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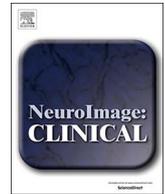
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## Risk for affective disorders is associated with greater prefrontal gray matter volumes: A prospective longitudinal study

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### ABSTRACT

**Background:** Major depression and bipolar disorders aggregates in families and are linked with a wide range of neurobiological abnormalities including cortical gray matter (GM) alterations. Prospective studies of individuals at familial risk may expose the neural mechanisms underlying risk transmission.

**Methods:** We used voxel based morphometry to investigate changes in regional GM brain volume, over a seven-year period, in 37 initially healthy individuals having a mono- or di-zygotic twin diagnosed with major depression or bipolar disorder (high-risk group; mean age 41.6 yrs.) as compared to 36 individuals with no history of affective disorders in the index twin and first-degree relatives (low-risk group; mean age 38.5 yrs.).

**Results:** Groups did not differ in regional GM volume changes over time. However, independent of time, high-risk twins had significantly greater GM volumes in bilateral dorsal anterior cingulate, inferior frontal gyrus and temporoparietal regions as compared to low-risk twins. Further, individuals who developed an affective disorder at follow-up (n = 12), had relatively the largest GM volumes, both at baseline and follow-up, in the right dorsal anterior cingulate cortex and right inferior frontal cortex compared to high- and low-risk twins who remained well at follow-up.

**Conclusion:** This pattern of apparently stable greater regional GM volume may constitute a neural marker of an increased risk for developing an affective disorder in individuals at familial risk.

### 1. Introduction

Major depressive disorder (MDD) and bipolar disorder (BD) have consistently been linked with neurophysiological impairments related to cognitive and executive function, and emotional processing (Gotlib and Joormann, 2010), with numerous neuroimaging studies reporting structural and functional abnormalities in the brain regions subserving these processes (Hamilton et al., 2013; Peng et al., 2016; Wise et al., 2016). The available neuroimaging data in depressed patients, reveal highly heterogeneous and widespread brain abnormalities, involving prefrontal, as well as temporal and subcortical brain structures. Studies of gray matter (GM) changes in various groups of MDD and BD patients have reported smaller volume in hippocampus, anterior cingulate cortex (ACC), and multiple regions in prefrontal cortex (PFC) such as subgenual and orbitofrontal cortex, and dorsolateral and dorsomedial PFC (Bora et al., 2012; Lai, 2013; Lorenzetti et al., 2009).

Studies in MDD patients investigated immediately after their first episode and in medication-naïve patients often report conflicting findings compared with studies in medicated and chronically ill patients (Peng et al., 2016). For instance, no volumetric differences in patients in the early course of depression relative to non-depressed participants were observed in amygdala, hippocampus or orbitofrontal cortex (OFC) (Arnone et al., 2012; Hastings et al., 2004; McKinnon et al., 2009; Pizzagalli et al., 2004; van Eijndhoven et al., 2009). Other studies reported larger amygdala volume (Frodl et al., 2003, 2002) or larger cortical volume, density and thickness in ACC (Adler et al., 2007; van Eijndhoven et al., 2013; Zhao et al., 2014), or OFC and dorsolateral PFC (Qiu et al., 2014). In conclusion, the described heterogeneity in the neuroimaging findings may well reflect the effects of distinct clinical factors such as chronicity, disorder progression, frequency of depression episodes, and/or medical treatment.

Studies in healthy individuals at increased risk for affective

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disorders are instrumental in identifying dysfunctional systems that precede onset of the disorders without being confounded by clinical factors. Having a first-degree relative with an affective disorder is a strong predictor of increased risk for depression (Gottesman et al., 2010; Oquendo et al., 2013; Sullivan et al., 2000). Our group has previously found reduced hippocampal volumes (Baaré et al., 2010) in healthy co-twins of patients with MDD ( $n = 59$ ) as compared to healthy twins with no family history of affective disorders ( $n = 53$ ). In line with this finding, Amico et al. (2011) reported smaller right hippocampal gray matter volumes in individuals with a positive family history for MDD compared to both matched controls with no family history and MDD patients. They further reported gray and white matter volume reductions in dorsolateral PFC in healthy high-risk individuals compared to the low-risk controls. MDD mothers and their healthy daughters showed reduced volume and cortical thickness in temporoparietal regions and the dorsomedial PFC compared to matched healthy mothers and their healthy daughters (Ozalay et al., 2016). In a study investigating a group of high-risk young adults at baseline and following a 2-year period, Pappmeyer et al. (2015) found reduced cortical thickness in the right parahippocampal and fusiform gyrus across both time points. However, contrasting previous findings of reduced volume and thickness, Romanczuk-Seiferth et al. (2014) reported greater GM volume in bilateral amygdala and hippocampus, and left dorsolateral PFC in healthy first-degree relatives of MDD patients compared to matched participants with no family history of psychiatric disorders. Peterson et al. (2009) observed both cortical thickening in subgenual, medial OFC, and anterior and posterior cingulate gyrus and cortical thinning across the lateral surface of the right cerebral hemisphere in children and grandchildren of depressed individuals compared to age matched individuals with no family risk of affective disorders.

The present longitudinal high-risk study investigated possible regional brain differences in GM volume in healthy co-twins of patients with MDD or BD as compared to healthy co-twins with no family history of affective disorders over a seven-year follow-up period. MDD and BD show genetic overlap (McGuffin et al., 2003; Smoller et al., 2013) and 10–15% of individuals with an index diagnosis of depression will subsequently develop a bipolar disorder (Kessing et al., 2017). We therefore combined relatives to MDD and BD to reveal gray matter changes related to increased risk in a continuum of affective disorders. Participants were part of a larger longitudinal study ( $n = 234$ ) on demographic and clinical risk markers for affective disorders, where we initially showed that increased affective symptoms, neuroticism and severe life events (Vinberg et al., 2013a), and impaired executive function and attention (Vinberg et al., 2013b) at baseline were predictive of subsequent onset of an affective disorder. Here we used voxel based morphometry to test the hypothesis that high- and low-risk participants will show a differential temporal development of regional GM volume in brain regions critically implicated in MDD and BD i.e. PFC, ACC and hippocampus. We further expected that longitudinal changes in GM volume in these regions would be most prominent in those individuals who developed an affective disorder during the 7-years follow-up.

## 2. Methods

### 2.1. Participants

73 healthy MZ and DZ twins, with no diagnosis of affective disorder prior to the baseline investigation, were included in the current high-risk structural MRI study. The participants were scanned at baseline and following a period of  $7.1 \pm 1.2$  years (mean  $\pm$  SD). The baseline scan took place between June 2003 and June 2004 and the follow-up scan between May 2011 and June 2012. All participants were recruited from a larger high-risk study ( $n = 234$ ) on demographic and clinical risk markers for developing depression (Christensen et al., 2007). At baseline, 174 participants out of the 234 participants took part in the MRI

investigation (for details please see Baaré et al., 2010). Of these 125 participants were considered suitable for follow-up and 49 participants were excluded due to: arterial malformations ( $n = 3$ ), hypertension, diabetes, epilepsy, head trauma or previous chemotherapy ( $n = 30$ ) or a family history of psychiatric illness other than MDD or BD ( $n = 16$ ). Of the 125 participants invited for the follow-up MRI scan 7 years later, 85 agreed to participate and 73 of them were scanned. The 73 included participants had a co-twin diagnosed and treated for either MDD or BD in a psychiatric hospital setting (high-risk twins,  $n = 37$ , 27 relatives of MDD and 10 of BD patients), or no history of affective disorders or other severe psychiatric illness in first-degree relatives (low-risk twins,  $n = 36$ ).

The high- and low-risk twins were identified through record linkage between the nation-wide Danish Twin Registry, the Danish Psychiatric Central Research Register and the Danish Civil registration system. Low-risk twins were matched for age, sex and zygosity with the high-risk twins. None of the included twins had a record in the Danish Psychiatric Central Research Register. All participants underwent a clinical interview using Schedules for Clinical Assessment in Neuropsychiatry (SCAN 2.1) (Wing et al., 1990) to ascertain that they did not have a personal history of affective disorders or severe depression episodes, schizoaffective disorders or schizophrenia, or any severe organic brain disease preventing compliance with the study protocol. The participants were further enquired about lifetime family psychiatric history based on the Brief Screening for Family Psychiatric History questionnaire (Weissman et al., 2000). The zygotic status was obtained from the Danish Twin Registry, which records the zygosity of same-sex twins based on mailed questionnaires.

Out of the 73 participants investigated over the follow-up period, 12 had an onset of affective disorder during follow-up (10 from the high-risk group and two from the low-risk group). The time of onset was in average  $1141 (\pm 758)$  days before the follow-up investigation, with diagnoses categorized according to the International Classification of Diseases (ICD-10) as follows: moderate ( $n = 3$ ; ICD 32.1) or severe depressive episode ( $n = 5$ ; ICD 32.2), panic disorder ( $n = 1$ ; ICD 41), prolonged depressive reaction ( $n = 1$ ; ICD 43.21), and mixed anxiety and depressive reaction ( $n = 2$ ; ICD 43.22). When comparing the 73 participants with the 161 participants from the initial cohort that were not included, the included group had a statistically significant lower mean age (40.6 vs. 45.7 years,  $p = 0.003$ ), higher education level (13.6 vs. 12.4 years,  $p = 0.005$ ), and a lower percentage of high-risk individuals (50.7% vs. 67%). Groups did not statistically significantly differ in sex distribution, zygosity and other clinical measurements.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Danish Ministry of Health and The Danish Regional Scientific Ethical Committee (KF-12-122/99 and KF-01-001/02), and the Data Inspection Agency. Written informed consent was obtained from all participants.

### 2.2. Demographic, socio-economic and clinical assessment

The participants underwent clinical interviews using Schedules for Clinical Assessment in Neuropsychiatry (SCAN 2.1) (Wing et al., 1990) performed by a trained psychiatrist (MV). At baseline, all participants were rated with the 17-item Hamilton (HamD) rating scale (Hamilton, 1980), self-rating of psychopathology using the 21-item Beck Depression Inventory (BDI-21) (Beck et al., 1961), and personality traits including neuroticism using the Eysenck Personality Questionnaire (EPQ) (Eysenck and Eysenck, 1975), the number of severe lifetime life events (prior LEs), (Kendler et al., 1995) and lifetime family psychiatric history based on the Brief Screening for Family Psychiatric History questionnaire (Weissman et al., 2000). Participants were asked to fill in the BDI-21 questionnaire every six-months, sent to them by mail, to monitor if they developed an affective disorder. Moreover, they annually filled in a questionnaire assessing experienced life events in the preceding 12 months, and a cumulative value during the entire follow-

up period was calculated (follow-up LEs). The 7-year follow-up assessments were conducted from January 2010 to May 2012. The assessment involved a telephone interview and a SCAN interview was performed in case the participants had: 1) any contact to a psychologist or psychiatrist, 2) been on sickness leave because of personal problems, 3) been prescribed any psychopharmacological medicine, or if 4) their answers in the depression questionnaires raised a suspicion of onset of psychiatric disorder, or 5) if they were listed with a first psychiatric diagnosis in the Danish Psychiatric Central Research Register during the follow-up period. For detailed descriptions please see (Christensen et al., 2007; Vinberg et al., 2013a).

The discordance time was calculated as the number of years between the date of the MRI follow-up investigation of a healthy high-risk twin and the date the ill co-twin was diagnosed with either MD or BD.

### 2.3. Image acquisition protocol

Baseline and follow-up MRI brain scans were performed on the same 3 Tesla MR scanner (Siemens Trio, Erlangen, Germany) using an eight-channel head array coil. Compatible MPRAGE T1 structural images were acquired at baseline and follow-up. Image acquisition settings—Baseline: TR = 1540 ms, TE = 3.93 ms, FA = 9°, matrix 256 × 256, 192 slices 1 × 1 × 1 mm voxels; Follow-up: TR = 1550 ms, TE = 3.04 ms, FA = 9°, matrix 256 × 256, 192 slices, 1 × 1 × 1 mm voxels, acquisition time 6.32 min.

### 2.4. Image processing

A good image quality at acquisition time was ascertained by visual inspection of all individual images. Using Voxel Based Morphometry (VBM), (Ashburner and Friston, 2000) the structural T1 images were preprocessed with the Computational Anatomy Toolbox (CAT12 r930) according to standard procedure. The CAT12 toolbox was implemented in SPM12 ([www.fil.ion.ucl.ac.uk/spm](http://www.fil.ion.ucl.ac.uk/spm)), which was run on MATLAB R2015a (The MathWorks, Inc., Natick, Massachusetts, United States). Shortly, during the automatized longitudinal processing stream in CAT12, baseline and follow-up T1 images were rigidly realigned to correct for differences in head position within-subject and a subject specific mean image was calculated and used as reference in a subsequent realignment of the baseline and follow-up T1 images. The realigned images were segmented in GM, white matter (WM) and cerebrospinal fluid (CSF) and corrected for signal inhomogeneities with regard to the reference mean image (bias correction). Resulting GM and WM tissue images were used to estimate between-subject spatial normalization deformation filed using the high dimensional Diffeomorphic Anatomic Registration Through Exponentiated Lie Algebra (DARTEL) warping algorithm (Ashburner, 2007). Resulting deformation fields were then applied to the individual bias-corrected tissue images at both acquisition time points. The resulting DARTEL *normalized* images were realigned again to the mean DARTEL normalized image to account for residual segmentation effects. To compare GM volume, DARTEL warped tissue images were multiplied i.e. modulated by the linear and non-linear components of the Jacobian determinant derived from the deformation fields. Finally, the resulting GM and WM segmentations were smoothed using a full-width to half maximum Gaussian smoothing kernel of 6 mm in all directions (Shen and Sterr, 2013).

The quality of the segmentations, within subject warping, and spatial normalization was ascertained through visual inspection of all individual images. We also checked for the sample homogeneity and identified outliers by visualizing the correlation values between all individual GM segmentations within each group using a boxplot and correlation matrices as implemented in the CAT12 software. We further calculated the individual GM, WM and CSF volumes in native space, total brain volume (TBV = GM + WM), and total intracranial volume (TIV) calculated based on tissue segmentations (Malone et al., 2015) to correct for differences in brain size and intracranial capacity. A mean

T1 image was calculated by averaging bias and noise corrected DARTEL normalized T1 images across all subjects using a 7th degree sinc interpolation for delineating volumes-of-interest (see below).

### 2.5. Definition of the volume of interest

Based on our a priori hypothesis, we initially limited our statistical search to a VOI that included bilateral PFC, ACC and hippocampal formation. The VOI was defined on the normalized study mean T1 image using FSLView 4.0.1. The ACC (29,524 voxels) and PFC (676,492 voxels) regions of interest (ROIs) were delineated bilaterally by editing cortical maps, thresholded at 30%, provided by the Harvard-Oxford cortical structural Atlas implemented in FSLView (Desikan et al., 2006). The PFC comprised the cortical regions anterior to the precentral sulcus: superior, middle and inferior frontal gyri, the frontal medial cortex and subgenual cortices and the frontal poles. The hippocampal formation (9916 voxels) was delineated bilaterally as described in Maller et al. (2006). A VOI mask was constructed by combining the three a priori ROIs. We visually verified that the VOI mask had a good fit with all individual spatial normalized T1 images and tissue maps.

### 2.6. Statistical analysis

#### 2.6.1. Demographic and clinical variables

Group differences between high- and low-risk groups were assessed using the SPSS 20 statistical software Sciences (IBM, Armonk, New York, United States). Continuous data (HamD, BDI-21, neuroticism, years of education, prior-LEs and follow-up LEs were checked for deviation from the normal distribution using the Shapiro-Wilk test. Group differences were assessed using either *t*-tests for normally distributed data, or non-parametric Mann-Whitney *U* tests when significant deviations from normality present. Pearson's chi-square tests were used for categorical demographic and socio-economic data (sex, zygosity, salary and employment data). We also used Mann-Whitney *U* tests to evaluate differences in affective symptoms between twins diagnosed with an affective disorder at follow up ( $n = 12$ ) and high-risk twins that remained well ( $n = 26$ ).

#### 2.6.2. Voxel-wise analyses

All statistical models were implemented in SPM12 using the individual modulated segmentations with a 0.1 threshold masking level, controlling for age, sex and TIV (Peelle et al., 2012) and adjusting for non-stationarity.

We first tested our hypothesis that high- and low-risk participants will show differential temporal development of regional GM volume in brain regions critically implicated in MDD and BD i.e. PFC, ACC and hippocampus. For this we used a repeated measure 2-by-2 factorial model, testing for differences in regional GM volume changes over time between low and high-risk groups (group-by-time interactions), as well as testing for main effects of risk group (between-subject factor: low-risk vs. high-risk) and time (within-subject factor: baseline vs. follow-up). Second, we investigated whether the twins diagnosed at follow-up with an affective disorder showed a differential pattern of GM volume changes compared to the high-risk and low-risk twins that remained well. In an analog model, we therefore split the group factor in three levels (diagnosed, high-risk well and low-risk well) and computed an *F*-test for group differences. Upon significant findings, we computed between groups *t*-tests ( $n = 6$ ). The search volume in the first and second model was initially confined to the generated VOI mask. We subsequently explored effects across the entire brain. We applied a cluster-forming threshold of  $p < 0.001$  uncorrected (Eklund et al., 2016). Clusters were considered significant at  $p < 0.05$  after correction for multiple comparison using the Family-Wise Error (FWE) method.

### 2.6.3. Planned follow-up analyses

Prompted by the results from testing the main hypothesis (first analysis described above), we performed several follow-up analyses in analog models using a statistical search volume limited to the clusters showing significant GM volume differences between the high and low-risk groups. First, we tested whether the observed group differences pertained participants with MDD probands only ( $n = 27$ ). Second, we investigated whether observed volume differences were specific to GM by testing for possible white matter volume difference in an analog model. Third, we tested if observed significant effects remained after additionally controlling for education level. Fourth, we investigated if observed differences were anatomical specific by using TBV instead of TIV as covariate in models. Fifth, we tested whether observed GM volume differences were independent of the clinical factors found to be predictive for disease onset e.g. HamD, BDI-21, neuroticism, prior LEs, and follow-up LEs. Sixth, we explored for possible risk group-by-zygosity interactions, and main effect of zygosity. Seventh, within the high-risk group, we explored for possible correlations between regional GM volume change and discordance time. The significance threshold for the follow-up analyses was the same as for the main voxel-wise analyses (see above).

## 3. Results

### 3.1. Demographic, socio-economic and clinical measurements

The distribution of age, sex, zygosity and the time between baseline and follow-up measurements did not differ significantly between the high- and low-risk groups (Table 1). The high-risk twins had a significantly lower level of education and salary status, and showed higher rates of subclinical affective symptoms and a greater number of average lifetime events (Table 1) in accordance with previous reports from the larger cohort from which current participants were recruited (Christensen et al., 2007). There were no statistically significant differences in affective symptoms between high-risk twins with a co-twin diagnosed with MDD or BD, respectively. Twins diagnosed with an affective disorder at follow-up had significantly higher BDI-21 ratings ( $p = 0.019$ ) compared with twins who remained well. The other tested affective symptoms did not differ significantly between these groups.

### 3.2. Voxel-wise analysis

We did not observe any significant risk group-by-time interactions within the predefined VOI mask or at the whole brain level, indicating that high-risk and low-risk groups did not differ in the temporal development of GM volume. However, when comparing high-risk and low-risk twins we found a main effect of risk within the a priori VOI, with high-risk twins having greater GM volumes in bilateral ACC and inferior frontal gyrus (IFG) clusters as compared to low-risk twins (Fig. 1, Table 2). Additionally, exploratory whole-brain analyses showed that, independent of time, high-risk twins compared to low-risk twins had significantly greater GM volumes in left supramarginal and right angular gyri and lower GM volumes in a cerebellum cluster (Table 2). We also found a main effect of time at the whole brain level, with regional decreases in dorsomedial PFC GM volume and regional increases in GM volume in several right hemisphere posterior cortical regions and left ventral striatum at follow-up compared to baseline (see Table 2).

The ANOVA model where the group factor had three levels (diagnosed, high-risk well, and low-risk well), revealed a significant main effect of group in the same bilateral ACC regions where high-risk twins showed increased GM volume compared to low-risk twins in the previous two-group comparison (see above). The twins diagnosed with an affective disorder at follow-up displayed the greatest GM volumes changes compared to high- and low-risk twins that remained well (Fig. 1, Table 2). Post-hoc two-group comparisons confirmed the

**Table 1**

Risk status, demographic and clinical data at baseline for the initial groups of high-risk and low-risk twins and for the twins diagnosed with an affective disorder at follow-up. Bold values represent significant group differences.

	Diagnosed	High-risk	Low-risk	Significance high vs. low risk
<b>Demographic data</b>				
Number of participants	12	37	36	
Time between scans (yrs.)	7.4 (0.9)	7.4 (0.8)	7.2 (0.8)	0.329 <sup>t</sup>
Age (baseline)	39.1 (10.5)	41.6 (11.1)	38.5 (10.7)	0.220 <sup>t</sup>
Sex (m/f)	2/10	15/22	13/23	0.697 <sup>p</sup>
Zygosity (MZ/DZ)	6/6	14/23	16/20	0.633 <sup>p</sup>
Education (yrs.)	12.2 (2.5)	12.8 (2.9)	14.4 (2.5)	<b>0.014<sup>t</sup></b>
<b>Employment status</b>				
Employed	9	32	35	0.095 <sup>p</sup>
Unemployed	3	5	1	
<b>Salary status</b>				
High earner	1	4	11	<b>0.033<sup>p</sup></b>
Low or medium earner	11	23	16	
<b>Clinical data</b>				
HamD baseline	4.1 (3.1)	3.6 (2.2)	1.7 (1.4)	<b>0.000<sup>u</sup></b>
BDI-21 baseline	3.3 (2.9)	2.6 (2.9)	1.0 (1.5)	<b>0.004<sup>t</sup></b>
BDI-21 follow-up	3.6 (3.8)	2.4 (3.1)	1.2 (1.5)	0.169 <sup>u</sup>
Neuroticism		5.3 (4.5)	4.8 (3.7)	<b>0.011<sup>u</sup></b>
Prior LEs	2.4 (1.8)	2.7 (1.7)	1.6 (1.2)	<b>0.000<sup>t</sup></b>
LEs follow-up	11.3 (6.0)	11.2 (6.8)	5.3 (4.5)	<b>0.001<sup>t</sup></b>
Discordance time	5.8 (5.6)	12.5 (8.5)		

Values for the demographic data are mean (SD) or frequency; values for the clinical data are mean (SD); values for employment and salary status are frequency;  $t = t$ -test;  $u =$  Mann-Whitney  $U$  test;  $p =$  Pearson's chi-square test;  $m =$  male;  $f =$  female; MZ = monozygotic; DZ = dizygotic; HamD = Hamilton Depression Scale; BDI-21 = 21-item Beck Depression Inventory; prior LE = number of previous severe life events. LEs follow-up = number of LEs during follow-up. Discordance time = number of years at follow-up between the MRI investigation of a healthy high-risk twin and diagnosis of the ill co-twin.

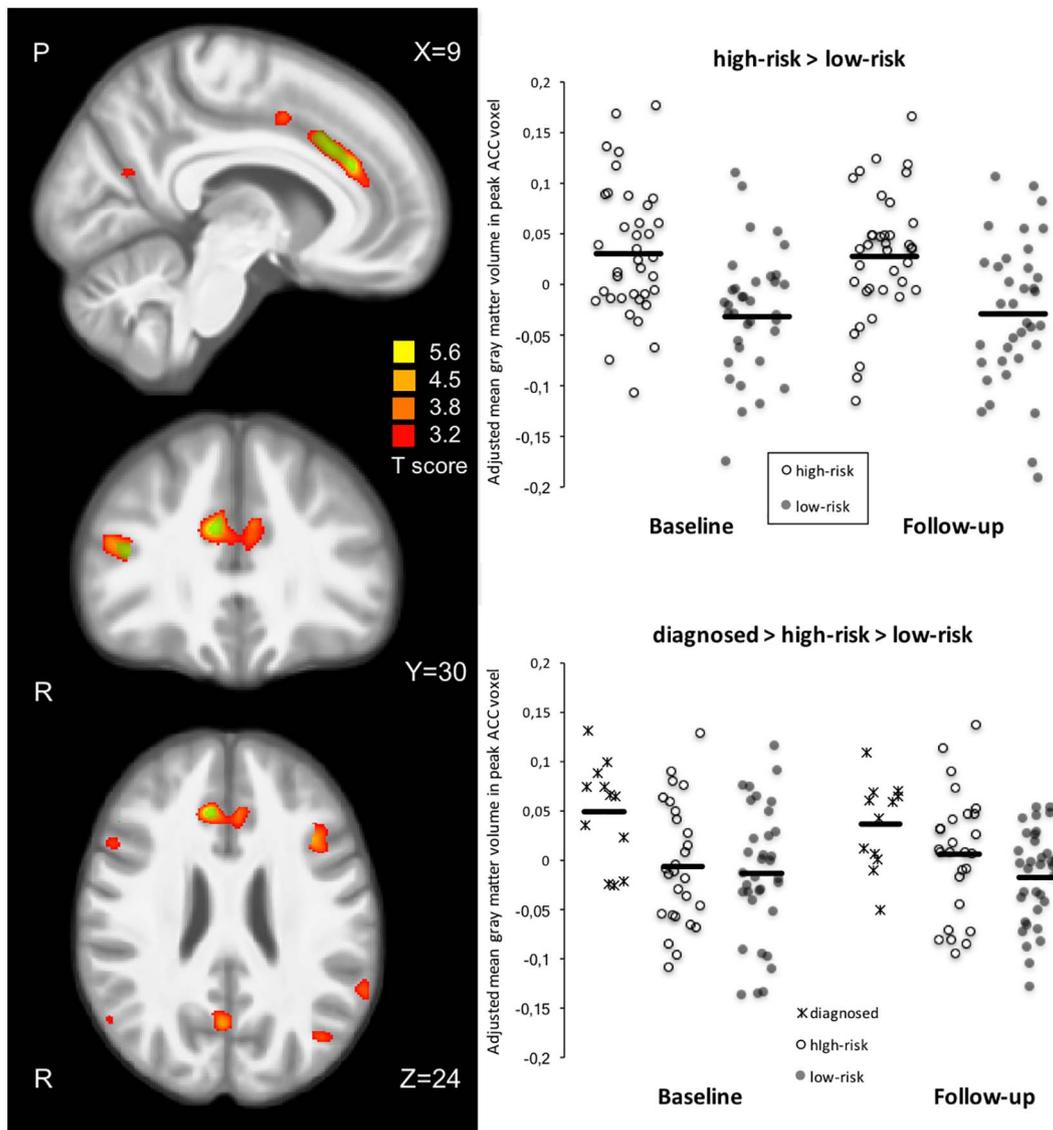
increased ACC volume in the high-risk twins compared to the low-risk twins that remained well. In addition, the diagnosed twins showed larger precuneus compared to the low-risk twins that remained well, and the low-risk twins further showed higher cerebellar volumes compared to the high-risk twins that remained well (Table 2).

### 3.3. Planned follow-up analyses

Follow-up tests restricted to those brain regions for which we found main group differences, independent of time, confirmed that all observed regional GM volume group differences remained statistically significant when including only the high-risk twins with a co-twin diagnosed with MDD. We did not find a significant risk group-by-zygosity interaction or a main effect of zygosity. Group differences also remained statistically significant when controlling for TBV, education level, and clinical factors (HamD, neuroticism and lifetime life events) in three separate analyses. Finally, we found a significant main effect of group in dorsal ACC and right IFG when comparing high-risk participants with an affective disorder at follow-up with high-risk individuals who remained well and low-risk participants.

## 4. Discussion

The current longitudinal high-risk study aimed to characterize differences in regional GM brain volume changes between healthy twins discordant for affective disorders and healthy twins with no familial history of affective disorders. In contrast to our hypothesis, we did not



**Fig. 1.** Time independent group differences between the high-risk, and low-risk groups in regional gray matter volume, adjusted for age, sex and total intracranial volume. Left panels: Clusters showing increased gray matter volume (FWE corrected  $p < 0.05$ ) in high-risk > low-risk (yellow > red). Overlaid light green indicate subregions where the gray matter volume was relatively highest for participants who developed an affective disorder during follow-up (diagnosed > high-risk > low-risk). Right panels: partial regression plots of peak ACC volumes adjusted for sex and age and total intracranial volume from the high-risk > low-risk analysis (top) and the diagnosed > high-risk > low-risk analyses (bottom). Abbreviations: R—right, P—posterior, ACC—anterior cingulate cortex. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

find that high-risk twins differed from low-risk twins in regional GM volume changes over a seven-year follow-up period, within the a priori volume of interest including PFC, ACC and hippocampal complex or at the whole brain level. However, we found that independent of time, high-risk twins had larger GM volumes in bilateral ACC, IFG and temporoparietal regions as compared to low-risk twins. This pattern did not change when controlling for zygotic group or when considering only co-twins of probands with MDD. Exploratory analyses revealed that the twins who developed an affective disorder during follow-up had the relatively largest right dorsal ACC and right IFG volumes.

Our observation of a statistically significant risk group effect and a lack of risk group by time interactions, suggests that larger GM volumes observed in the high-risk group occurred at an earlier time point in development and remained stable over time. To our knowledge, only one other high-risk cohort was previously investigated over time. Young adults with a family history of BD were examined at baseline (average age 21.0 years) and after a 2-year follow-up period (Pappmeyer et al., 2016, 2015). It was found that high-risk individuals who were

diagnosed with MDD at follow-up displayed left IFG thickening relative to high-risk individuals that remained well (Pappmeyer et al., 2015). Additionally, at both times of investigation, the two high-risk groups showed reduced cortical thickness compared to matched low-risk participants in the right parahippocampal and fusiform gyrus. In the same cohort, investigation of regional GM volumes in subcortical regions of interest yielded no significant group differences in GM volume changes over time or across the two measurement times (Pappmeyer et al., 2016). The findings in the young adults with a family history of BD suggest that increased risk and onset of illness is associated with distinct changes in cortical thickness. These findings may stand in contrast with our current data showing bilateral larger IFG volumes at both baseline and follow-up. These differences may be attributed to differences in sample age, diagnosis of index twin, and GM metrics.

The larger GM volume we observed in the high-risk group was located in the dorsal “cognitive” ACC region, which is part of a distributed attentional network playing a key role in the modulation of executive functions, error monitoring and motivation (Bush et al.,

**Table 2**  
Regional gray matter volume differences from the VBM analyses.

Region	Side	x	y	z	Z-stat voxel	Voxels in cluster	Cluster $P_{FWE}$
<b>Main effect of risk group</b>							
<b>High-risk &gt; low-risk (t-test)</b>							
Anterior cingulate	R	10	30	24	5.3	3286	< 0.001 (VOI)
Inferior frontal gyrus	R	40	26	30	4.5	846	0.047 (VOI)
	L	-36	18	24	4.6	958	0.020 (VOI)
Angular gyrus	R	56	-58	30	4.5	991	0.026
Supramarginal gyrus	L	-56	-47	38	4.4	2000	< 0.001
<b>Low-risk &gt; high-risk (t-test)</b>							
Cerebellum	R	10	-62	-14	4.7	3076	< 0.001
<b>Diagnosed vs. high-risk well vs. low-risk well (F-test)</b>							
Anterior cingulate	R	10	30	24	5.0	1251	0.001 (VOI)
	L	-2	28	22	4.2	1292	0.001 (VOI)
<b>High-risk well &gt; low-risk well (t-test)</b>							
Anterior cingulate	R	10	30	24	5.2	3835	< 0.001 (VOI)
	L	-2	28	22	4.7		
<b>Diagnosed &gt; low-risk well (t-test)</b>							
Precuneus	R	8	-54	66	4.5	903	0.04
Anterior cingulate	R	8	20	36	4.2	693	0.056 n.s.
<b>Low-risk well &gt; high-risk well (t-test)</b>							
Cerebellum	R	8	-64	-12	4.6	2707	< 0.001
<b>Main effect of time</b>							
<b>Follow-up &gt; baseline (t-test)</b>							
Ventral striatum	L	-4	8	-14	6.14	4923	< 0.001
Inferior temporal gyrus	R	52	-64	-8	5.61	2153	< 0.001
Temporal pole	R	54	12	-16	5.12	1198	< 0.001
Lingual gyrus	R	12	-54	0	5.03	1403	< 0.001
Precuneus	R	6	-46	18	4.51	575	0.028
<b>Baseline &gt; follow-up (t-test)</b>							
Dorsomedial prefrontal cortex	L	-4	42	48	4.50	963	0.004

Data shown for regional peaks with coordinates x, y, z in MNI standard stereotactic space, Z statistics, cluster size in voxels at an extent threshold of  $p < 0.001$  uncorrected and corrected cluster p. Data from volume of interest analyses are presented with small volume corrected p values (VOI). Abbreviations: L = left, R = Right, FWE = Family-Wise Error corrected, n.s. = not significant, VOI = volume of interest.

2000). This structural change may account for the enhanced functional response in this region to happy and fearful faces observed in a subsample of the present high-risk twins (Miskowiak et al., 2015). Our finding of larger GM volume in this region corroborates previous reports of greater cortical thickness in the dorsal ACC (among other regions showing both thinner and thicker cortex) in healthy individuals at familial risk for affective disorders (Peterson et al., 2009). In addition, larger regional GM volumes in high-risk individuals are further supported by previous reports in first-episode treatment-naïve patients with MDD showing widespread increases in cortical thickness in frontal, temporal and anterior and posterior cingulate cortex (van Eijndhoven et al., 2013; Qiu et al., 2014). In comparison with findings in high-risk individuals and first-episode MDD, meta-analyses across studies with various groups of MDD patients report GM alterations in the anterior “affective” ACC division, involved in emotional regulation e.g. (Lai, 2013).

The greater GM volumes we found in dorsal ACC and right IFG were stable over the follow-up period with the greatest differences observed in individuals that developed an affective disorder. These GM differences may therefore constitute a vulnerability biomarker for developing

affective disorders. The observed greater GM volumes in temporoparietal cortex were however only associated with high-risk but not with illness onset. Greater GM volumes in temporoparietal regions may therefore reflect resilience to illness, or may be of compensatory nature to e.g. offset deficits in cognitive performance observed in these high-risk twins (Vinberg et al., 2013b). We find the latter speculation more plausible since our high-risk cohort, despite being middle-aged, was still at elevated risk compared with low-risk individuals. Our longitudinal assessment revealed onset of an affective disorder in 10 high-risk compared to two in the low-risk group during the seven years' period between measurements. Similar proportions were found in our larger twin cohort ( $n = 234$ , mean age at baseline 39.4 and follow-up 44.8) where 21.2% ( $n = 31$ ) in the high-risk group vs. 5.7% ( $n = 5$ ) in the low-risk group had an onset of affective disorder at the 7 year follow-up.

The follow-up analysis did not reveal a significant effect of zygosity. It is possible that environmental factors may have contributed more to the observed differences than genetic risk factors. In line with such interpretation are the finding that high-risk twins had experienced significantly more severe life events compared to the low-risk group (Table 1) and that the experience of severe life events before baseline has been shown to be predictive for onset of affective disorder (Vinberg et al., 2013a).

The neurobiological underpinnings of the observed GM volume differences in high-risk and depressed individuals and how these changes related to functional changes remain unclear. In general, VBM is not specific to neural tissue and cannot offer direct information about the molecular and cellular processes leading to volumetric differences. For instance, changes in myelination, cell size and density, vascularization and neuroinflammatory processes may affect relaxation times and therefore voxel intensities of a T1-weighted image (Zatorre et al., 2012). Future studies are needed to provide a better insight into the neurobiological processes mediating the increased risk for affective disorders.

There are several potential limitations to our findings. The small sample of participants ( $n = 12$ ) that developed an affective disorder at follow-up resulted in a low statistical power to detect GM volume differences between this group and the high-risk and low-risk groups that remained well. Moreover, the follow-up MRI scan took place 7 years after the initial baseline assessment which resulted in intersubject differences in the time past between disorder onset and follow-up scan. Since we did not observe any group by time interaction, suggesting that groups did not develop differently over time, investigating individuals an earlier point in time and/or follow individuals over a longer period of time might have allowed to elucidate possible different GM volume trajectories in high-risk compared to low-risk twins. While we were able to confirm our findings in relatives of MDD only, our high-risk sample with an index twin diagnosed with BD was too small ( $n = 10$ ) to be able to detect subtle differences between relatives to BD and MDD patients. Caution should therefore be applied when interpreting the current findings in the context of relatives to BD patients. Further, we did not corroborate hippocampus reduction as a biomarker for risk, or the negative whole-brain VBM findings in our previous study performed in a larger cohort (59 high-risk and 53 low-risk) that included the baseline scans of the participants that took part in the current study (Baaré et al., 2010). These differences may be accounted by several factors. The Baaré et al. study performed an ROI analysis comparing mean hippocampal volume in a larger and more homogenous group of co-twins of unipolar patients only. In addition, the Baaré et al. study did not use a VOI approach for the prefrontal cortex in the VBM analysis and compared the groups only at baseline. Lastly, findings related to changes over time independent of risk group may be confounded by scanner and MRI sequence updates, despite adjusting for the time factor in the statistical models.

In conclusion, unaffected twins discordant for affective disorders showed greater GM volumes in five bilateral cortical regions located in

ACC, IFG and temporoparietal regions. These differences were stable at both times of investigation and appeared more pronounced in participants who developed an affective disorder at follow-up. This pattern in gray matter volume differences may be indicative of an increased vulnerability to affective disorders indexing as a potential endophenotype. Further studies in younger high-risk participants with a longer follow-up period may be more sensitive in differentiating between GM changes related to risk and GM changes predictive of illness onset.

### Declaration of interest

HRS has received honoraria as editor from Elsevier Publishers, Amsterdam, The Netherlands and Springer Publishing, Stuttgart, Germany, and has received a research fund from Biogen-idec. MV has within the last three years been a consultant for Lundbeck and Astra Zeneca. The other authors declare no competing financial interests.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.nicl.2017.12.011>.

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