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Hippocampal volume changes in healthy subjects at risk of unipolar depression

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ABSTRACT

Unipolar depression is moderately heritable. It is unclear whether structural brain changes associated with unipolar depression are present in healthy persons at risk of the disorder. Here we investigated whether a genetic predisposition to unipolar depression is associated with structural brain changes. A priori, hippocampal volume reductions were hypothesized. Using a high-risk study design, magnetic resonance imaging brain scans were obtained from 59 healthy high-risk subjects having a co-twin with unipolar depression, and 53 healthy low-risk subjects without a first-degree family history of major psychiatric disorder. High-risk twins had smaller hippocampal volumes than low-risk twins ($p < 0.04$). The finding was most pronounced in DZ twins. Groups did not differ on global brain tissue volumes or regional tissue volumes assessed in exploratory voxel-wise whole cerebrum analyses. In conclusion, hippocampal volume reduction may index a predisposition to develop depression and thus may be predictive of future onset of the disorder. Further studies are needed to elucidate the role of (shared) environmental and genetic factors.

1. Introduction

Unipolar depression is heritable with concordance rates from 0.23 to 0.67 for monozygotic (MZ) twins and from 0.14 to 0.43 for dizygotic (DZ) twins (Sullivan et al., 2000). Structural brain imaging studies in unipolar depression have reported increased prevalence of white matter hyperintensities and volume decreases/changes in hippocampus, amygdala, caudate, putamen and frontal cortex (for reviews see Sheline, 2003; Lorenzetti et al., 2009; Koolschijn et al., 2009). The presence of hippocampal volume reduction in patients with unipolar depression is underscored in recent meta-analyses (Videbech and Ravnkilde, 2004; Koolschijn et al., 2009). Familial major depressive disorder has been associated with subgenual frontal volume reduction (Drevets et al., 1997). Moreover, a recent study of young psychotropic-naive patients with familial major depressive disorder, showed that the 22 included patients had significantly smaller left and right hippocampal volumes than the 35 matched controls (MacMaster et al., 2008). It is at present unclear whether structural brain changes associated with unipolar depression can also be observed in healthy persons at risk of the disorder.

In the present study we examined healthy individuals who never experienced depressive episodes to look for neuroanatomical correlates of a genetic predisposition to unipolar depressive disorder. Identification of high and low-risk individuals was accomplished by linking records of the Danish Psychiatric Central Research Register and the Danish Twin Registry. High resolution magnetic resonance (MR) scans of the brain were obtained in healthy MZ and DZ twins with a co-twin diagnosed with unipolar depression, and healthy MZ and DZ twins with a co-twin never diagnosed with an affective disorder. That is, the present study is a *high-risk* study, not a "classical" twin study, as it was not possible to investigate the (ill) co-twins. A priori we hypothesized that high-risk twins would have smaller hippocampal volumes than low-risk twins. Additionally, we performed exploratory voxel-wise whole cerebrum analyses.

2. Methods and materials

2.1. Participants

In the present study 112 participants were included (Table 1): 59 healthy twins at risk of unipolar depression (high-risk – HR – twins) and 53 healthy twins without known personal or co-twin

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Table 1
Demographic and clinical data.[a]

	High-risk	Low-risk	*p*
Number	59	53	
Age	45.0 (13.5)	38.8 (12.1)	*0.01*
Sex (M/F)	27/32	21/32	0.51
Zygosity (MZ/DZ)	18/41	22/31	0.23
Education (years)	12.3 (3.3)	14.1 (2.7)	*0.01*
Weight	71.5(11.9)	71.7 (11.4)	0.96
Height	171.2 (8.9)	173.2 (8.5)	0.22
Handedness (R/L)	50/9	44/9	0.81
HAM-D	2.81 (1,61)	1.71 (1.38)	*0.001*
	3.00 (0–7)	2 .00 (0–5)	
BDI-21[b]	2.11 (2.88)	0.96 (1.47)	0.07
	1.00 (0–10)	0 (0–6)	
BDI-14[b]	1.35 (2.07)	0.70 (1.28)	0.154
	0 (0–9)	0 (0–6)	
Life events 12[b]	2.74 (2.82)	1.55 (1.67)	*0.031*
	2.00 (0–11)	1.00 (0–8)	
Lifetime life events[c]	2.07 (1.48)	1.59 (1.36)	0.08
	2.00 (0–6)	1.00 (0–5)	
Discordance time	7.12 (7.60)		
	4.5 (1–32.5)		
Age of proband at first discharge	37.87 (12.33)		
	34.08 (19.3–68.3)		

[a] Values for the demographic data are mean (SD) or frequency; Values for the clinical data are mean (SD) and median (range) respectively. M = male; F = female; MZ = monozygotic; DZ = dizygotic; R = right; L = left; HAM-D = Hamilton Depression Scale; BDI-21 = 21-item Beck Depression Inventory; BDI-14 = 14-item Beck Depression Inventory Anxiety Subscale; Life events 12 = Number of adverse life events in the 12 months preceding the MR scan; Lifetime life events = Number of adverse lifetime life events. Discordance time = Number of months between the date a healthy high-risk twin was scanned and the date that the ill co-twin was discharged from a psychiatric hospital with a diagnosis of depression or recurrent depression.

[b] Information on BDI-21, BDI-14, Life events 12 was missing for the same 2 high-risk subjects.

[c] Information on lifetime life events was missing for 2 low-risk and 3 high-risk subjects. Two of the latter were the same as under footnote "b".

history of hospital contact with affective disorder (low-risk – LR – twins). The healthy high-risk and low-risk twins were identified through record linkage between the Danish Twin Registry, the Danish Psychiatric Research Register and the Danish Civil Register (the registers are described in more detail in Section 2.3). This linkage identified same sex twin pairs in which one twin had been treated in a psychiatric hospital setting for a depressive episode (the proband) and one had not been treated for depressive disorder, the high-risk healthy co-twin. Probands were identified as twins who on their first admission, in the period between 1968 and 2005, were discharged from a psychiatric hospital with a diagnosis of depression or recurrent depression (ICD-8-codes: 296.09, 296.29, 296.89, 296.99; ICD-10-codes: F32-33.9). Low-risk healthy control twins were identified as twins without known personal or co-twin history of hospital contact with affective disorder, and were matched on age, sex and zygosity to a high-risk twin.

The participants are a subsample of a larger cohort included in a high-risk study on affective disorders. Participants and non-participants in the latter study have been described in detail elsewhere (Vinberg et al., 2007). The current study is the first report on structural MRI findings. The selection procedure identifying the 112 participants in the current study is detailed in the Section 2.4.

The study was approved by the Danish Ministry of Health, The Danish Regional Scientific Ethical Committee [(KF)-12-122/99 and (KF)-01-001/02], and the Data Inspection Agency. The study was conducted in accordance with the latest version of the Declaration of Helsinki. All procedures were carried out with adequate understanding and written informed consent of the participants.

2.2. Clinical assessment

Participants were rated by a trained psychiatrist in a face-to-face interview using semi-structured interviews: diagnoses were obtained using Schedules for Clinical Assessment in Neuropsychiatry (SCAN) version 2.1 (Wing et al., 1990). All persons with a lifetime (current or past) diagnosis of affective disorder, schizoaffective disorder or schizophrenia according to SCAN interviews were excluded from the study. Lifetime minor psychiatric diagnoses defined as non-organic, non-schizophrenic or non-affective SCAN diagnoses were not exclusion criteria. The Hamilton Depression Scale HAM-D, 17-item (Hamilton, 1960, 1967) was used to assess depressive symptoms. At the end of the interview, participants were interviewed about lifetime family psychiatric history of first-degree relatives (their biological parents, co-twin, siblings and offspring) based on the Brief Screening for Family Psychiatric History questionnaire (Weissman et al., 2000). They were asked specifically about depression, mania and schizophrenia among their first-degree relatives and questioned whether probands had been admitted to psychiatric hospital or received medical treatment for any psychiatric disorder. An additional exclusion criterion was any significant brain disease.

Further self-rating of psychopathology was assessed using Symptom Rating Scale for Depression and Anxiety including assessment of depressive symptoms using the 21-item Beck Depression Inventory (BDI-21) (Beck et al., 1961), manic symptoms using the 6-item Mania Subscale and anxiety symptoms using the 14-item Anxiety Subscale (BDI-14) (Beck et al., 1988). Life events in the last 12 months preceding the MR scan and lifetime were recorded using a Danish version (translated to Danish after permission from the author) of the questionnaires used by (Kendler et al., 1995; Vinberg et al., 2007). Participants were asked about 9 'personal' events, i.e. events that happened to the participant, and 22 'network' events, i.e. events that occurred primarily to, or in interaction with, an individual in the participant's social network. The 'personal' events, included assault, serious marital problems, divorce/break-up, job loss, and loss of a confidant, serious illness, major financial problem, being robbed, and serious legal problems. The 'network' events included death or severe illness of the participant's spouse, child, parent, co-twin, other sibling, other relative or other individuals close to the participant and serious trouble getting along with the participant's parent, child, co-twin, sibling, in-laws, other relative, neighbor, or close friend. The number of months between the dates that a healthy high-risk twin was MR scanned and the ill co-twin was discharged from a psychiatric hospital with a diagnosis of depression or recurrent depression defined the time of discordance. Zygosity was determined from anamnesis and/or from photographs of the twin pair. Disagreement in relation to the information from the Danish Twin Registry was found in 17 cases (7.2%), a slightly higher rate than the described error rate of 5% (Hauge, 1981). In case of doubt blood samples from both twins was taken for DNA-analyses.

2.3. The registers

The Danish Civil Registration System assigns a unique personal identification number for all Danish residents. This number is linked to information on name, address, and date of birth. All other Danish registers use the same unique identifier and thus Danish residents can be tracked in all the public registers through record linkage. *The Danish Psychiatric Central Research Register* is nationwide, with registration of all psychiatric admissions and (from 1995) outpatient hospital contacts in Denmark for the country's 5.3 million inhabitants (Munk-Jorgensen and Mortensen, 1997). From April 1969 to December 1993, diseases were classified according to the International Classification of Diseases, "8th"

(ICD-8), and from January 1994 according to the International Classification of Diseases, "10th" (ICD-10). *The Danish Twin Registry* was initiated in 1953 and contains information on 75,000 twin pairs born from 1870 to 2003. The completeness is close to 100% for the period after the Civil Registration System was established in 1968 (Kyvik et al., 1996; Harvald et al., 2004). The Twin Registry contains information about the zygosity of same-sexed twins based on mailed questionnaires. The questionnaire method used in the Danish Twin Register has been found to result in error rates of <5% when compared with serological and DNA methodology (Hauge, 1981; Christiansen et al., 2003).

2.4. Selection procedure

During the recruitment period (May 2003 to September 2005), 204 high-risk and 204 low-risk twins were invited to participate in the overarching high-risk study on affective disorders (Vinberg et al., 2007). Of the initial sample of 234 participants, 20 did not want to participate in a MR scan, 19 were not scanned because of physical contraindications (metal prosthesis, artificial heart valve, etc.), 8 became claustrophobic, and 3 scans were cancelled because of overweight or technical problems. Thus MRI scans were obtained in 175 participants. Of these, 3 participants were excluded because of incidental findings of severe arterial malformations. An additional 30 participants were excluded because of significant physical illness: 16 with hypertension, 2 with diabetes, 1 with epilepsy, 4 with a history of severe head trauma, 1 with brain damage thought to result from year long exposure to organic solvents, 4 who had previously received chemotherapy, and 2 with an ongoing history of substance abuse. Finally, 16 participants with a family history of psychiatric disorders other than affective disorder (Christensen et al., 2007) and 14 participants predisposed to bipolar depression were excluded from the present study in order to maintain a well-defined high-risk group predisposed to unipolar depression. When comparing the 59 participants who were not scanned with the 175 MR scanned participants, the scanned participants had a significantly ($F_{(2,234)} = 0.49$, $p < 0.01$) higher education level (mean years of education = 13.1, SD = 3.1) than those not scanned (mean years of education = 11.8, SD = 3.4. The two groups did not differ significantly with regard to age, gender balance or risk status.

2.5. Image acquisition and analysis

MR scans were generally performed on the same day as the clinical assessment. High-resolution 3D T1 weighted, sagittal, magnetization prepared rapid gradient echo (MPRAGE) scans of the head (echo time (TE)/repetition time (TR)/inversion time (TI) = 3.93/1540/800 ms; flip angle = 9°; field of view (FOV) = 256 mm; matrix 256 × 256; 1 × 1 × 1 mm voxels; 192 slices) and 2D T2 weighted, axial, Turbo Spin Echo (TSE) scans of the whole brain (TE1/TE2/TR = 17/100/9000 ms; flip angle = 150°; FOV = 220 mm, matrix = 256 × 256; GRAPPA: acceleration factor = 2; reference lines = 30; 0.9 × 0.9 × 3 mm voxels; 50 slices) acquired on a Siemens Magnetom Trio 3T MR scanner with an eight-channel head coil (In vivo, FL, USA) were used for analysis. MPRAGE, TSE and an additional acquired fluid-attenuated inversion recovery (FLAIR) scan were used for clinical evaluation by a neuroradiologist.

Both T1 and T2 images were corrected for spatial distortions due to non-linearity in the gradient system of the scanner (Jovicich et al., 2006) using the Gradient Non-Linearity Distortion Correction software distributed by the Biomedical Informatics Research Network (http://www.nbirn.net) and T1 images were reordered in transverse orientation. Resulting images are referred to as 'raw' T1 or T2 images. Additional image processing was done with the VBM5 toolbox (http://dbm.neuro.uni-jena.de/vbm/vbm5-for-spm5/) and the DARTEL ("Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra", Ashburner, 2007) tools implemented in SPM5 (Wellcome Department of Cognitive Neurology, University College London, UK). Images generated at each processing stage were visually checked to ensure the quality of the image processing. First, 'raw' T1 images were processed using the VBM5 toolbox to generate the gray (GM) and white matter (WM), and cerebral spinal fluid (CSF) tissue maps in native space and the spatial transformation (12 affine plus non-linear) from native to MNI space necessary for the DARTEL analysis. The VBM5 toolbox extends the unified segmentation algorithm of SPM5 (Ashburner and Friston, 2005) with the Hidden Markov Random Field (HMRF) approach based on (Cuadra et al., 2005) (settings: warping regularization = 1, warp frequency cutoff = 25, bias regularization = 0.0001, bias FWHM = 70 mm cutoff, sample distance = 3, HMRF weighting = 0.3, light clean of partitions). The unified segmentation method combines radio-frequency inhomogeneity correction, tissue classification and image registration in one generative model. The HMRF model uses spatial information in a 3 × 3 × 3 voxel neighborhood to remove isolated voxels of a certain tissue class and to close holes in clusters of connected voxels belonging to a certain tissue type, thereby minimizing the noise level of the resulting tissue classification. 'Raw' T2 weighted images were processed with the VBM5 toolbox to automatically create brain masks in native space (writing options → bias corrected → native space = yes; additional scalp editing = yes). The latter were applied to the GM, WM and CSF tissue probability maps to get rid of non brain/CSF tissue not cleaned by the cleaning step incorporated in the unified segmentation.

Next, DARTEL (using default settings) was used to perform a high-dimensional inter-subject registration (Fig. 1). Firstly, brain masked GM and WM tissue maps in native space were imported into DARTEL using corresponding spatial normalization transformation matrices. After import, rigidly aligned GM and WM images were used to estimate the non-linear deformations that best align all images together. The latter is achieved by alternating between building a template and registering individual tissue class images with the template. The initial template is the average of the imported images whereas the last is the average of the DARTEL registered data. Using the final flow fields that parameterize the deformations, brain masked GM, WM, and CSF images were warped into average image space ("DARTEL space") and modulated with the jacobian determinant of the applied deformation fields to correct for local volume changes following the high-dimensional inter-subject warping. Voxel-wise analyses thus test for differences in regional tissue volume (GM, WM and CSF). Modulated and warped GM, WM, and CSF images were then divided by the supratentorial (st) intracranial volume (ICV) to account for differences in head size. st-ICV estimates were acquired by integrating and adding the image intensity values of modulated and warped GM, WM and CSF images where the brainstem and cerebellum were masked out. To this end a brainstem and cerebellum mask was delineated on the last DARTEL GM template (i.e., the average of the DARTEL registered GM images). Finally, resulting tissue images were smoothed with a 4 mm FWHM Gaussian kernel.

To test our a priori hypothesis of decreased hippocampal volumes in subjects at risk for unipolar affective disorder, left and right hippocampal volumes of interest were delineated using FSLview (FMRIB Image Analysis Group University of Oxford, FMRIB Centre, Department of Clinical Neurology: http://www.fmrib.ox.ac.uk/fsl/fslview) on the last DARTEL GM template overlaid on an average of bias corrected T1 weighted images warped into DARTEL space using established criteria (Maller et al., 2006) (Fig. 2). By integrating the image intensity values within these VOI's in the modulated and warped GM images we extracted left and right hippocampal volumes for each subject.

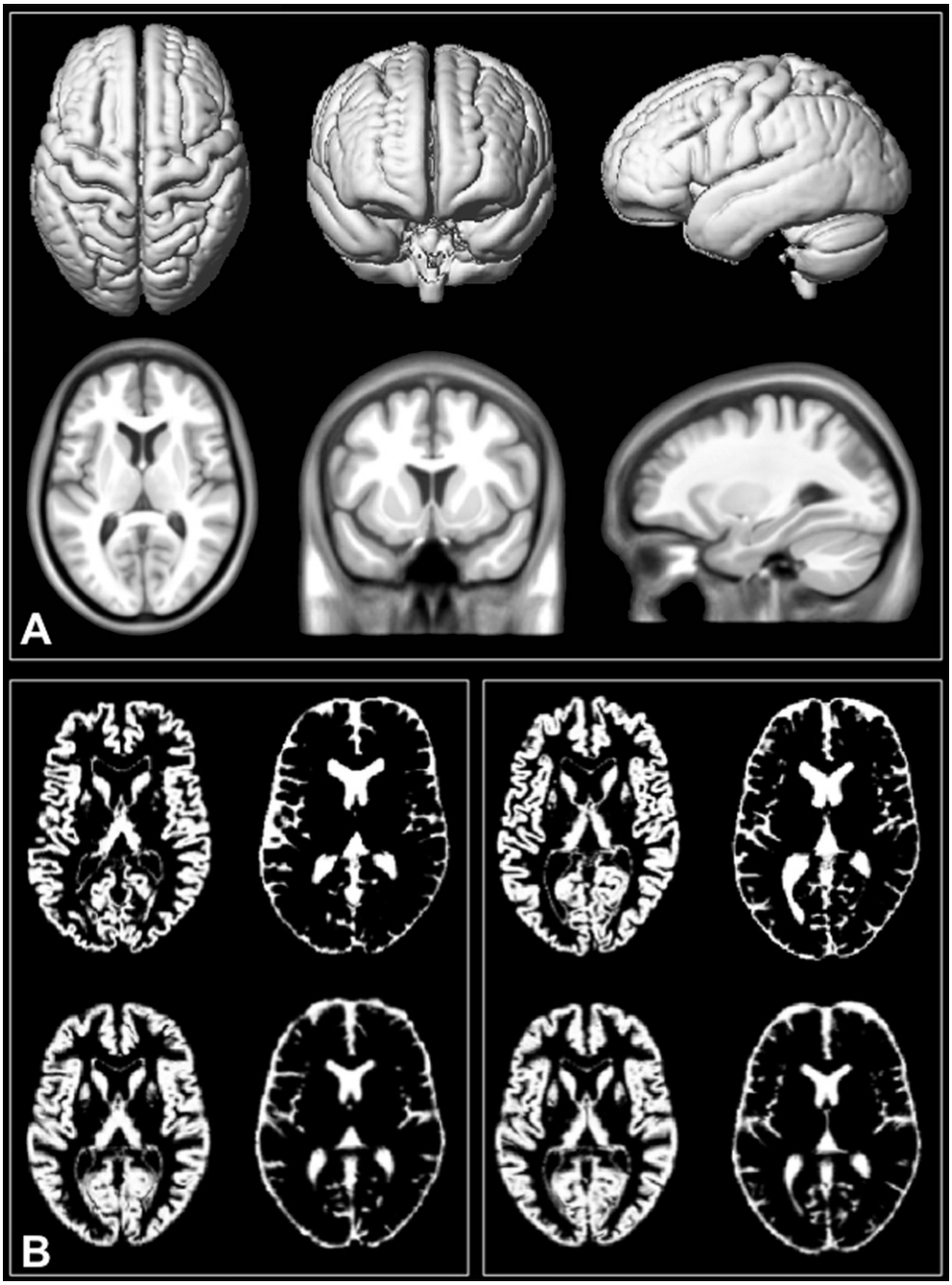

Fig. 1. DARTEL allows high-dimensional non-linear inter-subject registration. (A) Top row shows a rendering of the average GM map of the 112 subjects who were finally included in the study. The bottom row shows an axial, coronal and sagittal slice through the average of the 112 warped T1 weighted images. The clear definition of gyri and subcortical structures indicate that DARTEL successfully diminished inter-individual anatomical variation. (B) Each box depicts axial GM and CSF slices of two subjects before (top row) and after (bottom row) warping.

2.6. Statistical analyses

The Statistical Package for the Social Sciences (SPSS, version 15 for Windows) was used for statistical analyses of demographic data, and global brain and hippocampal volumes. One-way ANOVA and Pearson's chi-square tests were respectively used for continuous (age, education, weight, height) and categorical demographic (sex, zygosity, handedness) data. Group comparisons on HAM -D, BDI-21, BDI-14, number of adverse life events in the last 12 months and number of lifetime adverse life events were performed using nonparametric the Mann–Whitney U test. Global supratentorial (st) volumes i.e., st-GM, st-WM and st-CSF volumes, total brain volume (st-TBV = st-GM + st-WM), and intracranial volume (st-ICV = st-TBV + st-CSF), were derived from the DARTEL modulated and warped GM, WM, and CSF tissue images where the brainstem and cerebellum were masked out by integrating the image intensity values. With the exception of st-ICV, global volumes were expressed as a percentage of st-ICV volume to correct for differences in head size. Global brain measures were analyzed with ANCOVA using age and gender (and years of education in st-GM, st-WM, and st-TBV analyses) as covariates of no interest. Hippocampal volumes, expressed as percentage of st-ICV, were analyzed with repeated measures MANCOVA with Hemisphere (left and right hippocampal volume) as a within subject variable and Risk

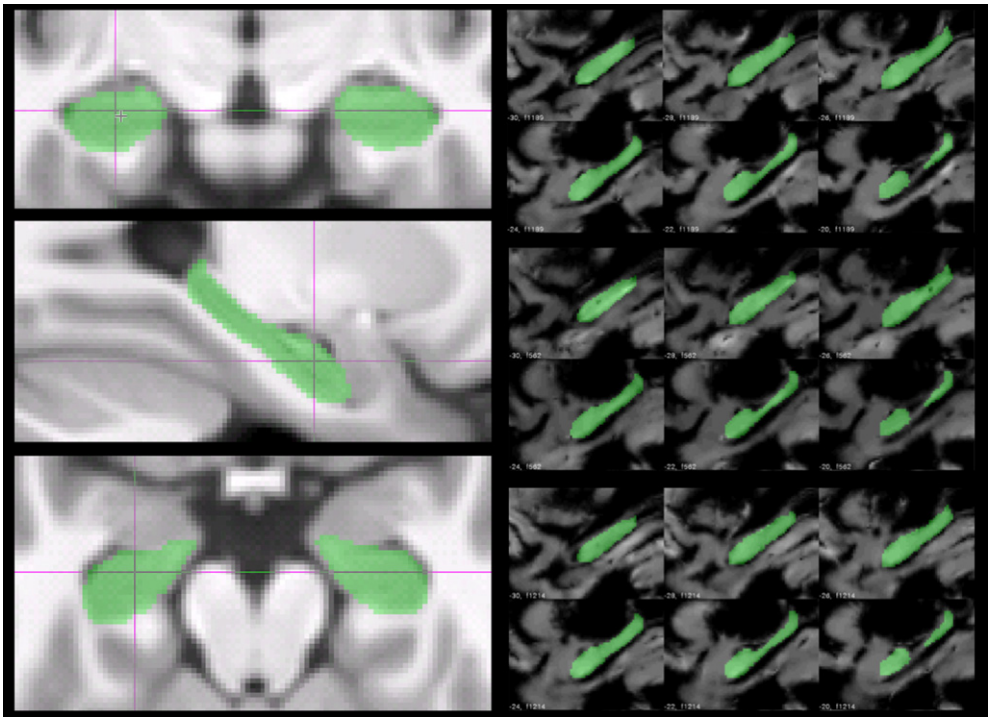

Fig. 2. At the left hand side of the image the left hippocampal volume of interest is overlaid on the average DARTEL warped T1 weighted images of the 112 subjects who were finally included in the study. At the right hand side of the image the left hippocampal volume of interest is overlaid on the modulated DARTEL warped GM maps of three participants.

(high-risk, low-risk) and Gender (male, female) as between group factors. Age and years of education were entered as covariates of no interest. Follow-up analyses with age squared and/or age cubic were performed to model potential non-linear age effects.

The general linear model (GLM) as implemented in SPM5 was used in the voxel-wise analyses of the smoothed, st-ICV weighted, modulated and warped tissue classes. Only voxels with a mean intensity >0.1 computed over all images were included in the computations (Ashburner and Friston, 2000). Four dummy variables in the design matrix represented group membership (e.g. MZ-HR, DZ-HR, MZ-LR and DZ-LR). Age, gender and years of education were entered as covariates of no interest in GM, WM analyses, while in the CSF analysis only age and gender were entered. Analyses were restricted to the cerebrum. T-contrasts were used to compare high-risk and low-risk groups. The main effect of Zygosity and the Zygosity × Risk interaction was examined using F-contrasts. Non-isotropic smoothness was corrected for by using the VBM5 toolbox (Hayasaka et al., 2004). A false discovery rate (FDR) threshold of 0.05 was used to correct for multiple comparisons (Genovese et al., 2002).

3. Results

3.1. Demographics and clinical data

Groups did not differ on sex, zygosity, height, weight, or handedness (Table 1). However, groups did differ significantly in age and education, with the high-risk twins being somewhat older and having received less years of education. The education effect remained significant after controlling for age.

High-risk twins scored significantly higher on the HAM-D and number of adverse life events in the past 12 months than low-risk twins. Moreover, there was a trend for high-risk twins to score higher than low-risk twins on the BDI-21 and the number of adverse lifetime life events. Groups did not differ on the BDI-14 scale. Concerning the probands diagnosis, 9 of the probands (15%; 2 MZ and 7 DZ) had an ICD-8 diagnoses and 50 (16 MZ and 34 DZ) received an ICD-10 diagnosis. Moreover, 6 subjects in the HR group (2 MZ, 4 DZ) and 1 subject (MZ) in the LR group had a minor psychiatric diagnoses related to anxiety and depression (ICD-10 diagnoses F41 and F43).

3.2. Global brain volumes

Mean raw supratentorial global brain volume measures are presented in Table 2. For completeness total (e.g. inclusive cerebellum and brain stem) are also reported. Note that in the analyses, with the exception of st-ICV, global volumes were expressed as a percentage of st-ICV volume to correct for differences in head size. Group comparisons did not reveal significant differences in supratentorial ICV, TBV, GM, WM or CSF volume (all p-values > .5). Entering age squared and/or age cubic to model non-linear age effects in the st-GM, st-WM and st-TBV analyses did not change results. Entering Zygosity did not reveal any main effect for Zygosity or Zygosity × Risk interaction effects (p-values > 0.24). However, MZ twins tended to have smaller st-ICV volumes then DZ twins ($F_{(1,106)} = 3.16$, $p = .08$).

3.3. Hippocampal volume

Mean raw left and right hippocampal volume measures are presented in Table 2. Note that in the analyses hippocampal volumes were expressed as percentage of st-ICV. As hypothesized we found a significant main effect of Risk ($F_{(1,106)} = 4.17$, $p = 0.04$), indicating that relative hippocampal volumes were significantly smaller in high-risk subjects (Fig. 3). A significant main effect of Gender ($F_{(1,106)} = 20.44$, $p < 0.001$), indicated relative hippocampal volume to be larger in females. There was no main effect of Hemisphere ($p > 0.27$) nor were there any significant interaction effects (p-values > .2). Results did not change when age squared and/or age

Table 2
Absolute global brain tissue and hippocampal volumes in healthy subjects at risk of unipolar depression and low-risk subjects.[a]

Structure	High-risk (N = 59)		Low-risk (N = 53)	
	MZ (N = 18)	DZ (N = 41)	MZ (N = 22)	DZ (N = 31)
GM	675.42 (57.09)	695.85 (64.24)	699.00 (55.95)	697.42 (92.03)
WM	452.27 (47.02)	489.05 (58.67)	464.26 (50.68)	476.19 (67.10)
CSF	297.38 (79.69)	333.79 (69.21)	282.16 (58.48)	308.83 (65.03)
TBV	1127.69 (91.70)	1184.89 (109.54)	1163.27 (103.05)	1173.61 (153.08)
ICV	1425.07 (134.97)	1518.68 (142.08)	1445.43 (121.43)	1482.44 (185.27)
st-GM	569.42 (51.25)	584.66 (58.77)	587.56 (47.67)	585.86 (82.54)
st-WM	408.91 (43.13)	441.97 (55.59)	418.40 (46.18)	429.64 (62.32)
st-CSF	260.48 (72.69)	292.57 (63.15)	241.85 (51.99)	267.50 (60.87)
st-TBV	978.33 (82.86)	1026.63 (101.67)	1005.95 (90.24)	1015.49 (138.91)
st-ICV	1238.82 (122.85)	1319.19 (131.58)	1247.80 (106.17)	1282.98 (169.19)
Hippocampus Left	3.491 (.30)	3.579 (.32)	3.519 (.32)	3.637 (0.39)
Hippocampus Right	3.355 (.28)	3.521 (.30)	3.453 (.30)	3.579 (0.39)

[a] Values are mean (SD) cm^3; MZ = monozygotic; DZ = dizygotic; GM = gray matter; WM = white matter; CSF = cerebral spinal fluid; TBV = total brain volume; ICV = intracranial volume; st = supratentorial.

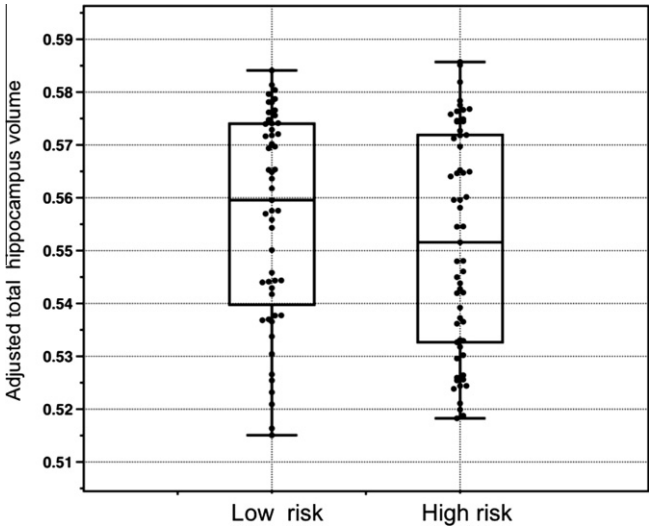

Fig. 3. Total hippocampal volume adjusted for age, gender, supratentorial intracranial volume and years of education by Risk.

cubic were added to model potential non-linear age effects. Likewise, results did not change when hippocampal volumes were expressed as percentage st-TBV (Risk: $F_{(1,106)} = 4.56$, $p < 0.04$; Gender: ($F_{(1,106)} = 6.9$, $p < 0.01$). Adding HAM-D scores and number of adverse life events in the past 12 months as additional covariates in the main analysis did not significantly change our results (Risk: $F_{(1,102)} = 3.94$, $p < 0.05$; Gender: $F_{(1,\ 102)} = 18.8$, $p < 0.001$). However, when we additionally excluded subjects with a minor psychiatric diagnoses related to depression and anxiety (ICD-10 F4x.xx) we observed a trend for the Risk effect ($F_{(1,95)} = 3.32$, $p < 0.07$).

Entering Zygosity in the main analysis – i.e., within subjects: Hemisphere (left and right hippocampal volume expressed as percentage of st-ICV); between subjects: Risk (high-risk, low-risk), Gender (male, female) and Zygosity (MZ, DZ); covariates: age and years of education – reduced the main effect for Risk to trend level ($F_{(1,102)} = 2.18$; $p < 0.14$) while the Gender effect was more pronounced ($F_{(1,102)} = 17.16$; $p < 0.001$). Neither the main effect for Zygosity ($p > 0.9$) nor the Zygosity × Risk interaction effect ($p > 0.16$) reached significance. Adding HAM-D scores and number of adverse life events in the past 12 months as additional covariates did not significantly change our results (Risk: $F_{(1,98)} = 2.24$, $p < 0.14$; Gender: $F_{(1,98)} = 16.2$, $p < 0.001$; Zygosity ($p > 0.9$) ; Zygosity × Risk interaction: $F_{(1,98)} = 2.24$, $p < 0.14$). When we additionally excluded subjects with minor depression/anxiety related disorders p-values slightly increased (Risk: $F_{(1,91)} = 1.59$, $p < 0.21$; Zygosity × Risk interaction: $F_{(1,91)} = 1.77$, $p < 0.19$).

Post hoc testing of the Risk effect separately for MZ and DZ groups, revealed that high-risk DZ twins had smaller hippocampal volumes as compared to low-risk DZ twins ($F_{(1,67)} = 5.96$, $p < 0.02$), while high-risk MZ twins did not differ from low-risk MZ twins ($F_{(1,35)} = 0.08$, $p > 0.7$). MZ and DZ high-risk groups did not differ from each other on age, education, height or weight (p's > 0.26), gender and handedness (p's > 0.17) or on HAM-D, BDI-21, BDI-14, life events 12, life events lifetime, discordance time or age at which proband was discharged for the first time (p's > 0.25). A post hoc power analysis indicated that given the observed effect size (partial $\eta^2 = 0.073$) for the Risk effect in the DZ group and an alpha of 0.05 the statistical power in the MZ group to detect a similar effect was 0.3 (Faul et al., 2007). Further controlling for HAM-D scores and number of adverse life events in the past 12 months did not significantly change our results (MZ: Risk: $F_{(1,32)} = 0.3$, $p > 0.6$; DZ: Risk: $F_{(1,62)} = 4.9$, $p < 0.03$). Nor did subsequent exclusion of subjects with minor depression/anxiety related disorders (MZ: Risk: $F_{(1,29)} = 0.10$, $p > 0.7$; DZ: Risk: $F_{(1,58)} = 4.73$, $p < 0.03$). Exploratory Spearman correlations between total hippocampus volume corrected for age, gender and st-ICV and clinical variables within the high-risk and low-risk groups did not reach significance (p's > 0.4).

3.4. Voxel-wise analyses

Exploratory post hoc analyses, with a FDR threshold of 0.05 to correct for multiple comparisons, restricted to the cerebrum did not reveal any significant differences in GM, WM or CSF volumes between the two risk groups. Nor did we observe any significant Zygosity or Zygosity × Risk effects.

4. Discussion

Consistent with our hypothesis, after correcting for supratentorial ICV, age, sex, and education, healthy high-risk subjects had smaller hippocampal volumes than healthy low-risk subjects. This effect remained significant after controlling for possible non-linear age effects, group differences on years of education, HAM-D scores and number of adverse life events in the past 12 months. However, excluding subjects with minor depression/anxiety related disorders reduced the Risk effect to trend level. Although the Zygosity by Risk interaction effect did not reach significance, follow-up analyses showed that while MZ and DZ twins, irrespective of risk, did not differ in hippocampal volume, DZ high-risk subjects exhibited significantly decreased hippocampal volumes as compared to DZ low-risk subjects whereas high and low-risk MZ subjects did not differ significantly from each other. Risk groups did not differ on global brain (tissue) volume measures nor did they differ from

each other in regional GM, WM, or CSF volumes in our exploratory voxel-wise analyses.

Our finding of decreased hippocampal volumes in a healthy high-risk sample suggests that hippocampal volume reduction may be part of the diathesis for unipolar depressive disorder and may have predictive value for future onset of depression. To the best of our knowledge no other MRI studies have investigated healthy subjects at genetic risk for depression. It has been argued that the consistent finding of hippocampal volume reductions in depression might be due to the fact that many studies investigated elderly, middle-aged or chronically ill populations (Savitz and Drevets, 2009). However, recent observations of hippocampal volume reductions in drug-naïve first-episode depressed subjects (Frodl et al., 2002; Kronmuller et al., 2009; MacMaster et al., 2008; Zou et al., 2009), suggest that the hippocampus might already be affected early in the disease, independent of chronicity, number of relapses, and medication. Nevertheless, hippocampal volume reductions are likely not specific to unipolar depression and have been reported in several neuropsychiatric disorders (Geuze et al., 2005). Notably, the hippocampus has been found reduced in schizophrenia patients in different stages of the disease as well as in their unaffected relatives (Ebdrup et al., 2010; Lawrie et al., 2008). Interestingly, a recent study directly comparing depressed and schizophrenia patients found that first-episode depressed and schizophrenia patients both had reduced hippocampus volumes as compared to healthy controls but did not differ from each other (Meisenzahl et al., 2009).

The present finding that the hippocampal volume reductions appeared more prominent among the DZ than the MZ twins is somewhat counterintuitive. However, the latter might be partly due to reduced statistical power in the smaller subgroup of MZ twins (40 MZ vs. 72 DZ). Because heritability estimates of hippocampal volume are low to moderate (Peper et al., 2007; Schmitt et al., 2007) it is possible that the observed hippocampal volume reductions are best explained by environmental rather than genetic influences. In line with such an interpretation are findings from a recent study of 10 MZ twin pairs discordant for the risk of anxiety and depression. The twins with high trait anxiety and depression had gray matter reductions in left posterior hippocampal regions compared to their less anxious and depressed co-twin. In the same study, no comparable differences could be demonstrated between MZ twins concordant for high anxiety/depression (7 pairs) relative to MZ twins concordant for low anxiety/depression (15 pairs) (de Geus et al., 2007). A possible mechanism for hippocampal tissue loss is exposure to repeated episodes of hypercortisolemia (Geuze et al., 2005). Nevertheless, we did not find any correlations between clinical variables and hippocampus volume nor did the high-risk MZ and DZ twins differ from each other on any of the clinical variables. High-risk twins did however score significantly higher on the HAM-D and the number of adverse life events in the past 12 months than low-risk twins and had life time more minor psychiatric diagnoses related to anxiety and depression. Given that unipolar depression has a moderate genetic component the present results are likely best explained by an interaction between genetic and environmental factors. Notably, the present study does not allow for inferences about gene/environment interactions, because affected co-twins were not included. The aim of the present study was rather to establish hippocampal volume reduction as a trait or state marker. Based on our data the volume reduction appears to be a trait marker. Further studies are needed to identify the separate roles of genetic and environmental factors.

Regarding the diagnosis of the probands, there are some disadvantages to using registers: the diagnoses are clinical, not research diagnoses and up until 1995 only hospitalized probands were included. As the diagnoses were given at the first discharge it is unknown whether the diagnosis of the proband has changed. Nevertheless, it has been shown that the diagnosis of affective disorders in the Danish Psychiatric Central Research Register was correct in 94 % of the cases when compared with ICD-10 diagnoses made on the basis of case notes using OPCRIT and research (SCAN) interviews (Kessing, 1998). At the same time, our sample was population based, and high and low-risk twins were identified systematically using registry linkage, which obviated the need to ask the proband for permission to contact the high-risk twin, thereby reducing selection bias and likely increasing the rate of study participation. Finally, one could argue that the long sampling period may have caused bias if the LR vs. HR participants and MZ vs. DZ groups were uneven recruited during the sampling period. However, the latter seemed not to be the case.

The image processing methods of the present study employed high-dimensional warping techniques to attempt to minimize misregistration errors associated with morphological variation between subjects' brains, thereby potentially increasing the sensitivity (over previous VBM methods) for observing "real" gray matter volume differences. Similar approaches have been used successfully in previous studies (see for example (Davatzikos et al., 2001). The DARTEL approach for defining ROIs has recently been shown comparable to manual ROI-based analyses for detecting hippocampal volume differences (Bergouignan et al., 2009; Yassa and Stark, 2009). An exploratory voxel-wise analysis surveying the whole cerebrum was conducted to address the possibility that the hippocampal alterations we observed were accompanied by effects elsewhere that were not the focus of the study. These exploratory tests, because of corrections for multiple comparisons, were much less powerful than the ROI analysis of the hippocampal volume, even within hippocampal voxels, and obviously, our failure to find any significant Risk effects in these analyses does not rule out their existence. Furthermore, warping algorithms in general are not "perfect", meaning that inter-individual differences, although minimized, are still present. Moreover, smoothing the modulated warped tissue images with a 4 mm FWHM Gaussian smoothing kernel sensitized our analyses to structural differences of around 6–7 mm spatial extent. Consequently, we cannot exclude the presence of subtle tissue volume differences. Finally, it has recently been shown that different genetic influences may underlie cortical thickness and cortical surface area measures (Panizzon et al., 2009). As cortical thickness and surface area define cortical volume it might very well be that cortical thickness and surface measures are more sensitive in detecting possible group differences.

In conclusion our findings suggest that hippocampal volume reduction may index a predisposition to develop depression and thus may be predictive of future onset of the disorder. However, to elucidate environmental and genetic contributions future studies are needed.

Contributors

Lars Vedel Kessing and Maj Vinberg designed the overall study and wrote the protocol. William F.C. Baaré, Terry L. Jernigan, Gitte M. Knudsen and Olaf B. Paulson designed the MR acquisition as well as image analysis part of the study. William F.C. Baaré and Annika R. Langkilde performed the MR image analyses. William F.C. Baaré and Maj Vinberg performed the literature searches and statistical analyses and wrote the first draft of the article. All authors contributed to and have approved the final manuscript.

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Conflict of interest

None declared.

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