK, Turrisi R (1990): The detection and interpretation of interaction effects between continuous variables in multiple regression. Multivariate Behav Res 25: 467–478.

Jenkins GM, Watts DG (1968): Spectral Analysis and Its Applications, Vol. xviii. San Francisco: Holden-Day, 525 p.

Kaiser RH, Andrews-Hanna JR, Wager TD, Pizzagalli DA (2015): Large-scale network dysfunction in major depressive disorder: A meta-analysis of resting-state functional connectivity. JAMA Psychiatry 72:603–611.

Kendler KS, Hettema JM, Butera F, Gardner CO, Prescott CA (2003): Life event dimensions of loss, humiliation, entrapment, and danger in the prediction of onsets of major depression and generalized anxiety. Arch Gen Psychiatry 60:789–796.

Kessler RC, Angermeyer M, Anthony JC, De Graaf R, Demyttenaere K, Gasquet I, De Girolamo G, Gluzman S, Gureje O, Haro JM, Kawakami N, Karam A, Levinson D, Medina Mora ME, Oakley Browne MA, Posada-Villa J, Stein DJ, Adley Tsang CH, Aguilar-Gaxiola S, Alonso J, Lee S, Heeringa S, Pennell BE, Berglund P, Gruber MJ, Petukhova M, Chatterji S, Ustun TB (2007): Lifetime prevalence and age-of-onset distributions of mental disorders in the World Health Organization's World Mental Health Survey Initiative. World Psychiatry 6:168–176.

Lee MH, Smyser CD, Shimony JS (2013): Resting-state fMRI: A review of methods and clinical applications. AJNR. Am J Neuroradiol 34:1866–1872.

Leonardo ED, Hen R (2006): Genetics of affective and anxiety disorders. Annu Rev Psychol 57:117–137.

Li S, Zou Q, Li J, Li J, Wang D, Yan C, Dong Q, Zang YF (2012): 5-HTTLPR polymorphism impacts task-evoked and resting-state activities of the amygdala in Han Chinese. PloS One 7: e36513

Liu J, Pearlson G, Windemuth A, Ruano G, Perrone-Bizzozero NI, Calhoun V (2009): Combining fMRI and SNP data to investigate connections between brain function and genetics using parallel ICA. Hum Brain Mapp 30:241–255.

Liu J, Ren L, Womer FY, Wang J, Fan G, Jiang W, Blumberg HP, Tang Y, Xu K, Wang F (2014): Alterations in amplitude of low frequency fluctuation in treatment-naive major depressive disorder measured with resting-state fMRI. Hum Brain Mapp 35: 4979–4988.

Liu W, Jamshidian M, Zhang Y (2004): Multiple comparison of several linear regression models. J Am Stat Assoc 99:395–403.

Lord A, Horn D, Breakspear M, Walter M (2012): Changes in community structure of resting state functional connectivity in unipolar depression. PloS One 7:e41282

Lundberg A (1998): The Environment and Mental Health: A Guide for Clinicians, Vol. xi. Mahwah, N.J.: Lawrence Erlbaum Associates, 233 p.

Lynn R, Meisenberg G (2010): National IQs calculated and validated for 108 nations. Intelligence 38:353–360.

Martin AK, Robinson G, Reutens D, Mowry B (2014): Copy number deletion burden is associated with cognitive, structural, and resting-state network differences in patients with schizophrenia. Behav Brain Res 272:324–334.

Mathieu JE, Aguinis H, Culpepper SA, Chen G (2012): Understanding and estimating the power to detect cross-level interaction effects in multilevel modeling. J Appl Psychol 97:951–966.

McCabe C, Mishor Z (2011): Antidepressant medications reduce subcortical-cortical resting-state functional connectivity in healthy volunteers. NeuroImage 57:1317–1323.

Moffitt TE, Caspi A, Harrington H, Milne BJ, Melchior M, Goldberg D, Poulton R (2007): Generalized anxiety disorder and depression: Childhood risk factors in a birth cohort followed to age 32. Psychol Med 37:441–452.

Mosing MA, Gordon SD, Medland SE, Statham DJ, Nelson EC, Heath AC, Martin NG, Wray NR (2009): Genetic and environmental influences on the co-morbidity between depression, panic disorder, agoraphobia, and social phobia: A twin study. Depress Anxiety 26:1004–1011.

Munafo MR, Brown SM, Hariri AR (2008): Serotonin transporter (5-HTTLPR) genotype and amygdala activation: A meta-analysis. Biolog Psychiatry 63:852–857.

Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, Aboyans V, Abraham J, Ackerman I, Aggarwal R, Ahn SY, Ali MK, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Bahalim AN, Barker-Collo S, Barrero LH, Bartels DH, Basanez MG, Baxter A, Bell ML, Benjamin EJ, Bennett D, Bernabe E, Bhalla K, Bhandari B, Bikbov B, Bin Abdulhak A, Birbeck G, Black JA, Blencowe H, Blore JD, Blyth F, Bolliger I, Bonaventure A, Boufous S, Bourne R, Boussinesq M, Braithwaite T, Brayne C, Bridgett L, Brooker S, Brooks P, Brugha TS, Bryan-Hancock C, Bucello C, Buchbinder R, Buckle G, Budke CM, Burch M, Burney P, Burstein R, Calabria B, Campbell B, Canter CE, Carabin H, Carapetis J, Carmona L, Cella C, Charlson F, Chen H, Cheng AT, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahiya M, Dahodwala N, Damsere-Derry J, Danaei G, Davis A, De Leo D, Degenhardt L, Dellavalle R, Delossantos A, Denenberg J, Derrett S, Des Jarlais DC, Dharmaratne SD, Dherani M, Diaz-Torne C, Dolk H, Dorsey ER, Driscoll T, Duber H, Ebel B, Edmond K, Elbaz A, Ali SE, Erskine H, Erwin PJ, Espindola P, Ewoigbokhan SE, Farzadfar F, Feigin V, Felson DT, Ferrari A, Ferri CP, Fevre EM, Finucane MM, Flaxman S, Flood L, Foreman K, Forouzanfar MH, Fowkes FG, Fransen M, Freeman MK, Gabbe BJ, Gabriel SE, Gakidou E, Ganatra HA, Garcia B, Gaspari F, Gillum RF, Gmel G, Gonzalez-Medina D, Gosselin R, Grainger R, Grant B, Groeger J, Guillemin F, Gunnell D, Gupta R, Haagsma J, Hagan H, Halasa YA, Hall W, Haring D, Haro JM, Harrison JE, Havmoeller R, Hay RJ, Higashi H, Hill C, Hoen B, Hoffman H, Hotez PJ, Hoy D, Huang JJ, Ibeanusi SE, Jacobsen KH, James SL, Jarvis D, Jasrasaria R, Jayaraman S, Johns N, Jonas JB, Karthikeyan G, Kassebaum N, Kawakami N, Keren A, Khoo JP, King CH, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Laden F, Lalloo R, Laslett LL, Lathlean T, Leasher JL, Lee YY, Leigh J, Levinson D, Lim SS, Limb E, Lin JK, Lipnick M, Lipshultz SE, Liu W, Loane M, Ohno SL, Lyons R, Mabweijano J, MacIntyre MF, Malekzadeh R, Mallinger L, Manivannan S, Marcenes W, March L, Margolis DJ, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGill N, McGrath J, Medina-Mora ME, Meltzer M, Mensah GA, Merriman TR, Meyer AC, Miglioli V, Miller M, Miller TR, Mitchell PB, Mock C, Mocumbi AO, Moffitt TE, Mokdad AA, Monasta L, Montico M, Moradi-Lakeh M, Moran A, Morawska L, Mori R, Murdoch ME, Mwaniki MK, Naidoo K, Nair MN, Naldi L, Narayan KM, Nelson PK, Nelson RG, Nevitt MC, Newton CR, Nolte S, Norman P, Norman R, O'Donnell M, O'Hanlon S, Olives C, Omer SB, Ortblad K, Osborne R, Ozgediz D, Page A, Pahari B, Pandian JD, Rivero AP , Patten SB, Pearce N, Padilla RP, Perez-Ruiz F, Perico N, Pesudovs K, Phillips D, Phillips MR, Pierce K, Pion S, Polanczyk GV, Polinder S, Pope CA, 3rd, Popova S, Porrini E, Pourmalek F,

Prince M, Pullan RL, Ramaiah KD, Ranganathan D, Razavi H, Regan M, Rehm JT, Rein DB, Remuzzi G, Richardson K, Rivara FP, Roberts T, Robinson C, De Leon FR, Ronfani L, Room R, Rosenfeld LC, Rushton L, Sacco RL, Saha S, Sampson U, Sanchez-Riera L, Sanman, Schwebel DC, Scott JG, Segui-Gomez M, Shahraz S, Shepard DS, Shin H, Shivakoti R, Singh D, Singh GM, Singh JA, Singleton J, Sleet DA, Sliwa K, Smith E, Smith JL, Stapelberg NJ, Steer A, Steiner T, Stolk WA, Stovner LJ, Sudfeld C, Syed S, Tamburlini G, Tavakkoli M, Taylor HR, Taylor JA, Taylor WJ, Thomas B, Thomson WM, Thurston GD, Tleyjeh IM, Tonelli M, Towbin JA, Truelsen T, Tsilimbaris MK, Ubeda C, Undurraga EA, van der Werf, MJ, van Os J, Vavilala MS, Venketasubramanian N, Wang M, Wang W, Watt K, Weatherall DJ, Weinstock MA, Weintraub R, Weisskopf MG, Weissman MM, White RA, Whiteford H, Wiebe N, Wiersma ST, Wilkinson JD, Williams HC, Williams SR, Witt E, Wolfe F, Woolf AD, Wulf S, Yeh PH, Zaidi AK, Zheng ZJ, Zonies D, Lopez AD, AlMazroa MA, Memish ZA (2012): Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2197–223.

Nakagawa S (2004): A farewell to Bonferroni: The problems of low statistical power and publication bias. Behav Ecol 15:1044–1045.

O'Reilly, J (1984): Sinusoidal Carrier Modulation. Telecommunication Principles. Houte, the Netherlands: Springer Netherlands. pp. 53–66.

Oathes DJ, Patenaude B, Schatzberg AF, Etkin A (2014): Neurobiological signatures of anxiety and depression in resting-state functional magnetic resonance imaging. Biol Psychiatry 77:385–393.

Paus T (2013): How environment and genes shape the adolescent brain. Horm Behav 64:195–202.

Perneger TV (1998): What's wrong with Bonferroni adjustments. BMJ 316:1236–1238.

Ponce-Alvarez A, Deco G, Hagmann P, Romani GL, Mantini D, Corbetta M (2015): Resting-state temporal synchronization networks emerge from connectivity topology and heterogeneity. PLoS Comput Biol 11:e1004100.

Power JD, Mitra A, Laumann TO, Snyder AZ, Schlaggar BL, Petersen SE (2014): Methods to detect, characterize, and remove motion artifact in resting state fMRI. NeuroImage 84:320–341.

Qin J, Wei M, Liu H, Yan R, Luo G, Yao Z, Lu Q (2014): Abnormal brain anatomical topological organization of the cognitive-emotional and the frontoparietal circuitry in major depressive disorder. Magn Reson Med 72:1397–1407.

R Development Core Team (2011): R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.

Ressler KJ, Mayberg HS (2007): Targeting abnormal neural circuits in mood and anxiety disorders: From the laboratory to the clinic. Nat Neurosci 10:1116–1124.

Richiardi J, Eryilmaz H, Schwartz S, Vuilleumier P, Van De Ville D (2011): Decoding brain states from fMRI connectivity graphs. NeuroImage 56:616–626.

Rose EJ, Donohoe G (2013): Brain vs behavior: An effect size comparison of neuroimaging and cognitive studies of genetic risk for schizophrenia. Schizophrenia Bull 39:518–526.

Roy AK, Shehzad Z, Margulies DS, Kelly AM, Uddin LQ, Gotimer K, Biswal BB, Castellanos FX, Milham MP (2009): Functional connectivity of the human amygdala using resting state fMRI. NeuroImage 45:614–626.

Rudie JD, Hernandez LM, Brown JA, Beck-Pancer D, Colich NL, Gorrindo P, Thompson PM, Geschwind DH, Bookheimer SY, Levitt P, Dapretto M (2012): Autism-associated promoter variant in MET impacts functional and structural brain networks. Neuron 75:904–915.

Ruiperez M, Ibáñez MI, Lorente E, Moro M, Ortet G (2001): Psychometric properties of the Spanish version of the BSI: Contributions to the relationship between personality and psychopathology. Eur J Psychol Assess 17:241

Sattler JM (2001): Assessment of children: Cognitive applications. San Diego: J.M. Sattler.

Schwarz AJ, McGonigle J (2011): Negative edges and soft thresholding in complex network analysis of resting state functional connectivity data. NeuroImage 55:1132–1146.

Shannon CE (1997): The mathematical theory of communication. 1963. M.D. Comput: Comput Med Practic 14:306–317.

Sheline YI, Barch DM, Price JL, Rundle MM, Vaishnavi SN, Snyder AZ, Mintun MA, Wang S, Coalson RS, Raichle ME (2009): The default mode network and self-referential processes in depression. Proc Natl Acad Sci USA 106:1942–1947.

Sheline YI, Price JL, Yan Z, Mintun MA (2010): Resting-state functional MRI in depression unmasks increased connectivity between networks via the dorsal nexus. Proc Natl Acad Sci USA 107:11020–11025.

Smith SM (2012): The future of FMRI connectivity. NeuroImage 62:1257–1266.

Smith SM, Vidaurre D, Beckmann CF, Glasser MF, Jenkinson M, Miller KL, Nichols TE, Robinson EC, Salimi-Khorshidi G, Woolrich MW, Barch DM, Ugurbil K, Van Essen DC (2013): Functional connectomics from resting-state fMRI. Trends Cogn Sci 17:666–682.

Sokunbi MO, Fung W, Sawlani V, Choppin S, Linden DE, Thome J (2013): Resting state fMRI entropy probes complexity of brain activity in adults with ADHD. Psychiatry Res 214:341–348.

Stamoulis C, Gruber LJ, Chang BS (2010): Network dynamics of the epileptic brain at rest. In: Conference Proceedings: Annual International Conference of the IEEE Engineering in Medicine and Biology Society. pp 150–153.

Sui J, Adali T, Pearlson GD, Calhoun VD (2009): An ICA-based method for the identification of optimal FMRI features and components using combined group-discriminative techniques. NeuroImage 46:73–86.

Sullivan PF, Neale MC, Kendler KS (2000): Genetic epidemiology of major depression: Review and meta-analysis. Am J Psychiatry 157:1552–1562.

Trachtenberg AJ, Filippini N, Ebmeier KP, Smith SM, Karpe F, Mackay CE (2012): The effects of APOE on the functional architecture of the resting brain. NeuroImage 59:565–572.

Tunbridge EM, Harrison PJ, Weinberger DR (2006): Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. Biol Psychiatry 60:141–151.

Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M (2002): Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. NeuroImage 15:273–289.

van den Heuvel MP, Hulshoff Pol HE (2010): Exploring the brain network: A review on resting-state fMRI functional connectivity. Eur Neuropsychopharmacol 20:519–534.

van den Heuvel MP, van Soelen IL, Stam CJ, Kahn RS, Boomsma DI, Hulshoff Pol HE (2013): Genetic control of functional brain network efficiency in children. Eur Neuropsychopharmacol 23:19–23.

Villain N, Fouquet M, Baron JC, Mezenge F, Landeau B, de La Sayette V, Viader F, Eustache F, Desgranges B, Chetelat G (2010): Sequential relationships between grey matter and white matter atrophy and brain metabolic abnormalities in early Alzheimer's disease. Brain 133:3301–3314.

Wang L, Dai Z, Peng H, Tan L, Ding Y, He Z, Zhang Y, Xia M, Li Z, Li W, Cai Y, Lu S, Liao M, Zhang L, Wu W, He Y, Li L (2014): Overlapping and segregated resting-state functional connectivity in patients with major depressive disorder with and without childhood neglect. Hum Brain Mapp 35:1154–1166.

Wang L, Song M, Jiang T, Zhang Y, Yu C (2011): Regional homogeneity of the resting-state brain activity correlates with individual intelligence. Neurosci Lett 488:275–278.

Wechsler D, Cordero Pando A, Yela Granizo M, Zimmerman IL, Woo-Sam JM, Glasser AJ (1997): WAIS escala de inteligencia de Wechsler para adultos. Madrid, Barcelona: TEA Ediciones.

White H (1982): Maximum likelihood estimation of misspecified models. Econometrica: J Econometric Soc 50:1–25.

Wittchen HU, Kessler RC, Beesdo K, Krause P, Hofler M, Hoyer J (2002): Generalized anxiety and depression in primary care: Prevalence, recognition, and management. J Clin Psychiatry 63 Suppl 8:24–34.

Zbozinek TD, Rose RD, Wolitzky-Taylor KB, Sherbourne C, Sullivan G, Stein MB, Roy-Byrne PP, Craske MG (2012): Diagnostic overlap of generalized anxiety disorder and major depressive disorder in a primary care sample. Depression Anxiety 29:1065–1071.

Zeng LL, Shen H, Liu L, Hu D (2014): Unsupervised classification of major depression using functional connectivity MRI. Hum Brain Mapp 35:1630–1641.

Zeng LL, Shen H, Liu L, Wang L, Li B, Fang P, Zhou Z, Li Y, Hu D (2012): Identifying major depression using whole-brain functional connectivity: A multivariate pattern analysis. Brain 135: 1498–507.

Zhang X, Zhu X, Wang X, Zhu X, Zhong M, Yi J, Rao H, Yao S (2014a): First-episode medication-naive major depressive disorder is associated with altered resting brain function in the affective network. PloS One 9:e85241.

Zhang Z, Liao W, Wang Z, Xu Q, Yang F, Mantini D, Jiao Q, Tian L, Liu Y, Lu G (2014b): Epileptic discharges specifically affect intrinsic connectivity networks during absence seizures. J Neurol Sci 336:138–145.

Advisor's report on the contribution of the Ph.D. candidate to the article

Prof. Dr. Lourdes Fañanás Saura, associate professor at the Faculty of Biology, University of Barcelona, and supervisor of the present doctoral thesis by Aldo Córdova Palomera, hereby certifies that the participation of the Ph.D. candidate in the article "Altered amygdalar resting-state connectivity in depression is explained by both genes and environment" included the following tasks:

- fMRI data pre- and post-processing.
- Participation in study design.
- Statistical analyses.
- Writing of the first manuscript draft.
- Critical revision of the article.

Prof. Dr. Lourdes Fañanás Saura

Barcelona, June 30th 2015.

5. GLOBAL SUMMARY OF RESULTS

The **Main hypothesis** was thus tested throughout the ten independent projects mentioned above. These ten results refer to the two **Specific hypotheses** as follows.

Specific hypothesis 1: ***[Depression and developmental plasticity.]*** Depression-related psychopathological phenotypes are induced by factors altering the early neurodevelopment, and these long-lasting changes can be assessed in adulthood. This was tested in five studies; some of their most relevant results are:

I. A first manuscript discussed how, if there were a statistical association between low birth weight and adult depression, it should be caused by genetic but not environmental factors affecting fetal growth (Cordova-Palomera *et al.*, 2014c).

II. The results of another manuscript support findings indicating that i) BW has a long-lasting effect on cortical SA, where some familial and environmental influences alter both fetal growth and brain morphology; ii) uniquely environmental factors affecting BW also alter SA; iii) higher IQ correlates with larger SA; and iv) these effects are not modified by anxious-depressive psychopathology (Cordova-Palomera *et al.*, 2015c).

III. Another publication discussed the relationship between birth weight, working memory, and DNA methylation signatures in *IGF2* and related genes (Cordova-Palomera *et al.*, 2014b). The findings are in agreement with previous evidence indicating that DNA methylation status may be related to prenatal stress and later neurocognitive phenotypes. While former reports independently detected associations between DNA methylation and either BW or WM, current results suggest that these relationships are not confounded by each other.

IV. By systematically reviewing and meta-analyzing new and existing data, an additional study aimed to determine whether there is evidence to support an association between winter season of birth (SOB) and subclinical psychosis in the general population (Cordova-Palomera *et al.*, 2015a). Overall, results indicate the association between winter SOB and increased subclinical psychosis may hold in children, but not in the

broad adult general population. Nevertheless, epidemiological and clinicopathological significance of winter SOB as a risk factor for subclinical psychosis will probably be slight due to the small effect sizes indicated by reports available to date.

V. Furthermore, another report supports previous findings indicative of cortical thickening in healthy individuals with high psychometrically assessed psychosis scores, probably in line with theories of compensatory aspects of brain features in non-clinical populations (Cordova-Palomera *et al.*, 2014a). Additionally, its findings suggest distinct patterns of cortical thickness–PEs relationships depending on birth seasonality. Familial factors underlying the presence of PEs may drive these effects.

Specific hypothesis 2: ***[Depression and activational plasticity.]*** The clinical manifestation of depression-related psychopathological phenotypes can be understood as activational plasticity deficits; these deficits can be assessed as neurobiological disease traits using novel epigenetic and neuroimaging techniques. This was tested in five studies; some of their most relevant results are:

I. Since monozygotic (MZ) twins may show larger or smaller intrapair phenotypic differences depending on whether their genetic background is more or less sensitive to environmental factors, a twin design was implemented to determine if particular polymorphisms in the *DNMT3B* gene may be linked to a better (worse) response to enriched (deprived) environmental factors. Results suggests that *DNMT3B* polymorphisms may allow determining which individuals are more prone to either improve their IQ after exposure to potentially enriched environments, or to have cognitive declines if not exposed to an appropriate environment (Cordova-Palomera *et al.*, 2015e).

II. In an additional project, intrapair DNA methylation differences in an intron of *DEPDC7* (chr11:33040743) were evaluated in relation with intrapair differences in current depressive symptoms. The findings indicate that *DEPDC7* hypomethylation in

peripheral blood DNA may be associated with recent depressive symptomatology, in line with previous results (Cordova-Palomera *et al.*, 2015f).

III. Moreover, a genome-wide DNA methylation study in twins suggested that both differential methylation and differential variability have a role in the etiology and clinical manifestation of depression, and provided clues on specific genomic loci of potential interest in the epigenetics of depression (Cordova-Palomera *et al.*, 2015d).

IV. Besides, an additional work showed alterations of the communication patterns between the hippocampus and the rest of the brain in depression, effects potentially driven by overall familial factors (genes plus shared twin environment) and modified by the *FKBP5* gene (Cordova-Palomera *et al.*, 2015b).

V. Finally, additional findings showed that both genes and environment modify different patterns the amygdala resting-state connectivity to increase depression risk. The genetic relationship between amygdalar connectivity and depression may be better elicited by examining analytic components of the brain resting-state fMRI signals (Cordova-Palomera *et al.*, 2015g).

6. DISCUSSION AND CONCLUSIONS

The present thesis was aimed at studying how several etiopathogenic mechanisms of depression-related phenotypes can be explained as disruptions of biobehavioral plasticity processes in response to the experience. To this end, a number of early neurodevelopmental pathways and putative neurophysiological markers of disease were investigated. Next, a brief discussion of the research findings of this work is presented, explained in the frame of both developmental and activational plasticity phenomena.

Depression and developmental plasticity. The neurodevelopmental hypothesis of depression typically explains an elevated disease risk for individuals who suffered stressful insults during their first years of –postnatal– life (Ansorge *et al.*, 2007). Nevertheless, associations between prenatal risk factors and risk for depression-related phenotypes are still inconclusive (Wojcik *et al.*, 2013). Further investigation on this topic is needed to determine the extent to which there could be a developmental plasticity hypothesis of depression.

[Manuscript 1] In view of this, a genetically informative design was implemented to evaluate the putative association between low birth weight and adult depression. Specifically, since both birth weight and adult depression have genetic and environmental components, this work evaluated whether genetic or environmental factors altering birth weight may also alter the risk for adult depression (Cordova-Palomera *et al.*, 2014c). Based on the findings of this and other studies, it was proposed that if there was a link between low birth weight and adult depression, it should be caused by genetic factors regulating neurobiological pathways underlying both phenotypes.

[Manuscript 2] Furthermore, the results of another report indicated an association between birth weight and adult brain cortical surface, which seemed driven by environmental factors (Cordova-Palomera *et al.*, 2015c). As noticed in previous results by Raznahan *et al.* (2012), environmental differences in intrauterine conditions (as assessed by MZ intrapair quantitative discordance) can lead to cortical surface area disruptions. This finding by Raznahan *et al.* (2012) is thus partly replicated in a subset of the UB Twin Registry. Of note, the participants from the UB Twin subset constitute a sample older than those previously studied (Raznahan *et al.*, 2012;

Walhovd *et al.*, 2012; Haukvik *et al.*, 2014), suggesting that the association between some environmentally-induced fetal growth alterations and brain cortical surface area remains valid even more than three decades after birth. But perhaps the most remarkable finding of this work is the fact that environmentally-induced birth weight decreases are associated with reduced adult cortical surface area, regardless of depression-related psychopathology. This would somehow complement the first work of this thesis (Cordova-Palomera *et al.*, 2014c), in which the results indicate that the environmental factors leading to low birth weight may be independent of those increasing depression risk. Namely, these new imaging genetics findings would suggest that there are some specific environmental factors altering fetal growth and leading to long-lasting cortical morphology alterations; nevertheless, these brain morphology changes are not directly linked to depressive psychopathology.

[Manuscript 3] A third work incorporated molecular biology data on DNA methylation signatures to be analyzed in relation to birth weight and neurocognitive changes (Cordova-Palomera *et al.*, 2014b). The insulin-like growth factor 2 (*IGF2*) and its binding protein genes (*IGF2BP1-3*) were studied, since there is evidence indicating that prenatal neurodevelopmental insults may fix long-lasting DNA methylation signatures in these genes. DNA methylation of a CpG site in *IGF2BP1* was associated with both birth weight and adult depression. The association between birth weight and *IGF2BP1* methylation seemed due to unique environmental factors influencing fetal growth. This very role for the environment may perhaps explain the lack of sound evidence (to the best of our knowledge) associating *IGF2* DNA methylation –which is seemingly changed by the prenatal environment– and depressive disorder. But there was an additional finding of this work: familial factors modifying DNA methylation of the *IGF2BP1* may be related to adult neurocognition. Thus, one could perhaps speculate that there is some genetic regulation of DNA methylation of this set of genes which influences adult neurocognition, but that other environmental factors modify fetal health through methylation of the same loci. This may in some way be related to the notion of genetic pleiotropy in psychiatry (Cross-Disorder Group of the Psychiatric Genomics, 2013), though at the level of epigenetics and, perhaps, gene-environment interactions.

Two additional research works investigated winter SOB –one of the most replicated prenatal neurodevelopmental risk factors for psychosis– with regard to depression-related phenotypes. In these works, the phenotype of interest was subclinical psychosis, which is highly prevalent in depression (Wigman *et al.*, 2012) and, as discussed in section 1.3.3, may help elicit the relationship(s) between the risk factors for psychosis and those leading to depression.

[Manuscript 4] Initially, a systematic review and meta-analysis of new and existing data was aimed to determine whether there is evidence to support an association between winter SOB and subclinical psychosis in the general population (Cordova-Palomera *et al.*, 2015a). The results indicate that there may be an association between winter SOB and subclinical psychosis in children but not in adults. However, since the effect sizes are small, this association may perhaps have only minor clinical relevance. In a way, this (null) finding would support the existence of psychopathology-specific risk factors for clinical and subclinical psychosis, which may ultimately lead to infer differential risk specificity for depression and subclinical psychosis (Kounali *et al.*, 2014), as well as for depression and clinical psychosis. The fact that this meta-analytic work assessed an environmental risk exposure should certainly be kept in mind: though the findings are suggestive of risk specificity, the reports on genetic pleiotropy across psychiatric disorders (Gorwood, 2004; Cross-Disorder Group of the Psychiatric Genomics, 2013) may provide additional clues. Namely, perhaps this apparent environmental risk factor specificity may share an etiological scenario with genetic non-specificity (i.e., pleiotropy). Back to the first manuscript of this thesis, genetic factors altering neurodevelopmental trajectories may increase the (non-specific) risk for psychopathology, though environmentally-induced prenatal alterations may not be related to depression risk.

[Manuscript 5] Complementarily, a fifth research project analyzed the putative relationship between cortical thickness and (subclinical) psychotic experiences (Cordova-Palomera *et al.*, 2015a). In line with previous evidence (Kuhn *et al.*, 2012), overall results showed that high (psychometric) psychotic experiences correlate with cortical thickening of brain regions typically affected by cortical thickness reductions in schizophrenia. Additionally, other cortical regions had

different thickness-psychopathology relationships (i.e., thickening or thinning) depending on birth seasonality. Individuals born during the risk SOB displayed a correlation between cortical thinning (at some regions) and psychosis, as occurs in the schizophrenia phenotype (Rimol *et al.*, 2012). Familial factors (i.e., genetics plus shared environment) determining cortical morphology seemed to mediate this association. From these results, one may speculate that individuals born during summer (i.e., those without exposure to prenatal insults) can trigger a genetic compensatory mechanism. By this means, they may have a compensatory mechanism (i.e., a thick cortex) to avoid transitioning to a schizophrenic phenotype. With these results, it is thus likely hypothesizing that the interaction between the genetic program of development for the cortex and the early environmental insults caused by the SOB may be linked to different psychopathological profiles. Namely, perhaps the genes (somehow pleiotropic) are likely to produce a psychotic phenotype when interacting with this neurodevelopmental risk factor.

Taken together, the evidences from these five manuscripts would be in favor of a genetic pleiotropy plus an environmental risk-specificity to link early neurodevelopment and adult depression. More precisely, these results allow hypothesizing that some of the genetic factors conferring a pleiotropic load for psychiatric disorders may similarly modulate early neurodevelopmental trajectories. While they could, by themselves, largely influence disease risk, other particular environmental factors may play an important role in determining the final phenotype outcome (i.e., the particular disease risk).

Rather than concluding, the present findings may hence help supporting previous evidence and proposing new alternatives for the developmental plasticity hypothesis of depression. They could indicate that some experiences (particularly those genetically-driven), which regulate early neurodevelopmental trajectories are able to generate a wide range of phenotypes to adapt to a lifelong changing environment, but the presence of adverse environmental experiences may, in turn, rise pathology-prone organisms. In addition, these findings may indicate that some early neurodevelopmental experiences driven by genetic factors may influence depression risk, though this genetic disruption of the prenatal environment seems relatively infrequent.

Depression and activational plasticity. Several depression-related psychopathological phenotypes have been explained across the literature as neural plasticity deficits. Nevertheless, novel brain imaging and epigenetic techniques assessing the neurobiological structure and function underlying the clinical manifestation of depression could provide new ways to understand potential neuroplastic disruptions. Considering this point, five additional research projects were developed.

[Manuscript 6] First, a genetically informative design using only MZ twins was implemented to test whether genetic variation in the epigenetic gene *DNMT3B* is be linked to a better or worse neurocognitive response to enriched or deprived environmental factors (Cordova-Palomera *et al.*, 2015e). *DNMT3B* has long been recognized to play an chief role in methylation of centromeric minor satellite repeats, and some mutations of its DNA sequence have been found in developmental disorders (Okano *et al.*, 1999). This and other neuropsychiatric findings led to hypothesizing that it may serve as a plasticity marker of cognitive response. The results of this work indicate that a *DNMT3B* polymorphism may allow determining which individuals are more prone to either improving or worsening their intelligence in response to either enriched or deprived environments. This may have indirect implications in psychiatric research considering the cognitive impairments typically found in depression (Marazziti *et al.*, 2010).

[Manuscript 7] Then, a novel molecular biology technique was used to analyze DNA methylation signatures potentially related to recent depressive symptomatology (Cordova-Palomera *et al.*, 2015f). A previous report had shown hypomethylation of an intron of *DEPDC7* (chr11:33040743) in both saliva of adolescent twins and post-mortem brain tissue of major depressive disorder patients (Dempster *et al.*, 2014). In line with the former findings, it was found that the intrapair difference in DNA methylation levels of the same CpG in peripheral blood of MZ twins correlated with their intrapair differences in depression symptoms during the last 30 days. This result expands on the previous study by proposing that *DEPDC7* (chr11:33040743) DNA methylation may dynamically vary depending on the depressive traits observed in an individual.

[Manuscript 8] To complement the previous report, a genome-wide DNA methylation analysis was conducted. Its results showed that both differential methylation and differential variability have a role in the etiology and clinical manifestation of depression, and provided clues on specific genomic loci of potential interest in the epigenetics of depression (Cordova-Palomera *et al.*, 2015d).

The previous three manuscripts are particularly relevant as indicators of an epigenetic regulation of (activational) plasticity in depression-related traits and symptoms. These results are relevant in view of the evidence supporting a link between DNA methylation and cognitive plasticity (Miller *et al.*, 2008), and particularly since phenotypic plasticity is largely determined by epigenetic factors (Schlichting and Wund, 2014). Additionally, they complement previous evidence of stochastic epigenetic variation as a chief factor in plasticity and disease (Feinberg, 2007; Feinberg and Irizarry, 2010), by signaling some genomic loci of potential interest to understand depressive psychopathology.

[Manuscript 9] Further research integrated molecular genetic information on the *FKBP5* gene, which participates in the epigenetic response to stress (Provencal and Binder, 2015), with structural connectivity data to try to determine some neurobiological mechanisms underlying depression risk. In this work, specific alterations of the communication patterns between the hippocampus and the rest of the brain were found in depression; these effects were modulated by the *FKBP5* gene (Cordova-Palomera *et al.*, 2015b). This finding would somehow support the idea that the epigenetic processes modulated by DNA sequence changes could alter the information transfer patterns between brain regions.

[Manuscript 10] Finally, to further expand on brain communicational deficits in depression, an additional research project investigated the role of genetic and environmental factors in shaping the amygdalar synchrony with the rest of the (depressed) brain (Cordova-Palomera *et al.*, 2015g). This work showed an important role for unique environmental factors in determining neural synchrony deficits in the amygdala of depressed individuals; furthermore, by implementing a novel analytical approach to fMRI time-series analysis, it was showed that the individual genetic background may alter the synchrony of the amygdala's oscillatory amplitude envelope (at the low-frequency BOLD

fluctuations). The former finding suggests that the genetically-driven alterations of the amygdalar resting-state activity in depression may be better elicited by examining the analytical representation (amplitude envelope) of the brain's BOLD fMRI activity.

In summary, these five projects somehow indicate the presence of activational plasticity deficits in depression. They show putative neurobiological mechanisms underneath the daily-life manifestation of depression (i.e., transversal traits). They also set forth novel epigenetic and brain imaging mediators of system-wide disruptions linked to depression. Namely, these findings suggest that some physiological disruptions in depression are detectable not only in through brain information, but also by analyzing epigenetic signatures in DNA extracted from peripheral tissue. This is suggested by indicating putative genetic markers of neurocognitive plasticity and by proposing some DNA methylation signatures potentially correlated with the clinical manifestation of depressive psychopathology. Similarly, the last two of them propose novel brain information transfer mechanisms –mainly through the hippocampus and the amygdala– that could be altered in depression. These two mechanisms are seemingly driven by genetic and epigenetic factors.

In general, these reports propose novel genetic, epigenetic and brain physiology mechanisms as candidate markers of activational plasticity deficits in depression-related phenotypes. Once more, rather than concluding, this research is proposing feasible biological pathways to understand the clinical manifestation of depressive psychopathology.

In conclusion, these ten reports provide support to the *neuroplasticity hypothesis* of depression, from both developmental and activational perspectives. Developmentally, they suggest putative etiopathogenic pathways leading from an altered early neurodevelopment to an increased risk for depression-related phenotypes. By exploring and combining genetic, environmental and psychopathologic concepts, the feasibility of these results has been explained by combining the popular genetic pleiotropy hypothesis in psychiatry (Gorwood, 2004; Cross-Disorder Group of the Psychiatric Genomics, 2013) with a notion of disease-specificity liability driven by the environment. With regards to activational plasticity, this work has proposed novel genetic and epigenetic

signatures potentially underlying the clinical manifestation of neuropsychiatric and neurocognitive features of depression (i.e., the genetics of *DNMT3B* and the epigenetics of *DEPDC7*); additionally, it has proposed new putative neurobiological mechanisms to explain depressive traits (i.e., a combination of differential and variable methylation, a genetically-mediated hippocampal communication deficit, and a new amygdalar synchrony failure driven by the genes).

As stated in all the manuscripts, the limitations of these reports should certainly be considered. Nine out of these ten research projects –all but the meta-analysis– have been carried out using a relatively small twin sample which may have constrained the statistical power. Thus, the findings require replication. However, being able to detect biologically-feasible effects may argue in favor of moderate or large effect sizes for the mentioned associations. Additionally, latent clinical heterogeneity of the participants may have also been an issue across these reports. This must definitely be addressed by future studies, though it should be noted that perhaps some neurobiological homogeneity may have allowed detecting statistical associations despite the clinical divergences. Other specific limitations have been further discussed within each article.

7. REFERENCES

Abdolmaleky, H. M., Smith, C. L., Faraone, S. V., Shafa, R., Stone, W., Glatt, S. J. & Tsuang, M. T. (2004) 'Methylomics in psychiatry: Modulation of gene-environment interactions may be through DNA methylation', *Am J Med Genet B Neuropsychiatr Genet*, **127B**(1), pp. 51-59.

Aguilera, O., Fernandez, A. F., Munoz, A. & Fraga, M. F. (2010) 'Epigenetics and environment: a complex relationship', *J Appl Physiol (1985)*, **109**(1), pp. 243-251.

Aldao, A. & Nolen-Hoeksema, S. (2012) 'When are adaptive strategies most predictive of psychopathology?', *J Abnorm Psychol*, **121**(1), p. 276.

Alemany, S., Arias, B., Aguilera, M., Villa, H., Moya, J., Ibanez, M. I., Vossen, H., Gasto, C., Ortet, G. & Fananas, L. (2011) 'Childhood abuse, the BDNF-Val66Met polymorphism and adult psychotic-like experiences', *Br J Psychiatry*, **199**(1), pp. 38-42.

Alemany, S., Arias, B., Fatjo-Vilas, M., Villa, H., Moya, J., Ibanez, M. I., Ortet, G., Gasto, C. & Fananas, L. (2014) 'Psychosis-inducing effects of cannabis are related to both childhood abuse and COMT genotypes', *Acta Psychiatr Scand*, **129**(1), pp. 54-62.

Alisch, R. S., Chopra, P., Fox, A. S., Chen, K., White, A. T., Roseboom, P. H., Keles, S. & Kalin, N. H. (2014) 'Differentially methylated plasticity genes in the amygdala of young primates are linked to anxious temperament, an at risk phenotype for anxiety and depressive disorders', *J Neurosci*, **34**(47), pp. 15548-15556.

Alvarado, S., Tajerian, M., Suderman, M., Machnes, Z., Pierfelice, S., Millecamps, M., Stone, L. S. & Szyf, M. (2015) 'An epigenetic hypothesis for the genomic memory of pain', *Front Cell Neurosci*, **9**, p. 88.

Andersen, S. L. (2003) 'Trajectories of brain development: point of vulnerability or window of opportunity?', *Neurosci Biobehav Rev*, **27**(1-2), pp. 3-18.

Ansorge, M. S., Hen, R. & Gingrich, J. A. (2007) 'Neurodevelopmental origins of depressive disorders', *Curr Opin Pharmacol*, **7**(1), pp. 8-17.

Arslan, A. (2015) 'Genes, brains, and behavior: imaging genetics for neuropsychiatric disorders', *J Neuropsychiatry Clin Neurosci*, **27**(2), pp. 81-92.

Ashby, W. R. (1956) *An introduction to cybernetics*, New York,, J. Wiley.

Ayroles, J. F., Buchanan, S. M., O'Leary, C., Skutt-Kakaria, K., Grenier, J. K., Clark, A. G., Hartl, D. L. & de Bivort, B. L. (2015) 'Behavioral idiosyncrasy reveals genetic control of phenotypic variability', *Proc Natl Acad Sci U S A*.

Baker-Andresen, D., Ratnu, V. S. & Bredy, T. W. (2013) 'Dynamic DNA methylation: a prime candidate for genomic metaplasticity and behavioral adaptation', *Trends Neurosci*, **36**(1), pp. 3-13.

Ballestar, E. (2010) 'Epigenetics lessons from twins: prospects for autoimmune disease', *Clin Rev Allergy Immunol*, **39**(1), pp. 30-41.

Barbier, E., Tapocik, J. D., Juergens, N., Pitcairn, C., Borich, A., Schank, J. R., Sun, H., Schuebel, K., Zhou, Z., Yuan, Q., Vendruscolo, L. F., Goldman, D. & Heilig, M. (2015) 'DNA methylation in the medial prefrontal cortex regulates alcohol-induced behavior and plasticity', *J Neurosci*, **35**(15), pp. 6153-6164.

Bavamian, S., Mellios, N., Lalonde, J., Fass, D. M., Wang, J., Sheridan, S. D., Madison, J. M., Zhou, F., Rueckert, E. H., Barker, D., Perlis, R. H., Sur, M. & Haggarty, S. J. (2015) 'Noncoding RNAs connect genetic risk factors to the neurodevelopmental basis of bipolar disorder', *Mol Psychiatry*, **20**(5), p. 548.

Begg, M. D. & Parides, M. K. (2003) 'Separation of individual-level and cluster-level covariate effects in regression analysis of correlated data', *Stat Med*, **22**(16), pp. 2591-2602.

Belsky, J., Jonassaint, C., Pluess, M., Stanton, M., Brummett, B. & Williams, R. (2009) 'Vulnerability genes or plasticity genes?', *Mol Psychiatry*, **14**(8), pp. 746-754.

Belsky, J. & Pluess, M. (2013) 'Beyond risk, resilience, and dysregulation: phenotypic plasticity and human development', *Dev Psychopathol*, **25**(4 Pt 2), pp. 1243-1261.

Berg, K. (1988) 'Variability gene effect on cholesterol at the Kidd blood group locus', *Clin Genet*, **33**(2), pp. 102-107.

Bigos, K. L. & Weinberger, D. R. (2010) 'Imaging genetics--days of future past', *Neuroimage*, **53**(3), pp. 804-809.

Bondy, B. (2011) 'Genetics in psychiatry: are the promises met?', *World J Biol Psychiatry*, **12**(2), pp. 81-88.

Borgwardt, S. J., Picchioni, M. M., Ettinger, U., Toulopoulou, T., Murray, R. & McGuire, P. K. (2010) 'Regional gray matter volume in monozygotic twins concordant and discordant for schizophrenia', *Biol Psychiatry*, **67**(10), pp. 956-964.

Bradshaw, A. D. (1965) 'Evolutionary significance of phenotypic plasticity in plants', *Advances in genetics*, **13**, pp. 115-155.

Brans, R. G., Kahn, R. S., Schnack, H. G., van Baal, G. C., Posthuma, D., van Haren, N. E., Lepage, C., Lerch, J. P., Collins, D. L., Evans, A. C., Boomsma, D. I. & Hulshoff Pol, H. E. (2010) 'Brain plasticity and intellectual ability are influenced by shared genes', *J Neurosci*, **30**(16), pp. 5519-5524.

Bunge, S. A. & Wright, S. B. (2007) 'Neurodevelopmental changes in working memory and cognitive control', *Curr Opin Neurobiol*, **17**(2), pp. 243-250.

Burrows, E. L. & Hannan, A. J. (2013) 'Decanalization mediating gene-environment interactions in schizophrenia and other psychiatric disorders with neurodevelopmental etiology', *Front Behav Neurosci*, **7**, p. 157.

Bylsma, L. M., Morris, B. H. & Rottenberg, J. (2008) 'A meta-analysis of emotional reactivity in major depressive disorder', *Clin Psychol Rev*, **28**(4), pp. 676-691.

Calabrese, F., Molteni, R., Racagni, G. & Riva, M. A. (2009) 'Neuronal plasticity: a link between stress and mood disorders', *Psychoneuroendocrinology*, **34 Suppl 1**, pp. S208-216.

Calkins, K. & Devaskar, S. U. (2011) 'Fetal origins of adult disease', *Curr Probl Pediatr Adolesc Health Care*, **41**(6), pp. 158-176.

Carlin, J. B., Gurrin, L. C., Sterne, J. A., Morley, R. & Dwyer, T. (2005) 'Regression models for twin studies: a critical review', *Int J Epidemiol*, **34**(5), pp. 1089-1099.

Casey, B. J., Oliveri, M. E. & Insel, T. (2014) 'A neurodevelopmental perspective on the research domain criteria (RDoC) framework', *Biol Psychiatry*, **76**(5), pp. 350-353.

Caspi, A., McClay, J., Moffitt, T. E., Mill, J., Martin, J., Craig, I. W., Taylor, A. & Poulton, R. (2002) 'Role of genotype in the cycle of violence in maltreated children', *Science*, **297**(5582), pp. 851-854.

Castren, E. & Rantamaki, T. (2010) 'The role of BDNF and its receptors in depression and antidepressant drug action: Reactivation of developmental plasticity', *Dev Neurobiol*, **70**(5), pp. 289-297.

Caswell, H. (1983) 'Phenotypic plasticity in life-history traits: demographic effects and evolutionary consequences', *American Zoologist*, **23**(1), pp. 35-46.

Cattaneo, A., Macchi, F., Plazzotta, G., Veronica, B., Bocchio-Chiavetto, L., Riva, M. A. & Pariante, C. M. (2015) 'Inflammation and neuronal plasticity: a link between childhood trauma and depression pathogenesis', *Front Cell Neurosci*, **9**, p. 40.

Cicchetti, D. & Cohen, D. J. (2006) *Developmental psychopathology*, Hoboken, N.J., John Wiley & Sons.

Cicchetti, D. & Walker, E. F. (2003) *Neurodevelopmental mechanisms in psychopathology*, Cambridge, UK ; New York, Cambridge University Press.

Cordova-Palomera, A., Alemany, S., Falcon, C., Bargallo, N., Goldberg, X., Crespo-Facorro, B., Nenadic, I. & Fananas, L. (2014a) 'Cortical thickness correlates of psychotic experiences:

examining the effect of season of birth using a genetically informative design', *J Psychiatr Res*, **56**, pp. 144-149.

Cordova-Palomera, A., Alemany, S., Fatjo-Vilas, M., Goldberg, X., Leza, J. C., Gonzalez-Pinto, A., Nenadic, I. & Fananas, L. (2014b) 'Birth weight, working memory and epigenetic signatures in IGF2 and related genes: a MZ twin study', *PLoS One*, **9**(8), p. e103639.

Cordova-Palomera, A., Calati, R., Arias, B., Ibanez, M. I., Moya, J., Ortet, G., Crespo-Facorro, B. & Fananas, L. (2015a) 'Season of birth and subclinical psychosis: systematic review and meta-analysis of new and existing data', *Psychiatry Res*, **225**(3), pp. 227-235.

Cordova-Palomera, A., De Reus, M. A., Fatjó-Vilas, M., Falcon, C., Bargallo, N., Van den Heuvel, M. P. & Fananas, L. (2015b) 'Polymorphic variation in FKBP5 interacts with hippocampal connectivity to influence the risk for depression: a twin design study', *(Submitted)*.

Cordova-Palomera, A., Fatjo-Vilas, M., Falcon, C., Bargallo, N., Alemany, S., Crespo-Facorro, B., Nenadic, I. & Fananas, L. (2015c) 'Birth weight and adult IQ, but not anxious-depressive psychopathology, are associated with cortical surface area: a study in twins', *PLoS ONE (in press)*.

Cordova-Palomera, A., Fatjo-Vilas, M., Gasto, C., Navarro, V., Krebs, M. O. & Fananas, L. (2015d) 'Genome-wide methylation study on depression: differential methylation and variable methylation in monozygotic twins', *Transl Psychiatry*, **5**, p. e557.

Cordova-Palomera, A., Fatjo-Vilas, M., Kebir, O., Gasto, C., Krebs, M. O. & Fananas, L. (2015e) 'Polymorphic variation in the epigenetic gene DNMT3B modulates the environmental impact on cognitive ability: a twin study', *Eur Psychiatry*, **30**(2), pp. 303-308.

Cordova-Palomera, A., Fatjo-Vilas, M., Palma-Gudiel, H., Blasco-Fontecilla, H., Kebir, O. & Fananas, L. (2015f) 'Further evidence of DEPDC7 DNA hypomethylation in depression: A study in adult twins', *Eur Psychiatry*.

Cordova-Palomera, A., Goldberg, X., Alemany, S., Nenadic, I., Gasto, C. & Fananas, L. (2014c) 'Letter to the editor: low birth weight and adult depression: eliciting their association', *Psychol Med*, **44**(5), pp. 1117-1119.

Cordova-Palomera, A., Tornador, C., Falcon, C., Bargallo, N., Nenadic, I., Deco, G. & Fananas, L. (2015g) 'Altered amygdalar resting-state connectivity in depression is explained by both genes and environment', *Hum Brain Mapp*.

Cross-Disorder Group of the Psychiatric Genomics, C. (2013) 'Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis', *Lancet*, **381**(9875), pp. 1371-1379.

Chen, F., Wegener, G., Madsen, T. M. & Nyengaard, J. R. (2013) 'Mitochondrial plasticity of the hippocampus in a genetic rat model of depression after antidepressant treatment', *Synapse*, **67**(3), pp. 127-134.

Choi, S., Han, K. M., Won, E., Yoon, B. J., Lee, M. S. & Ham, B. J. (2015) 'Association of brain-derived neurotrophic factor DNA methylation and reduced white matter integrity in the anterior corona radiata in major depression', *J Affect Disord*, **172C**, pp. 74-80.

Day, J. J. & Sweatt, J. D. (2010) 'DNA methylation and memory formation', *Nat Neurosci*, **13**(11), pp. 1319-1323.

de Kloet, E. R., Joels, M. & Holsboer, F. (2005) 'Stress and the brain: from adaptation to disease', *Nat Rev Neurosci*, **6**(6), pp. 463-475.

Dempster, E. L., Pidsley, R., Schalkwyk, L. C., Owens, S., Georgiades, A., Kane, F., Kalidindi, S., Picchioni, M., Kravariti, E., Toulopoulou, T., Murray, R. M. & Mill, J. (2011) 'Disease-associated epigenetic changes in monozygotic twins discordant for schizophrenia and bipolar disorder', *Hum Mol Genet*, **20**(24), pp. 4786-4796.

Dempster, E. L., Wong, C. C., Lester, K. J., Burrage, J., Gregory, A. M., Mill, J. & Eley, T. C. (2014) 'Genome-wide Methylomic Analysis of Monozygotic Twins Discordant for Adolescent Depression', *Biol Psychiatry*, **76**(12), pp. 977-983.

Dong, E., Dzitoyeva, S. G., Matrisciano, F., Tueting, P., Grayson, D. R. & Guidotti, A. (2015) 'Brain-derived neurotrophic factor epigenetic modifications associated with schizophrenia-like phenotype induced by prenatal stress in mice', *Biol Psychiatry*, **77**(6), pp. 589-596.

Draisma, H. H. M. (2011) 'Analysis of metabolomics data from twin families', Division of Analytical Biosciences, Leiden/Amsterdam Center for Drug Research (LACDR), Faculty of Science, Leiden University.

Dukas, R. (1998) *Cognitive ecology : the evolutionary ecology of information processing and decision making*, Chicago, University of Chicago Press.

Dunn, E. C., McLaughlin, K. A., Slopen, N., Rosand, J. & Smoller, J. W. (2013) 'Developmental timing of child maltreatment and symptoms of depression and suicidal ideation in young adulthood: results from the National Longitudinal Study of Adolescent Health', *Depress Anxiety*, **30**(10), pp. 955-964.

Estrada, G., Fatjo-Vilas, M., Munoz, M. J., Pulido, G., Minano, M. J., Toledo, E., Illa, J. M., Martin, M., Miralles, M. L., Miret, S., Campanera, S., Bernabeu, C., Navarro, M. E. & Fananas, L. (2011) 'Cannabis use and age at onset of psychosis: further evidence of interaction with COMT Val158Met polymorphism', *Acta Psychiatr Scand*, **123**(6), pp. 485-492.

Ettinger, U., Schmechtig, A., Toulopoulou, T., Borg, C., Orrells, C., Owens, S., Matsumoto, K., van Haren, N. E., Hall, M. H., Kumari, V., McGuire, P. K., Murray, R. M. & Picchioni, M. (2012) 'Prefrontal and striatal volumes in monozygotic twins concordant and discordant for schizophrenia', *Schizophr Bull*, **38**(1), pp. 192-203.

Fananas, L., Moral, P. & Bertranpetit, J. (1990) 'Quantitative dermatoglyphics in schizophrenia: study of family history subgroups', *Hum Biol*, **62**(3), pp. 421-427.

Fananas, L., van Os, J., Hoyos, C., McGrath, J., Mellor, C. S. & Murray, R. (1996) 'Dermatoglyphic a-b ridge count as a possible marker for developmental disturbance in schizophrenia: replication in two samples', *Schizophr Res*, **20**(3), pp. 307-314.

Fatjo-Vilas, M., Pomarol-Clotet, E., Salvador, R., Monte, G. C., Gomar, J. J., Sarro, S., Ortiz-Gil, J., Aguirre, C., Landin-Romero, R., Guerrero-Pedraza, A., Papiol, S., Blanch, J., McKenna, P. J. & Fananas, L. (2012) 'Effect of the interleukin-1beta gene on dorsolateral prefrontal cortex function in schizophrenia: a genetic neuroimaging study', *Biol Psychiatry*, **72**(9), pp. 758-765.

Feinberg, A. P. (2007) 'Phenotypic plasticity and the epigenetics of human disease', *Nature*, **447**(7143), pp. 433-440.

Feinberg, A. P. & Irizarry, R. A. (2010) 'Evolution in health and medicine Sackler colloquium: Stochastic epigenetic variation as a driving force of development, evolutionary adaptation, and disease', *Proc Natl Acad Sci U S A*, **107 Suppl 1**, pp. 1757-1764.

Foley, D. L., Craig, J. M., Morley, R., Olsson, C. A., Dwyer, T., Smith, K. & Saffery, R. (2009) 'Prospects for epigenetic epidemiology', *Am J Epidemiol*, **169**(4), pp. 389-400.

Forsman, A. (2014) 'Rethinking phenotypic plasticity and its consequences for individuals, populations and species', *Heredity (Edinb)*.

Fresco, D. M., Williams, N. L. & Nugent, N. R. (2006) 'Flexibility and negative affect: Examining the associations of explanatory flexibility and coping flexibility to each other and to depression and anxiety', *Cognitive Therapy and Research*, **30**(2), pp. 201-210.

Freund, J., Brandmaier, A. M., Lewejohann, L., Kirste, I., Kritzler, M., Kruger, A., Sachser, N., Lindenberger, U. & Kempermann, G. (2013) 'Emergence of individuality in genetically identical mice', *Science*, **340**(6133), pp. 756-759.

Gilbert, S. L., Dobyns, W. B. & Lahn, B. T. (2005) 'Genetic links between brain development and brain evolution', *Nat Rev Genet*, **6**(7), pp. 581-590.

Gill, H. C. (1883) 'II. Twins Suffering from Mania', *The British Journal of Psychiatry*, **28**(124), pp. 540-544.

Gliga, T., Jones, E. J., Bedford, R., Charman, T. & Johnson, M. H. (2014) 'From early markers to neuro-developmental mechanisms of autism', *Dev Rev*, **34**(3), pp. 189-207.

Goldberg, X., Fatjo-Vilas, M., Alemany, S., Nenadic, I., Gasto, C. & Fananas, L. (2013) 'Gene-environment interaction on cognition: a twin study of childhood maltreatment and COMT variability', *J Psychiatr Res*, **47**(7), pp. 989-994.

Goldstein, G., Minshew, N. J., Allen, D. N. & Seaton, B. E. (2002) 'High-functioning autism and schizophrenia: a comparison of an early and late onset neurodevelopmental disorder', *Arch Clin Neuropsychol*, **17**(5), pp. 461-475.

Gorwood, P. (2004) 'Generalized anxiety disorder and major depressive disorder comorbidity: an example of genetic pleiotropy?', *Eur Psychiatry*, **19**(1), pp. 27-33.

Gottesman, II & Gould, T. D. (2003) 'The endophenotype concept in psychiatry: etymology and strategic intentions', *Am J Psychiatry*, **160**(4), pp. 636-645.

Grayson, D. R. & Guidotti, A. (2013) 'The dynamics of DNA methylation in schizophrenia and related psychiatric disorders', *Neuropsychopharmacology*, **38**(1), pp. 138-166.

Greenough, W. T., Black, J. E. & Wallace, C. S. (1987) 'Experience and brain development', *Child Dev*, **58**(3), pp. 539-559.

Gunnar, M. R. & Nelson, C. A. (1992) *Developmental behavioral neuroscience*, Hillsdale, N.J., Lawrence Erlbaum Associates.

Hall, J. G. (2003) 'Twinning', *Lancet*, **362**(9385), pp. 735-743.

Halligan, P. W. & David, A. S. (2001) 'Cognitive neuropsychiatry: towards a scientific psychopathology', *Nat Rev Neurosci*, **2**(3), pp. 209-215.

Harkness, K. L., Hayden, E. P. & Lopez-Duran, N. L. (2015) 'Stress sensitivity and stress sensitization in psychopathology: an introduction to the special section', *J Abnorm Psychol*, **124**(1), pp. 1-3.

Hashimoto, R., Numakawa, T., Ohnishi, T., Kumamaru, E., Yagasaki, Y., Ishimoto, T., Mori, T., Nemoto, K., Adachi, N., Izumi, A., Chiba, S., Noguchi, H., Suzuki, T., Iwata, N., Ozaki, N., Taguchi, T., Kamiya, A., Kosuga, A., Tatsumi, M., Kamijima, K., Weinberger, D. R., Sawa, A. & Kunugi, H. (2006) 'Impact of the DISC1 Ser704Cys polymorphism on risk for major depression, brain morphology and ERK signaling', *Hum Mol Genet*, **15**(20), pp. 3024-3033.

Haukvik, U. K., Rimol, L. M., Roddey, J. C., Hartberg, C. B., Lange, E. H., Vaskinn, A., Melle, I., Andreassen, O. A., Dale, A. & Agartz, I. (2014) 'Normal birth weight variation is related to cortical morphology across the psychosis spectrum', *Schizophr Bull*, **40**(2), pp. 410-419.

Heijmans, B. T., Tobi, E. W., Stein, A. D., Putter, H., Blauw, G. J., Susser, E. S., Slagboom, P. E. & Lumey, L. H. (2008) 'Persistent epigenetic differences associated with prenatal exposure to famine in humans', *Proc Natl Acad Sci U S A*, **105**(44), pp. 17046-17049.

Heim, C. & Binder, E. B. (2012) 'Current research trends in early life stress and depression: review of human studies on sensitive periods, gene-environment interactions, and epigenetics', *Exp Neurol*, **233**(1), pp. 102-111.

Heim, C., Shugart, M., Craighead, W. E. & Nemeroff, C. B. (2010) 'Neurobiological and psychiatric consequences of child abuse and neglect', *Dev Psychobiol*, **52**(7), pp. 671-690.

Henikoff, S. & Matzke, M. A. (1997) 'Exploring and explaining epigenetic effects', *Trends Genet*, **13**(8), pp. 293-295.

Herpfer, I., Hezel, H., Reichardt, W., Clark, K., Geiger, J., Gross, C. M., Heyer, A., Neagu, V., Bhatia, H., Atas, H. C., Fiebich, B. L., Bischofberger, J., Haas, C. A., Lieb, K. & Normann, C. (2012) 'Early life stress differentially modulates distinct forms of brain plasticity in young and adult mice', *PLoS One*, **7**(10), p. e46004.

Hu, F. B., Goldberg, J., Hedeker, D. & Henderson, W. G. (1998) 'Modelling ordinal responses from co-twin control studies', *Stat Med*, **17**(9), pp. 957-970.

Hyman, S. E. (2007) 'Can neuroscience be integrated into the DSM-V?', *Nat Rev Neurosci*, **8**(9), pp. 725-732.

Japyassú, H. F. & Caires, R. A. (2008) 'Hunting tactics in a cobweb spider (Araneae-Theridiidae) and the evolution of behavioral plasticity', *Journal of Insect Behavior*, **21**(4), pp. 258-284.

Japyassu, H. F. & Malange, J. (2014) 'Plasticity, stereotypy, intra-individual variability and personality: handle with care', *Behav Processes*, **109 Pt A**, pp. 40-47.

Johnstone, A. L., Wahlestedt, C. & Silva, J. P. (2013) 'To Eat or Not To Eat: The Neurobiological Substrates Guiding Maladaptive Decision-Making in Obesity', *J Addict Med Ther*, **1**, p. 1002.

Joshi, S. H., Espinoza, R. T., Pirnia, T., Shi, J., Wang, Y., Ayers, B., Leaver, A., Woods, R. P. & Narr, K. L. (2015) 'Structural Plasticity of the Hippocampus and Amygdala Induced by Electroconvulsive Therapy in Major Depression', *Biol Psychiatry*.

Kaminsky, Z., Petronis, A., Wang, S. C., Levine, B., Ghaffar, O., Floden, D. & Feinstein, A. (2008) 'Epigenetics of personality traits: an illustrative study of identical twins discordant for risk-taking behavior', *Twin Res Hum Genet*, **11**(1), pp. 1-11.

Kaminsky, Z. A., Tang, T., Wang, S. C., Ptak, C., Oh, G. H., Wong, A. H., Feldcamp, L. A., Virtanen, C., Halfvarson, J., Tysk, C., McRae, A. F., Visscher, P. M., Montgomery, G. W., Gottesman, II, Martin, N. G. & Petronis, A. (2009) 'DNA methylation profiles in monozygotic and dizygotic twins', *Nat Genet*, **41**(2), pp. 240-245.

Kashdan, T. B. & Rottenberg, J. (2010) 'Psychological flexibility as a fundamental aspect of health', *Clin Psychol Rev*, **30**(7), pp. 865-878.

Kashdan, T. B. & Steger, M. F. (2006) 'Expanding the topography of social anxiety. An experience-sampling assessment of positive emotions, positive events, and emotion suppression', *Psychol Sci*, **17**(2), pp. 120-128.

Kelleher, I. & Cannon, M. (2011) 'Psychotic-like experiences in the general population: characterizing a high-risk group for psychosis', *Psychol Med*, **41**(1), pp. 1-6.

Killackey, H. P. (1990) 'Neocortical Expansion: An Attempt toward Relating Phylogeny and Ontogeny', *J Cogn Neurosci*, **2**(1), pp. 1-17.

Klengel, T., Pape, J., Binder, E. B. & Mehta, D. (2014) 'The role of DNA methylation in stress-related psychiatric disorders', *Neuropharmacology*, **80**, pp. 115-132.

Knickmeyer, R. C., Wang, J., Zhu, H., Geng, X., Woolson, S., Hamer, R. M., Konneker, T., Lin, W., Styner, M. & Gilmore, J. H. (2014) 'Common variants in psychiatric risk genes predict brain structure at birth', *Cereb Cortex*, **24**(5), pp. 1230-1246.

Kolb, B. (1995) *Brain plasticity and behavior*, Mahwah, N.J., Lawrence Erlbaum Associates.

Korzus, E. (2010) 'Manipulating the brain with epigenetics', *Nat Neurosci*, **13**(4), pp. 405-406.

Kounali, D., Zammit, S., Wiles, N., Sullivan, S., Cannon, M., Stochl, J., Jones, P., Mahedy, L., Gage, S. H., Heron, J. & Lewis, G. (2014) 'Common versus psychopathology-specific risk factors for psychotic experiences and depression during adolescence', *Psychol Med*, **44**(12), pp. 2557-2566.

Kuhn, M., Popovic, A. & Pezawas, L. (2014) 'Neuroplasticity and memory formation in major depressive disorder: an imaging genetics perspective on serotonin and BDNF', *Restor Neurol Neurosci*, **32**(1), pp. 25-49.

Kuhn, S., Schubert, F. & Gallinat, J. (2012) 'Higher prefrontal cortical thickness in high schizotypal personality trait', *J Psychiatr Res*, **46**(7), pp. 960-965.

Kuratomi, G., Iwamoto, K., Bundo, M., Kusumi, I., Kato, N., Iwata, N., Ozaki, N. & Kato, T. (2008) 'Aberrant DNA methylation associated with bipolar disorder identified from discordant monozygotic twins', *Mol Psychiatry*, **13**(4), pp. 429-441.

LaPlant, Q., Vialou, V., Covington, H. E., 3rd, Dumitriu, D., Feng, J., Warren, B. L., Maze, I., Dietz, D. M., Watts, E. L., Iniguez, S. D., Koo, J. W., Mouzon, E., Renthal, W., Hollis, F., Wang, H., Noonan, M. A., Ren, Y., Eisch, A. J., Bolanos, C. A., Kabbaj, M., Xiao, G., Neve, R. L., Hurd, Y. L., Oosting, R. S., Fan, G., Morrison, J. H. & Nestler, E. J. (2010) 'Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens', *Nat Neurosci*, **13**(9), pp. 1137-1143.

Lee, Y.-A., Yamaguchi, Y. & Goto, Y. (2015) 'Neurodevelopmental Plasticity in Pre-and Postnatal Environmental Interactions: Implications for Psychiatric Disorders from an Evolutionary Perspective', *Neural Plasticity*, **2015**.

Linden, D. J. (1994) 'Long-term synaptic depression in the mammalian brain', *Neuron*, **12**(3), pp. 457-472.

Linscott, R. J. & van Os, J. (2010) 'Systematic reviews of categorical versus continuum models in psychosis: evidence for discontinuous subpopulations underlying a psychometric continuum. Implications for DSM-V, DSM-VI, and DSM-VII', *Annu Rev Clin Psychol*, **6**, pp. 391-419.

Liu, J., Chen, J., Ehrlich, S., Walton, E., White, T., Perrone-Bizzozero, N., Bustillo, J., Turner, J. A. & Calhoun, V. D. (2013) 'Methylation Patterns in Whole Blood Correlate With Symptoms in Schizophrenia Patients', *Schizophr Bull*.

Lupien, S. J., McEwen, B. S., Gunnar, M. R. & Heim, C. (2009) 'Effects of stress throughout the lifespan on the brain, behaviour and cognition', *Nat Rev Neurosci*, **10**(6), pp. 434-445.

Machin, G. (2009) 'Non-identical monozygotic twins, intermediate twin types, zygosity testing, and the non-random nature of monozygotic twinning: a review', *Am J Med Genet C Semin Med Genet*, **151C**(2), pp. 110-127.

Marazziti, D., Consoli, G., Picchetti, M., Carlini, M. & Faravelli, L. (2010) 'Cognitive impairment in major depression', *Eur J Pharmacol*, **626**(1), pp. 83-86.

Marco, E. M., Macri, S. & Laviola, G. (2011) 'Critical age windows for neurodevelopmental psychiatric disorders: evidence from animal models', *Neurotox Res*, **19**(2), pp. 286-307.

Masi, G. & Brovedani, P. (2011) 'The hippocampus, neurotrophic factors and depression', *CNS Drugs*.

Mastroeni, D., McKee, A., Grover, A., Rogers, J. & Coleman, P. D. (2009) 'Epigenetic differences in cortical neurons from a pair of monozygotic twins discordant for Alzheimer's disease', *PLoS One*, **4**(8), p. e6617.

McEwen, B. S. (2001) 'Plasticity of the hippocampus: adaptation to chronic stress and allostatic load', *Ann N Y Acad Sci*, **933**, pp. 265-277.

Mery, F. & Burns, J. G. (2010) 'Behavioural plasticity: an interaction between evolution and experience', *Evolutionary Ecology*, **24**(3), pp. 571-583.

Miettinen, O. S. (1983) 'The need for randomization in the study of intended effects', *Stat Med*, **2**(2), pp. 267-271.

Miller, C. A., Campbell, S. L. & Sweatt, J. D. (2008) 'DNA methylation and histone acetylation work in concert to regulate memory formation and synaptic plasticity', *Neurobiol Learn Mem*, **89**(4), pp. 599-603.

Miller, C. A. & Sweatt, J. D. (2007) 'Covalent modification of DNA regulates memory formation', *Neuron*, **53**(6), pp. 857-869.

Moran, N. A. (1992) 'The evolutionary maintenance of alternative phenotypes', *American Naturalist*, pp. 971-989.

Nenadic, I., Maitra, R., Basmanav, F. B., Schultz, C. C., Lorenz, C., Schachtzabel, C., Smesny, S., Nothen, M. M., Cichon, S., Reichenbach, J. R., Sauer, H., Schlosser, R. G. & Gaser, C. (2015)

'ZNF804A genetic variation (rs1344706) affects brain grey but not white matter in schizophrenia and healthy subjects', *Psychol Med*, **45**(1), pp. 143-152.

Nikolova, Y. S. & Hariri, A. R. (2015) 'Can we observe epigenetic effects on human brain function?', *Trends Cogn Sci*.

Okano, M., Bell, D. W., Haber, D. A. & Li, E. (1999) 'DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development', *Cell*, **99**(3), pp. 247-257.

Pardes, H., Kaufmann, C. A., Pincus, H. A. & West, A. (1989) 'Genetics and psychiatry: past discoveries, current dilemmas, and future directions', *Am J Psychiatry*, **146**(4), pp. 435-443.

Paus, T., Keshavan, M. & Giedd, J. N. (2008) 'Why do many psychiatric disorders emerge during adolescence?', *Nat Rev Neurosci*, **9**(12), pp. 947-957.

Petronis, A. (2006) 'Epigenetics and twins: three variations on the theme', *Trends Genet*, **22**(7), pp. 347-350.

Petronis, A. (2010) 'Epigenetics as a unifying principle in the aetiology of complex traits and diseases', *Nature*, **465**(7299), pp. 721-727.

Pike, A., Reiss, D., Hetherington, E. M. & Plomin, R. (1996) 'Using MZ differences in the search for nonshared environmental effects', *J Child Psychol Psychiatry*, **37**(6), pp. 695-704.

Pishva, E., Drukker, M., Viechtbauer, W., Decoster, J., Collip, D., van Winkel, R., Wichers, M., Jacobs, N., Thiery, E., Derom, C., Geschwind, N., van den Hove, D., Lataster, T., Myin-Germeys, I., van Os, J., Rutten, B. P. & Kenis, G. (2014) 'Epigenetic genes and emotional reactivity to daily life events: a multi-step gene-environment interaction study', *PLoS One*, **9**(6), p. e100935.

Pittenger, C. (2013) 'Disorders of memory and plasticity in psychiatric disease', *Dialogues Clin Neurosci*, **15**(4), pp. 455-463.

Pittenger, C. & Duman, R. S. (2008) 'Stress, depression, and neuroplasticity: a convergence of mechanisms', *Neuropsychopharmacology*, **33**(1), pp. 88-109.

Plomin, R. & Davis, O. S. (2009) 'The future of genetics in psychology and psychiatry: microarrays, genome-wide association, and non-coding RNA', *J Child Psychol Psychiatry*, **50**(1-2), pp. 63-71.

Posthuma, D., Beem, A. L., de Geus, E. J., van Baal, G. C., von Hjelmborg, J. B., Iachine, I. & Boomsma, D. I. (2003) 'Theory and practice in quantitative genetics', *Twin Res*, **6**(5), pp. 361-376.

Posthuma, D. & Boomsma, D. I. (2000) 'A note on the statistical power in extended twin designs', *Behav Genet*, **30**(2), pp. 147-158.

Provencal, N. & Binder, E. B. (2015) 'The neurobiological effects of stress as contributors to psychiatric disorders: focus on epigenetics', *Curr Opin Neurobiol*, **30**, pp. 31-37.

Raine, A. (2006) 'Schizotypal personality: neurodevelopmental and psychosocial trajectories', *Annu Rev Clin Psychol*, **2**, pp. 291-326.

Rapoport, J. L., Giedd, J. N. & Gogtay, N. (2012) 'Neurodevelopmental model of schizophrenia: update 2012', *Mol Psychiatry*, **17**(12), pp. 1228-1238.

Raser, J. M. & O'Shea, E. K. (2005) 'Noise in gene expression: origins, consequences, and control', *Science*, **309**(5743), pp. 2010-2013.

Raznahan, A., Greenstein, D., Lee, N. R., Clasen, L. S. & Giedd, J. N. (2012) 'Prenatal growth in humans and postnatal brain maturation into late adolescence', *Proc Natl Acad Sci U S A*, **109**(28), pp. 11366-11371.

Relton, C. L. & Davey Smith, G. (2010) 'Epigenetic epidemiology of common complex disease: prospects for prediction, prevention, and treatment', *PLoS Med*, **7**(10), p. e1000356.

Rijsdijk, F. V. & Sham, P. C. (2002) 'Analytic approaches to twin data using structural equation models', *Brief Bioinform*, **3**(2), pp. 119-133.

Rimol, L. M., Nesvag, R., Hagler, D. J., Jr., Bergmann, O., Fennema-Notestine, C., Hartberg, C. B., Haukvik, U. K., Lange, E., Pung, C. J., Server, A., Melle, I., Andreassen, O. A., Agartz, I. & Dale, A.

M. (2012) 'Cortical volume, surface area, and thickness in schizophrenia and bipolar disorder', *Biol Psychiatry*, **71**(6), pp. 552-560.

Rolls, E. T. (2012) *Neuroculture : on the implications of brain science*, Oxford ; New York, Oxford University Press.

Rosenzweig, M. R. & Bennett, E. L. (1996) 'Psychobiology of plasticity: effects of training and experience on brain and behavior', *Behav Brain Res*, **78**(1), pp. 57-65.

Royer, S. & Pare, D. (2003) 'Conservation of total synaptic weight through balanced synaptic depression and potentiation', *Nature*, **422**(6931), pp. 518-522.

Sabunciyan, S., Aryee, M. J., Irizarry, R. A., Rongione, M., Webster, M. J., Kaufman, W. E., Murakami, P., Lessard, A., Yolken, R. H., Feinberg, A. P., Potash, J. B. & Gen, R. E. D. C. (2012) 'Genome-wide DNA methylation scan in major depressive disorder', *PLoS One*, **7**(4), p. e34451.

Sanderson, T. M., Cotel, M. C., O'Neill, M. J., Tricklebank, M. D., Collingridge, G. L. & Sher, E. (2012) 'Alterations in hippocampal excitability, synaptic transmission and synaptic plasticity in a neurodevelopmental model of schizophrenia', *Neuropharmacology*, **62**(3), pp. 1349-1358.

Savage, G. H. (1883) 'I. Twins Suffering from Similar Attacks of Melancholia', *The British Journal of Psychiatry*, **28**(124), pp. 539-540.

Schall, J. D. (2004) 'On building a bridge between brain and behavior', *Annu. Rev. Psychol.*, **55**, pp. 23-50.

Schlichting, C. D. & Wund, M. A. (2014) 'Phenotypic plasticity and epigenetic marking: an assessment of evidence for genetic accommodation', *Evolution*, **68**(3), pp. 656-672.

Simpson, J. A., Weiner, E. S. C. & Oxford University Press. (1989) *The Oxford English dictionary*, Oxford

Oxford ; New York, Clarendon Press ;

Oxford University Press.

Snell-Rood, E. C. (2013) 'An overview of the evolutionary causes and consequences of behavioural plasticity', *Animal Behaviour*, **85**(5), pp. 1004-1011.

Sultan, S. E. (2000) 'Phenotypic plasticity for plant development, function and life history', *Trends Plant Sci*, **5**(12), pp. 537-542.

Talens, R. P., Boomsma, D. I., Tobi, E. W., Kremer, D., Jukema, J. W., Willemsen, G., Putter, H., Slagboom, P. E. & Heijmans, B. T. (2010) 'Variation, patterns, and temporal stability of DNA methylation: considerations for epigenetic epidemiology', *FASEB J*, **24**(9), pp. 3135-3144.

Tau, G. Z. & Peterson, B. S. (2010) 'Normal development of brain circuits', *Neuropsychopharmacology*, **35**(1), pp. 147-168.

Teare, M. D. (2011) *Genetic epidemiology*, New York, NY, Humana Press.

Tobi, E. W., Lumey, L. H., Talens, R. P., Kremer, D., Putter, H., Stein, A. D., Slagboom, P. E. & Heijmans, B. T. (2009) 'DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific', *Hum Mol Genet*, **18**(21), pp. 4046-4053.

Tost, H., Böhringer, A. & Meyer-Lindenberg, A. (2014) 'Imaging Genetics: Unraveling the Neurogenetic Risk Architecture of Mental Illness', *MRI in Psychiatry*, Springer.

Turecki, G., Ota, V. K., Belangero, S. I., Jackowski, A. & Kaufman, J. (2014) 'Early life adversity, genomic plasticity, and psychopathology', *The Lancet Psychiatry*, **1**(6), pp. 461-466.

Van Buskirk, J. (2002) 'Phenotypic lability and the evolution of predator-induced plasticity in tadpoles', *Evolution*, **56**(2), pp. 361-370.

van Dongen, J., Slagboom, P. E., Draisma, H. H., Martin, N. G. & Boomsma, D. I. (2012) 'The continuing value of twin studies in the omics era', *Nat Rev Genet*, **13**(9), pp. 640-653.

Visscher, P. M. & Posthuma, D. (2010) 'Statistical power to detect genetic Loci affecting environmental sensitivity', *Behav Genet*, **40**(5), pp. 728-733.

Walhovd, K. B., Fjell, A. M., Brown, T. T., Kuperman, J. M., Chung, Y., Hagler, D. J., Jr., Roddey, J. C., Erhart, M., McCabe, C., Akshoomoff, N., Amaral, D. G., Bloss, C. S., Libiger, O., Schork, N. J., Darst, B. F., Casey, B. J., Chang, L., Ernst, T. M., Frazier, J., Gruen, J. R., Kaufmann, W. E., Murray, S. S., van Zijl, P., Mostofsky, S., Dale, A. M., Pediatric Imaging, N. & Genetics, S. (2012) 'Long-term influence of normal variation in neonatal characteristics on human brain development', *Proc Natl Acad Sci U S A*, **109**(49), pp. 20089-20094.

Walsh, M. R., Cooley, F. t., Biles, K. & Munch, S. B. (2015) 'Predator-induced phenotypic plasticity within- and across-generations: a challenge for theory?', *Proc Biol Sci*, **282**(1798), p. 20142205.

Waltereit, R., Banaschewski, T., Meyer-Lindenberg, A. & Poustka, L. (2014) 'Interaction of neurodevelopmental pathways and synaptic plasticity in mental retardation, autism spectrum disorder and schizophrenia: implications for psychiatry', *World J Biol Psychiatry*, **15**(7), pp. 507-516.

Wells, J. C. & Stock, J. T. (2011) 'Re-examining heritability: genetics, life history and plasticity', *Trends Endocrinol Metab*, **22**(10), pp. 421-428.

Whitfield-Gabrieli, S. & Ford, J. M. (2012) 'Default mode network activity and connectivity in psychopathology', *Annu Rev Clin Psychol*, **8**, pp. 49-76.

Widiger, T. A. (2011) 'Personality and psychopathology', *World Psychiatry*, **10**(2), pp. 103-106.

Wiers, C. E. (2012) 'Methylation and the human brain: towards a new discipline of imaging epigenetics', *Eur Arch Psychiatry Clin Neurosci*, **262**(3), pp. 271-273.

Wigman, J. T., van Nierop, M., Vollebergh, W. A., Lieb, R., Beesdo-Baum, K., Wittchen, H. U. & van Os, J. (2012) 'Evidence that psychotic symptoms are prevalent in disorders of anxiety and depression, impacting on illness onset, risk, and severity--implications for diagnosis and ultra-high risk research', *Schizophr Bull*, **38**(2), pp. 247-257.

Wojcik, W., Lee, W., Colman, I., Hardy, R. & Hotopf, M. (2013) 'Foetal origins of depression? A systematic review and meta-analysis of low birth weight and later depression', *Psychol Med*, **43**(1), pp. 1-12.

Wolfensberger, S. P., Veltman, D. J., Hoogendijk, W. J., Boomsma, D. I. & de Geus, E. J. (2008) 'Amygdala responses to emotional faces in twins discordant or concordant for the risk for anxiety and depression', *Neuroimage*, **41**(2), pp. 544-552.

Wong, A. H., Gottesman, II & Petronis, A. (2005) 'Phenotypic differences in genetically identical organisms: the epigenetic perspective', *Hum Mol Genet*, **14 Spec No 1**, pp. R11-18.

Yu, C. C., Furukawa, M., Kobayashi, K., Shikishima, C., Cha, P. C., Sese, J., Sugawara, H., Iwamoto, K., Kato, T., Ando, J. & Toda, T. (2012) 'Genome-wide DNA methylation and gene expression analyses of monozygotic twins discordant for intelligence levels', *PLoS One*, **7**(10), p. e47081.

Zammit, S., Wiles, N. & Lewis, G. (2010) 'The study of gene–environment interactions in psychiatry: limited gains at a substantial cost?', *Psychol Med*, **40**(05), pp. 711-716.

Zhao, J., Goldberg, J., Bremner, J. D. & Vaccarino, V. (2013) 'Association between promoter methylation of serotonin transporter gene and depressive symptoms: a monozygotic twin study', *Psychosom Med*, **75**(6), pp. 523-529.