FKBP5 modulates the hippocampal connectivity deficits in depression: a study in twins

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Abstract The hippocampus is a key modulator of stress responses underlying depressive behavior. While 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 depression. 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.

Keywords FKBP5 · Hippocampus · Depression · Brain network · MZ twins · DWI

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, both genetic variation and brain phenotypes measured with magnetic resonance imaging (MRI) have independently been associated with depression (Graham et al. 2013; Northoff 2013; Parasuraman and Jiang 2012; Van Horn 2014), and studies combining these two data sources provide a valuable tool to elicit the complex etiology of this psychopathology. Overall, neuroimaging genetic studies constitute a robust method to investigate how some genetic factors may alter brain activity and lead to behavioral and psychopathological outcomes (Dinov et al. 2014; Thompson et al. 2014). While one of the most recent approaches to study brain structure in psychopathology is the analysis of wiring patterns between different brain regions using graph theory and diffusion weighted imaging (DWI) (Fornito and Bullmore 2015; Hulshoff Pol and Bullmore 2013; Leistedt and Linkowski 2013), 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 previous findings 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 several depression risk factors and hippocampal features (Binder et al. 2008; Fani 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; Pagliaccio et al. 2014; Fani et al. 2014). Though this may be related to genetic factors underlying a communicational deficit of the hippocampus with other brain areas in individuals with depressive psychopathology, 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

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.

As shown in a previous report using this general population sample (Cordova-Palomera et al. 2014), prevalence estimates for lifetime depressive psychopathology in the whole group (230 individuals) was in agreement with other literature reports (Kessler et al. 2005). Using the previously collected data, a subset of 54 individuals (27 MZ twin pairs) was selected from the group of MZ twins, as they were informative for psychopathological traits and gave consent to participate in the MRI part of the present study. These 54 individuals were carefully chosen to have similar-sized twin subgroups with different genetic and environmental psychopathology predisposition (concordant, discordant and healthy pairs). They met the following criteria: i) age at scan between 21 and 53 years, ii) both twins right-handed, iii) none of the twins met diagnostic criteria for DSM-IV-R psychiatric diagnoses other than depression and/or anxiety (see below, Psychometric measures), and iv) no twin had a medical history of neurological disturbance, sensory or motor alterations, or substance misuse or dependence. None of the participants had a family history of liability for psychiatric disorders other than depression or anxiety in first degree relatives. The 54-individual sample included in all statistical analyses consisted of 20 males and 34 females, with a mean age of 34.8 years. Further
demographic information on this sample can be found in Table 1 and 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 had previously received 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; Ruipérez et al. 2001). Descriptive data from the current sample is summarized in Table 1. Total BSI scores for the affected co-twins from the ten discordant pairs had a slightly larger upper range limit (range in healthy discordant: 6–42 points; range in affected discordant: 4–45 points), although the present sample subset was underpowered to conduct formal statistical testing. The lack of marked score differences may be due to both the small sample size (10 discordant pairs) and the transversal character of the BSI (contrasting with the lifetime character of the DSM-IV SCID interview). As expected, in the overall sample (27 pairs), 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. Since both the DSM-IV SCID and the BSI questionnaire were in agreement with each other, individuals were classified as “depression-affected” only following the categorical conventions of the DSM-IV. Namely, subjects were considered “affected” if they met DSM-IV criteria for either depressive (F32.x), anxious (F40.x, F41.x) or comorbid anxious-depressive psychopathology. In the diagnostic-concordant subset (12 individuals), there were 4 depression, 5 anxiety and 4 comorbid diagnoses, whereas in the 10-individual subset of affected co-twins from diagnostic-discordant pairs there were 6 depression, 1 anxiety and 3 comorbid diagnoses. As shown in Table 1, there were no disease-associated cognitive differences.

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

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic, psychopathological and genotypic data for DSM-IV diagnostic concordant, discordant and healthy MZ twin pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1360780 genotype</td>
<td><strong>CONCORDANT</strong> (12 subjects, 10 female)</td>
</tr>
<tr>
<td></td>
<td>Frequency</td>
</tr>
<tr>
<td>CC</td>
<td>8</td>
</tr>
<tr>
<td>CT</td>
<td>2</td>
</tr>
<tr>
<td>TT</td>
<td>2</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>Range</td>
</tr>
<tr>
<td>Age</td>
<td>37 (12.1)</td>
</tr>
<tr>
<td>IQ</td>
<td>99.5 (14.6)</td>
</tr>
<tr>
<td>Total BSI</td>
<td>37.3 (26.7)</td>
</tr>
<tr>
<td>Depressive symptoms (BSI subscale)</td>
<td>8.7 (7.5)</td>
</tr>
</tbody>
</table>

<sup>a</sup>standard deviation; <sup>b</sup>intellectual quotient; <sup>c</sup>Brief Symptom Inventory

*statistically significant p-value

<sup>a</sup>Fisher’s exact test for count data

<sup>b</sup>Kruskal-Wallis X-squared, as these variables were continuous
blood samples were available. The common functional variant rs1360780, within the FKB5 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 intergroup differences in genotype frequency distribution across concordant, discordant and healthy pairs (Table 1).

MRI acquisition and pre-processing The images were acquired at the MRI Unit of the Image Platform (IDIBAPS, Hospital Clinic de Barcelona), using a TIM TRIO 3 T 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 mm³ 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 v5.1.0 (freesurfer-Linux-centos4 x86_64-stable-pub-v5.1.0; 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 Fig. 1. Complementarily, in recognition that
the NOS across each pair of brain of regions may be volume dependent (van den Heuvel and Sporns 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 and Sporns 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).

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 and Everett 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.e., nodal 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). A summary of the centrality measures obtained for the concordant, discordant and healthy groups is shown in Table 2.

Table 2 Descriptive data of the three nodal centrality measures analyzed for both left and right hippocampal ROIs, for DSM-IV diagnostic concordant, discordant and healthy MZ twin pairs

<table>
<thead>
<tr>
<th>Nodal Centrality Measure</th>
<th>Brain Hemisphere</th>
<th>CONCORDANT (12 subjects, 10 female)</th>
<th>DISCORDANT (20 subjects, 14 female)</th>
<th>HEALTHY (22 subjects, 10 female)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (S.D.) Range</td>
<td>Mean (S.D.) Range</td>
<td>Mean (S.D.) Range</td>
</tr>
<tr>
<td>Strength</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>7093.5 (2285.1)</td>
<td>3351–11,059</td>
<td>7099.2 (2003.6) 4046–11,344</td>
<td>8161.5 (2594.2) 4972–16,038</td>
</tr>
<tr>
<td>Right</td>
<td>5830 (1223.4)</td>
<td>3909–8306</td>
<td>7229.1 (2537) 3957–12,024</td>
<td>7280.7 (2498.2) 3728–12,651</td>
</tr>
<tr>
<td>Betweenness centrality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>479.2 (195.4)</td>
<td>244–924</td>
<td>457.1 (176.6) 176–874</td>
<td>432.6 (167.3) 44–744</td>
</tr>
<tr>
<td>Right</td>
<td>332.3 (212.7)</td>
<td>30–786</td>
<td>410.3 (126.6) 168–682</td>
<td>353.4 (164.2) 20–712</td>
</tr>
<tr>
<td>Eigenvector centrality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>0.04 (0.02)</td>
<td>0.03–0.08</td>
<td>0.03 (0.01) 0.01–0.06</td>
<td>0.04 (0.02) 0.01–0.09</td>
</tr>
<tr>
<td>Right</td>
<td>0.03 (0.01)</td>
<td>0.01–0.05</td>
<td>0.03 (0.02) 0.01–0.07</td>
<td>0.03 (0.02) 0.01–0.08</td>
</tr>
</tbody>
</table>

Values calculated from the number of streamlines (NOS)-weighted matrices

SD standard deviation

Statistical analysis All inter-subject analyses were conducted using logistic regression models using R’s software packages rms and mztwinreg (Harrel 2013; R Development Core Team 2011; Córdova-Palomera 2015). 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.

\[
\logit(\pi) = \beta_0 + \beta_1(\text{gender}) + \beta_2(\text{age}) + \beta_3(\text{rs1360780}) + \beta_4(\text{strength}) + \beta_5(\text{bet.}) + \beta_6(\text{eigenv.})
\]

was fitted. Here, \( \pi \) stands for the probability of an individual being depressed, and the left or right hippocampal metrics are introduced 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 tuned using, along with the familial-level covariates gender, age and genotype,

\[ \logit(\pi_{ij}) = \beta_0 + \beta_B \mu_i + \beta_W (X_{ij} - \mu_i) \]

in order to obtain estimates of both a) familial factors (genetic plus shared environment, \(\beta_W\) and b) unique environmental influences (from non-shared events within a pair, \(\beta_W\)) on every graph-theoretical nodal centrality measure (i.e., strength, betweenness centrality and eigenvector centrality). Subindex \(i \in \{1, \ldots, n\}\) stands for pair number (here, \(n = 27\) MZ pairs) and \(j \in \{1, 2\}\) refers to co-twin number (randomly assigned). \(\pi_{ij}\) stands for the probability that co-twin \(j\) from the \(i\)-th pair has of being affected by depression. \(\beta_0\) 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. None of the statistical model fits used for this study gave evidence of high multicollinearity: all variance inflation factors for the independent variables were clearly below the conventional threshold of 10 (O’Brien 2007). Additionally, the variance-covariance matrices of the model fits were adjusted to correct for correlated responses from twin pairs, using the Huber-White method as implemented by the robcov function from R’s rms package (Harrell 2013).

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). This test was implemented using the standard function \texttt{lrtest} from R’s rms package, which allows assessing pairs of nested models (Harrell 2013).

Power analysis estimations for these multiple regression models were conducted following standard protocols (Champely 2012; Cohen 1988). After including all covariates, the most saturated model considered here had 8 numerator and 40 denominator degrees of freedom. Using the conventional significance level of 0.05, the present sample has a power of 77.4 % 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). This power estimate can be considered acceptable in behavioral research (Cohen 1988), and the estimates for the rest of the models reached equally acceptable values, well above 80 %.

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 of biological and behavioral data in research protocols including various multivariate regression models in the same setting (Benjamini and Hochberg 1995; Cook and Farewell 1996; Glickman et al. 2014; Liu et al. 2004; Nakagawa 2004; Pemeger 1998).

### 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 (\(\beta = -0.61, \text{S.E.} = 0.4, p = 0.128\)). 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: \(\beta = -0.99, \text{S.E.} = 0.39, p = 0.011\); eigenvector centrality: \(\beta = 0.92, \text{S.E.} = 0.37, p = 0.013\) (Table 3). 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) (Fig. 2b). 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 (Fig. 2d).

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 \( \text{FKBP5} \) genotype and the right hippocampal centrality in the same regression model suggested an additive interaction effect (likelihood ratio tests: \( X^2 = 10.84, \text{d.f.} = 3, p = 0.013 \) full model vs. only genotype; \( X^2 = 5.97, \text{d.f.} = 1, p = 0.015 \) full model vs. only the hippocampal metrics) (Table 3). 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 (Fig. 2c). Likewise, the results in Table 3 suggest that C homozygotes with high (right) hippocampal eigenvector centrality show an increased probability of depression as compared to CT and TT genotype individuals (Fig. 2c). Additionally, Table 3 shows no evidence of interaction effects between the left hippocampal metrics and the \( \text{FKBP5} \) genotype (likelihood ratio tests: \( X^2 = 6.05, \text{d.f.} = 3, p = 0.109 \) full model vs. only genotype; \( X^2 = 3.84, \text{d.f.} = 1, p = 0.05 \) full model vs. only hippocampal metrics).

Additionally, Table 3 shows no evidence of interaction effects between the left hippocampal metrics and the \( \text{FKBP5} \) genotype (likelihood ratio tests: \( X^2 = 6.05, \text{d.f.} = 3, p = 0.109 \) full model vs. only genotype; \( X^2 = 3.84, \text{d.f.} = 1, p = 0.05 \) full model vs. only hippocampal metrics).

Discussion

In this work, a genetically-informative design was implemented to evaluate putative relationships between graph theoretical measures of hippocampal centrality, the common functional \( \text{FKBP5} \) 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 \( \text{FKBP5} \) 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 depression

The first result of this work is the association between depression and both hippocampal centrality and \( \text{FKBP5} \) genotype.
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 3). 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 (Fig. 2b). 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 hippocampus of individuals with depression.

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 (Misis et al. 2014). The current data show that the right hippocampus has a more central position in the brain network of depressed individuals 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 3 and Fig. 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 4).

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 (Tables 3 and 4). 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 (Figs. 2c and f, and 3b 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. 2014b; Fujii et al. 2014a; 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). 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

### Table 4 Logistic regression test results for the association between depression, the FKBP5 genotype and both familial and environmental factors altering right hippocampal centrality

<table>
<thead>
<tr>
<th>Centrality</th>
<th>Independent model (either graph metrics or FKBP5)</th>
<th>Additive model</th>
<th>Likelihood ratio test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>S.E.</td>
<td>p (&gt;</td>
</tr>
<tr>
<td><strong>RIGHT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strength (fam.)</td>
<td>−0.84</td>
<td>0.41</td>
<td>0.039*</td>
</tr>
<tr>
<td>Strength (env.)</td>
<td>−0.73</td>
<td>0.47</td>
<td>0.121</td>
</tr>
<tr>
<td>Betweenness c. (fam.)</td>
<td>0.35</td>
<td>0.46</td>
<td>0.448</td>
</tr>
<tr>
<td>Betweenness c. (env.)</td>
<td>−0.01</td>
<td>0.38</td>
<td>0.987</td>
</tr>
<tr>
<td>Eigenvector c. (fam.)</td>
<td>0.75</td>
<td>0.41</td>
<td>0.069</td>
</tr>
<tr>
<td>Eigenvector c. (env.)</td>
<td>0.73</td>
<td>0.37</td>
<td>0.049*</td>
</tr>
<tr>
<td><strong>FKBP5</strong></td>
<td>−0.61</td>
<td>0.4</td>
<td>0.128</td>
</tr>
</tbody>
</table>

SE standard error; df degrees of freedom; c centrality
*p-value <0.05
**statistically significant p-value after FDR multiple testing adjustment
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 (Lekman et al. 2008; Lavebratt et al. 2010).

The present results for the \textit{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 (Tables 3 and 4), in partial agreement with previous literature reports (Binder et al. 2004; Zou et al. 2010). However, the current findings indicate that the familial liability for hippocampal changes that cause depression could be modulated by rs1360780’s T allele (Table 4 and Fig. 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 (Fig. 2b and c), making this brain region more responsive to therapeutic factors (Figs. 2c and f, and 3).

\textbf{Fig. 3} Familial factors altering right hippocampal centrality associate with increased risk of depression, and the \textit{FKBP5} gene moderates this association. For simplicity, \textit{FKBP5} genotype effects are represented with two levels: C homozygotes and T (minor frequency allele) carriers. \textbf{a} the \textit{FKBP5} rs1360780 genotype interacts with the familial factors altering right hippocampal nodal strength to increase depression risk; \textbf{b} the \textit{FKBP5} rs1360780 genotype interacts with the familial factors altering right hippocampal eigenvector centrality to increase depression risk.
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 (Fuji 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 (Tables 3 and 4) may support the presence of relatively strong effects. Also, the youngest depression concordant and discordant pairs are 21 and 23 years old, which makes them prone to suffering psychiatric disorders other than depression (i.e., bipolar disorder) later in life. However, as mentioned above, none of the participants had a family history of liability for those disorders in first degree relatives. Besides, 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 connectomes 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.

Additionally, the age range of this study sample is relatively large (21–53 years), which may have influenced the network connectivity measures (Koenis et al. 2015; Lim et al. 2015; Stevens et al. 2009). Nevertheless, three features of the current sample make the results robust to this potential confounding. First, previous research shows that the brain network topology changes may be more pronounced from childhood to adulthood due to the transition through adolescent maturation, and the current sample ages range from early to middle adulthood. Secondly, the effects of the maturational brain network topology changes are usually reflected in cognitive profiles, but in the current sample there were no disease-associated cognitive difference (Table 1). And thirdly, all the analyses have been internally adjusted by age, and the associations remain statistically significant.

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Compliance with ethical standards

Conflict of interest Aldo Córdova-Palomera, Marcel A. de Reus, Mar Fatjo-Vilas, Carles Falcon, Nuria Bargalló, Martijn P. van den Heuvel, and Lourdes Pañanés declare that they have no conflict of interest.

Informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, and the applicable revisions at the time of the investigation. Informed consent was obtained from all subjects for being included in the study.

References


