TITLE: Mood episodes are associated with increased cortical thinning: a longitudinal study of bipolar disorder type II

AUTHORS: Nathalia Zak[1,2]; Erlend Bøen[3]; Birgitte Boye[4,5]; Ole A. Andreassen[1,2]; Nhat Trung Doan[1]; Ulrik F. Malt[2,6]; Lars T. Westlye[1,7]; Torbjørn Elvsåshagen[1,2,8]

AUTHOR AFFILIATIONS: [1]Norwegian Centre for Mental Disorders Research (NORMENT), KG Jebsen Centre for Psychosis Research, Oslo University Hospital, Oslo, Norway; [2]Institute of Clinical Medicine, University of Oslo, Oslo, Norway; [3]Department of Psychiatry, Diakonhjemmet Hospital, Oslo, Norway; [4]Section of Psychosocial Oncology, Division of Cancer Medicine, Oslo University Hospital, Oslo, Norway; [5]Department of Behavioural Sciences in Medicine, University of Oslo, Oslo, Norway; [6]Department of Research and Education, Oslo University Hospital, Oslo, Norway; [7]Department of Psychology, Faculty of Social Sciences, University of Oslo, Oslo, Norway; [8]Department of Neurology, Oslo University Hospital, Oslo, Norway

CORRESPONDING AUTHOR: Nathalia Zak, M.D.; Norwegian Centre for Mental Disorders Research (NORMENT), Oslo University Hospital, 0424 Oslo, Norway; nathalia.zak@studmed.uio.no

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Abstract

Objectives: Previous studies found evidence for thinner frontotemporal cortices in bipolar disorder (BD), yet whether this represents a stable disease trait or an effect of mood episodes remains unknown. Here we assessed the reproducibility of thinner frontotemporal cortices in BD type II, compared longitudinal changes in cortical thickness between individuals with BD type II and healthy controls (HCs), and examined the effect of mood episodes on cortical thickness change.

Methods: Thirty-three HCs and 29 individuals with BD type II underwent 3T magnetic resonance imaging at baseline, as published previously, and 2.4 years later, at follow-up. Cross-sectional and longitudinal analyses of cortical thickness were performed using Freesurfer, and relationships with mood episodes from baseline to follow-up were assessed.

Results: Individuals with BD type II had thinner left and right prefrontal and left temporal cortex clusters at follow-up (all corrected $P<.001$), consistent with baseline results. Both groups showed widespread longitudinal cortical thinning, and patients had increased thinning in a left temporal cortex cluster compared to HCs (corrected $P<.001$). Patients with more (>2) depressive episodes between baseline and follow-up had greater left temporal cortical thinning than patients with fewer depressive episodes (corrected $P<.05$). In addition, patients with more depressive episodes had greater thinning in bilateral ventromedial prefrontal clusters relative to HCs (uncorrected $P<.05$), yet these results did not survive correction for multiple comparisons.

Conclusions: Together, these findings support reduced frontotemporal cortical thickness in BD type II and provide the first preliminary evidence for an association between depressive episodes and increased cortical thinning.

KEY WORDS: bipolar disorder, depressive episodes, cortical thickness, cortical thinning, longitudinal study, progressive changes

1 | INTRODUCTION

Bipolar disorder (BD) is a chronic psychiatric illness characterized by episodic disturbances in mood and activity levels, and affects 2-3 % of the population worldwide.[1] BD type I is defined by at least one manic episode, while BD type II is characterized by alternating episodes of hypomania and depression. The clinical significance of both subtypes are comparable in terms of illness burden, role impairments, and suicide attempts.[1] Depressive symptoms dominate the longitudinal course of BD, and account for most of patients' lifetime disability and suffering.[2] Individuals with BD can experience a progressive illness course with shortening of inter-episode intervals and impaired treatment response.[3] Although the neural underpinnings of illness progression remain poorly understood, it has been suggested that mood episodes can cause lasting neurobiological alterations.[4]

Recent neuroimaging studies found thinner prefrontal and temporal cortices in BD type I and II.[5,6] These cortical regions are believed to play central roles in processing and regulation of emotions.[7-9] One recent study found a positive correlation between cortical thickness in medial prefrontal regions and executive function in individuals with BD type II, but not in BD type I,[10] yet there is still a scarcity of neuroimaging studies in BD type II. There are also few longitudinal cortical thickness studies in BD, and whether frontotemporal cortical thinning is a stable trait predisposing to illness development or an effect of mood episodes remains to be clarified. Only one longitudinal study has examined the effects of mood episodes on cortical thinning in BD and found increased frontal thinning in patients who experienced manic episodes during follow-up.[11] Two cross-sectional studies of cortical thickness in BD type I and II suggested thinner prefrontal cortices in individuals with higher lifetime number of depressive episodes.[12,13] One cross-sectional study of major depressive disorder (MDD),[14] but not others,[13,15,16] observed a negative association between number of previous depressive episodes and medial prefrontal and temporal cortical thickness.

Furthermore, a recent longitudinal study found that individuals with depressive symptoms at baseline had increased thinning in left frontal and bilateral temporal cortices and temporoparietal junctions compared to a control group.[17]

Here, we analyzed structural magnetic resonance imaging (MRI) data from individuals with BD type II and healthy controls (HCs) at baseline and 2.4 years later, at follow-up. At baseline, we found thinner bilateral prefrontal and temporal cortices in BD type II, but no group differences in surface area, as reported previously.[18] The aims of the present longitudinal study were to assess the reproducibility of thinner frontotemporal cortices in BD type II at follow-up, to compare changes in cortical thickness from baseline to follow-up between individuals with BD type II and HCs, and to examine whether changes in cortical thickness were associated with mood episodes in the patients.

2 | MATERIALS AND METHODS

Study Design and Participants

Seventy-eight subjects underwent MRI at baseline, as previously reported.[18] Sixty-two participants (33HCs and 29 individuals with BD type II) were reexamined on average 2.4 years later, and were included in longitudinal and cross-sectional analyses, at follow-up. Reasons for not participating in the follow-up study were: moved out of the area (1 patient, 5 HCs), exclusion at follow-up due to a newly diagnosed premalign condition (1 HC), did not attend or withdrew consent (6 patients, 2 HCs), claustrophobia (2 patients), and pregnancy (1 HC). In addition, seven new patients and two new HCs underwent MRI at follow-up; thus, the cross-sectional sample at follow-up comprised 71 participants.

Patients were recruited from psychiatric outpatient clinics in the Oslo area, and clinical examinations at baseline and follow-up were carried out by three senior psychiatrists (i.e., authors EB, BB, and UFM) at a university department specializing in evaluation and treatment of mood disorders. Axis I diagnoses and psychiatric comorbidities were determined at both time points using the Mini-International Neuropsychiatric Interview (MINI), Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria version 5.0.[19] Demographic and supplementary information was obtained through a semi-structured interview using the Stanley Foundation Network Entry Questionnaire.[20] Number of mood episodes from baseline to follow-up was assessed during this interview. Alcohol and drug abuse were assessed with the Alcohol Use Scale and the Drug Use Scale,[21] respectively. Mood state at the time of MRI scanning was determined by the Young Mania Rating Scale (YMRS)[22] and the Montgomery–Asberg Depression Rating Scale (MADRS).[23] There is no general agreement in the literature about the cut-off score of MADRS for euthymia and the suggested cut-off scores have varied between 6/7 (Snaith *et al,* 1986)[24] and 12/13 (e.g. Montgomery *et al,* 1993)[25]. As a compromise we defined euthymia as a MADRS score <11 as

suggested by Ventura *et al* (2007)[26] and by Norwegian guidelines.[27] However, we cannot rule out that some of our patients classified as euthymic might have been considered mildly depressed ("sub-clinical depression") by other authors.

Controls were recruited through local advertising and had no previous or current psychiatric illness. They underwent a full clinical examination similar to that of the patients. The exclusion criteria for all subjects were: age <18 or >50 years, previous head injury with loss of consciousness for >1 min, history of neurological or other severe chronic somatic disorder, and pregnancy. The Regional Ethical Committee of South-Eastern Norway approved the study, and all subjects provided written informed consent to participate after receiving written and oral information about the study.

MRI Acquisition and Analysis

Imaging at baseline and follow-up was performed on the same 3T Philips Achieva Scanner (Philips Healthcare, Eindhoven, the Netherlands) using an eight-channel SENSE head coil. Structural brain images were acquired using a T1-weighted three-dimensional turbo field echo (TFE) sequence [repetition time (TR)/echo time (TE)=8.4msec/2.3msec, field of view (FOV)=256mm × 256mm × 220mm, 1mm isotropic resolution, TA= 7min 40sec).

All data sets were processed blindly and analyzed at the Neuroimaging Analysis Lab at the Norwegian Centre of Mental Disorders Research at Oslo University Hospital. Freesurfer software version 5.3 was used to automatically reconstruct cortical surfaces from T1-weighted MRI-images, details regarding the surface-based analysis are provided elsewhere.[28-30] Briefly, processing steps include removal of non-brain tissue,[31] automated Talairach transformation, and intensity correction. Information about intensity and continuity from the three-dimensional volume is used in segmentation and deformation procedures to reconstruct a gray/white matter boundary across the brain.[28] Cortical surfaces are then

inflated, registered to a spherical atlas, and gyral and sulcal regions are identified automatically.[31] Reconstructed data sets were visually inspected for accuracy at several points along the processing pipeline, and segmentation errors were manually corrected, re-processed, and re-inspected. Cortical thickness maps were obtained for each participant by calculating the distance between the gray and white matter surfaces at each vertex, and compared across groups.

For longitudinal analyses the implemented longitudinal stream in Freesurfer was used to prepare surfaces for comparisons of baseline and follow-up examinations.[32] This procedure processes longitudinal data with common information from a template created for each subject, thereby reducing measurement noise and increasing precision. Temporal data within each subject was then reduced to rate of change maps (mm/year= thick2-thick1/time2-time1) using the *long_mris_slopes* function in Freesurfer´s longitudinal two-stage model, and these were compared across groups. Before statistical analysis, cortical thickness maps processed for cross-sectional and longitudinal analyses were smoothed with a full width of half maximum Gaussian kernel of 20mm, as in the previously published baseline analyses. [18]

Statistical Analyses

Cross-sectional and longitudinal analyses of cortical thickness

Cross-sectional and longitudinal surface-based analyses were performed using vertex-wise general linear models (GLMs). To reduce the probability of type I errors, all surface-based analyses were corrected for multiple comparisons using cluster size inference by means of *Z* Monte Carlo simulations, as implemented in Freesurfer. Here, clusters were tested against an empirical null distribution of maximum cluster size built using synthesized *Z* distributed data across 10,000 permutations, yielding clusters fully corrected for multiple comparisons across the surface. The initial cluster-forming threshold employed was $P<.05$. Longitudinal analyses

of thickness change (mm/year) were performed using the *mri_glmfit* function in Freesurfer´s longitudinal two-stage model.[32] Main group effects on cortical thickness and thickness change were tested by contrasting subjects with BD type II and HCs, while controlling for age and sex. The longitudinal patient sample was then split in two groups based on the median number of depressive and hypomanic episodes during the follow-up period (few vs. many episodes), as number of mood episodes was assessed retrospectively, and therefore prone to recall bias. Further longitudinal surface-based analyses were conducted pairwise contrasting the three groups: HCs vs. patients with few mood episodes, HCs vs. patients with many mood episodes, and patients with few vs. many mood episodes. For the sake of completeness, we also examined the effect of absolute number of mood episodes on cortical thinning in the patient group, and the results are shown in Supporting Figure S6. One patient was excluded from these analyses, as number of mood episodes was missing.

Analyses of demography and effects of clinical variables

Additional statistical analyses were performed using SPSS, version 24.0 for Windows (SPSS, Chicago, IL, USA). A two-tailed *P* value of <.05 was considered statistically significant. To test for differences in demographic and clinical variables between patients and HCs, Student's *t*-test, the χ^2 test, and the Fisher's exact test were performed. To examine the relationship between cortical thickness changes in BD type II and number of mood episodes between baseline and follow-up, mean rates of thickness change (mm/year) for each participant in clusters showing significant between-group differences were computed, and these values were also used to calculate effect sizes (Cohen's *d*). GLMs, with Bonferroni-corrected post-hoc tests, were then employed to test the effect of mood episodes (many vs. few vs. HCs) on cluster mean values. Effects of depressive episodes, when present, were then corrected for hypomanic episodes, and vice versa. In exploratory analyses, we assessed the effects of illness

duration (years since first mood episode), medication (use vs. no use of antidepressants, mood stabilizers, antipsychotic medications, and any psychotropic medication; see Table 1 and 2 for details), and family history of BD (patients with vs. patients without first-degree relatives with BD), on cluster mean values in the patient group, while controlling for age and sex. Analyses were also run while excluding subjects with alcohol abuse, cigarette smoking, and non-euthymic patients. In order to test for possible outlier effects we reran the analyses after removing subjects with studentized residuals of $>|2.0|$.

3 | RESULTS

Characteristics and Analyses of the Cross-Sectional Sample at Follow-up

Sample characteristics

Demographic and clinical characteristics of the study participants (n=71) at follow-up are shown in Table 1. There were no significant differences in age or sex distributions between patients (n=36, mean [SD] age, 36.4 [7.5] years; 26 women) and HCs (n=35, 35.0 [9.4] years; 19 women). Patients had lower educational level and higher body mass index (BMI) than HCs. No subjects had substance abuse, while three patients abused alcohol. Twenty-six of the patients were medicated, and mood stabilizers (n=21) and antidepressants (n=10) were the most frequently used medications. Panic disorder and social phobia were common comorbid diagnoses. Illness duration was 18.0±6.8 years. Twenty patients were euthymic (MADRS score <11 and YMRS score <8), three patients were hypomanic (YMRS score 8), nine patients were mildly depressed (MADRS score range 12-18), and four patients were moderately depressed (MADRS score range 21-27) at the time of MRI.

Cortical thickness

Patients versus HCs

Patients had significantly thinner cortices at follow-up relative to HCs in 1) a left prefrontal cluster comprising dorsolateral, ventromedial, and dorsomedial prefrontal cortices, 2) a right prefrontal cluster comprising dorsolateral, ventromedial, and dorsomedial prefrontal cortices and perigenual anterior cingulate cortex, and 3) a left anterior temporal cluster comprising the superior, middle and inferior temporal gyri (Figure 1A and Table 3A, all corrected P=.0001; see Supporting Figure S1C for uncorrected results). The group differences in mean cortical thickness in the left, right prefrontal and left temporal clusters were 4.6% (mean±SD, 2.22±0.12mm in patients vs. 2.32±0.13mm in HCs, Cohen's d=.87), 5.0% (2.18±0.12mm vs.

2.30±0.13mm, Cohen's *d*=.91), and 5.4% (2.64±0.15mm vs. 2.79±0.15mm, Cohen's *d*=.99), respectively . No region showed significantly thicker cortex in patients compared to controls.

Follow-up analyses

Group differences remained significant after controlling for educational level and BMI (all $P \leq .006$). There were no thickness differences in these clusters between patients who used and patients who did not use psychotropic medication, antidepressants, mood stabilizers, and antipsychotics (all $P > 05$). Furthermore, group differences remained significant after excluding the three patients with alcohol abuse and after excluding six patients and four controls who were smokers (all $P \leq .004$). Group differences remained significant when only euthymic patients (n=20) were included in the analyses (all $P < .003$). Similar group differences were found when using a cluster-forming threshold of <.01 (Supporting Figure S1D).

Characteristics and Analyses of the Longitudinal Sample

Sample characteristics

Demographic and clinical data for the longitudinal sample (n=62) are shown in Table 2. There were no significant differences in age or sex between patients (n=29, 33.3 [6.8] years; 20 women) and HCs (n=33, 32.5 [9.4] years; 18 women). There was a small, but significant difference in the interval between scans (2.3 years in patients vs. 2.4 years in HCs). This was accounted for in the analyses, which were performed on the annual change (mm/year) of cortical thickness. Patients had higher BMI than controls, and panic disorder and social phobia were frequent comorbid conditions. Twenty-six patients were using psychotropic drugs at both time points. Eighteen patients were euthymic (MADRS score <11 and YMRS score <8), one patient was hypomanic (YMRS score 8), seven patients were mildly depressed

(MADRS score range 12-18), and three patients were moderately depressed (MADRS score range 21-27) at the time of MRI. No subjects had substance abuse, while two patients abused alcohol. Illness duration was 17.5±6.8 years. Median number of depressive episodes between baseline and follow-up was two. Fifteen patients had 0-2 depressive episodes, while 13 patients had 3-10 episodes. Median number of hypomanic was four; twelve patients had 0-3 hypomanic episodes, while 16 patients had 4-20 episodes. There was a significant positive correlation between number of hypomanic and depressive episodes during the follow-up period (r=.43, P=.022). There were no significant differences in age, sex, scan interval, or clinical variables between patients with few and many depressive or hypomanic episodes (see Supporting Table S1 for details).

Cortical thickness change

Patients versus HCs

Patients and HCs showed widespread cortical thinning from baseline to follow-up; no regions showed significant increase in cortical thickness (Figure 1B and C, see also Supporting Figure S2C and D for maps showing rate of change). There were no significant sex differences in cortical thinning in the total sample, or within the two groups (Supporting Figure S5). Patients had significantly greater thinning than HCs in a left temporal cluster, mainly involving the middle and inferior temporal gyri, superior temporal sulcus, and the fusiform gyrus (Figure 1D and Table 3B, corrected P=.0001; see Supporting Figure S2A for uncorrected results). The mean rates of thickness change within this cluster were -.025±0.04mm/year for patients and .002±0.03mm/year for HCs (Cohen's d=-.79).

Follow-up analyses

The group difference remained significant after controlling for BMI (*P*=.007). Group differences remained significant after removing subjects with studentized residuals of >|2.0| (two patients and one HC, *P*=.003), after excluding two patients with alcohol abuse (*P*=.005) and after excluding six patients and four HCs who were smokers (*P*=.001). Moreover, the group difference remained significant when only including currently euthymic patients (n=18) in the analysis (*P*=.002). Furthermore, there was no significant effect of illness duration, family history of BD, current psychotropic medication use, antidepressant use, use of mood stabilizers, or use of antipsychotics on thinning within the left temporal cluster (all *P*>.05). Similar group difference was found when using a cluster-forming threshold of <.01 (Supporting Figure S2B).

Effect of mood episodes on left temporal cortical thinning

Effects of depressive episodes on cortical thinning

There was a significant effect of depressive episodes (patients with many episodes (>2) vs. patients with few (≤2) vs. HCs) on mean rate of thinning within the left temporal difference cluster (*P*=.001), and this effect remained significant after adjusting for hypomanic episodes (many (≥4) vs. few (<4) vs. HCs, *P*=.023). Post hoc analyses showed that mean thinning rate was significantly greater in patients with many depressive episodes relative to patients with few episodes (Bonferroni-corrected *P*=.039) and HCs (Bonferroni-corrected *P*=.001; Figure 1E); no significant difference was found between patients with few depressive episodes and HCs (*P*=.855).

Effects of hypomanic episodes on cortical thinning

There was also a significant effect of hypomanic episodes (many vs. few. vs. HCs) on mean rate of cortical thickness change in the left temporal difference cluster (*P*=.014), and post hoc analysis showed that patients with few hypomanic episodes had greater mean thinning rate compared to controls (Bonferroni-corrected *P*=.039), however this result was no longer significant after adjusting for depressive episodes (many vs. few vs. HCs, *P*=.603). There were no significant correlations between mean thinning rate in the left temporal cluster and absolute number of hypomanic, depressive or total number of mood episodes between baseline and follow-up. There was no sign of multicollinearity and no significant interaction effect of depressive and hypomanic episodes (absolute number and dichotomized) on mean rate of thinning in the left temporal difference cluster.

Whole-brain analysis of thickness changes and mood episodes

Based on the finding of significant effect of many depressive episodes on thickness change in the left temporal difference cluster, we then ran a longitudinal whole-brain thickness analysis pairwise contrasting the three groups (many depressive episodes vs. few vs. HCs; Figure 2).

Many depressive episodes versus few depressive episodes

We found significantly greater cortical thinning in an overlapping left temporal region in patients with many depressive episodes relative to patients with few episodes (corrected *P*=.0013, Figure 2A; -.048±0.01mm/year vs. <.001±0.01mm/year, Cohen's *d*=-1.17), which remained significant after adjusting for hypomanic episodes (many vs. few, *P*=.01). Details regarding the cluster are shown in Table 3B.Furthermore, patients with many depressive episodes had greater thinning in a right prefrontal cluster relative to patients with few depressive episodes (Supporting Figure S3D; -.027±0.02mm/year vs. .036±0.01mm/year, *P*

=.018, Cohen's *d*=-.86), however, this result did not survive correction for multiple comparisons.

Patients with many depressive episodes versus HCs

Patients with many depressive episodes had increased thinning in a left temporal cluster (corrected *P*=.0001, Figure 2B; -.044±0.01mm/year vs. -.005±0.01mm/year, Cohen's *d*=-1.14) and a small left parietal cluster (corrected *P*=.0353, -.063±0.01mm/year vs. -.028±0.01mm/year, Cohen's *d*=-.84) compared to HCs. Details regarding the clusters are shown in Table 3B. Furthermore, patients with many depressive episodes had increased thinning rate in left and right ventromedial prefrontal clusters relative to HCs (Figure 2C; -.032±0.01mm/year vs. .017±0.01mm/year, *P*=.014, Cohen's *d*=-.91and -.053±0.02mm/year vs. -.001±0.01mm/year, *P*=.022, Cohen's *d*=-.77, respectively), yet these results did not survive correction for multiple comparisons.

Patients with few depressive episodes versus HCs

There were no significant differences in cortical thinning between patients with few depressive episodes and HCs.

Effects of hypomanic episodes

In exploratory analyses of potential effects of hypomanic episodes on cortical thinning, we ran longitudinal whole-brain analyses pairwise contrasting patients with many hypomanic episodes between baseline and follow-up, patients with few hypomanic episodes, and HCs (Figure 3).

Few hypomanic episodes versus many hypomanic episodes

The analyses indicated that patients with few hypomanic episodes had increased rate of thinning in bilateral temporal cortex clusters compared to patients with many hypomanic episodes (Figure 3A; right cluster: corrected *P*=.0001, -.048±0.01mm/year vs. -.008±0.01mm/year, Cohen's *d*=-.94; and left cluster: corrected *P*=.0307, -.048±0.01mm/year vs. .001±0.02mm/year, Cohen's *d*=-.89), and these findings remained significant after adjustment for depressive episodes (many vs. few, *P*=.048 and *P* =.017, respectively).

Patients with few hypomanic episodes versus HCs

These exploratory analyses also indicated that patients with few hypomanic episodes had increased rate of thinning in bilateral temporal cortex clusters (Figure 3B; right cluster: corrected *P*=.0001, -.048±0.01mm/year vs. -.010±0.01mm/year, Cohen's *d*=-.99; and left cluster: corrected *P*=.0001, -.037±0.01mm/year vs. -.009±0.005mm/year, Cohen's *d*=-.70) and in a right ventromedial prefrontal cortex cluster (corrected *P*=.0023, -.053±0.02mm/year vs. -.007±0.01mm/year, Cohen's *d*=-.98) compared to HCs. Details regarding the clusters are shown in Supporting Table S2.

Patients with many hypomanic episodes versus HCs

There were no significant differences between patients with many hypomanic episodes and HCs.

Effects of total number of mood episodes

Whole-brain analyses of relationships between cortical thickness changes and absolute number of depressive, hypomanic and total number of mood episodes were also run and there

were no significant associations after corrections for multiple analyses (See Supporting Figure S6 for uncorrected results).

Cortical thickness at baseline for the longitudinal subsample

We also ran cross-sectional thickness analyses for the longitudinal subsample at baseline (n=62) and found thinner prefrontal cortices bilaterally in patients (see Supporting Figure 1A; all corrected $P<.05$), consistent with the results of the whole baseline sample (n=78), as published previously.[18] We then examined whether cortical thickness within these prefrontal regions could predict number of mood episodes in the patients between baseline and follow-up, but found no significant associations (all $P>.05$).

4 | DISCUSSION

The present longitudinal study of cortical thickness in BD type II had three main findings. First, consistent with the baseline results,[18] we found thinner cortices in frontotemporal regions in BD type II compared to HCs at follow-up, involving bilateral dorsolateral, ventromedial, and dorsomedial prefrontal cortices, right perigenual anterior cingulate cortex, and left temporal cortices. Second, whereas both groups exhibited widespread cortical thinning over 2.4 years, patients had greater thinning over time in left posterior and inferior temporal cortices. Third, individuals with higher number of depressive episodes between baseline and follow-up had greater left temporal cortical thinning over time than patients with fewer depressive episodes and HCs. In addition, patients with more depressive episodes had increased thinning of bilateral ventromedial prefrontal cortices from baseline to follow-up, yet these effects did not remain significant after correcting for analyses across the cortex. Together, these findings provide additional support for bilateral frontotemporal cortical thinning in BD type II and, although preliminary, the first longitudinal evidence linking depressive episodes to increased cortical thinning in mood disorders.

The present study suggests that depressive episodes are associated with increased thinning of temporal and ventromedial prefrontal cortices. These finding are consistent with some,[12-14] but not all,[15,33,34] previous cross-sectional studies of cortical thickness in BD and MDD. In addition, one study of individuals with treatment-resistant depression found that non-remitters had greater frontal cortical thinning than remitters.[35] Furthermore, longitudinal voxel- and tensor-based morphometric studies support an association between depressive episodes and frontal and temporal gray matter (GM) loss in BD[36,37] and MDD.[38] Interestingly, some previous studies found an association between depressive episodes and GM loss and cortical thinning in left inferior temporal regions overlapping with the regions found in this study.[14,36,37] Moreover, thinner left temporal cortices is one of the most consistent findings

across cortical thickness studies in BD.[5] Although the mechanisms underlying the potential lateralization effects of BD remain unknown, genes involved in handedness and language lateralization were associated with depression and BD in a recent study.[39] Importantly, however, uncorrected results of our analyses also indicated an association between depressive episodes and right temporal cortical thinning (Supporting Figure 2A and E) and the laterality of our results should therefore be interpreted with caution.

The precise mechanisms underlying the association between depressive episodes and increased cortical thinning, as indicated by the present study, remain to be clarified. However, depressive episodes can cause severe and long-lasting psychological stress, glucocorticoid increases, and hypothalamic-pituitary-adrenal axis dysregulation.[40] A recent longitudinal study found that subjects with depressive symptoms at baseline had increased cortical thinning in left frontal and bilateral superior temporal and supramarginal regions compared to controls, that thinning rate in these regions correlated with cortisol levels, and that cortisol levels were associated with thinning in widespread cortical regions in a mixed sample of depressed and non-depressed individuals.[17] In addition, BD is associated with increased levels of pro-inflammatory cytokines, and reduced levels of protective neurotrophic factors are found at later disease stages.[41] Together, these effects may result in loss of cortical GM.[42] In support of this notion, previous studies found loss of synapses and altered expression of genes involved in synapse morphology and formation in prefrontal cortices of depressed patients.[43,44] Moreover, rodent studies reported reduced dendrite branching and synapse formation after chronic stress.[42]

There are three previous longitudinal studies of cortical thickness in BD. The first longitudinal study found a tendency towards thinning of dorsolateral prefrontal and inferior frontal cortices in patients with BD type I who had at least one manic episode during a follow-up period of six years, and a significant decrease in cortical thickness in these regions,

compared to those with no manic episode.[11] The second study examined change in subcortical and cortical structures over one year in patients with first-episode psychosis and HCs, and found no significant group differences; however, only a subgroup of the patients had BD.[45] The third study assessed cortical structural changes over 7.5 years in twins who were either concordant or discordant for BD, and found no significant group differences.[46] Further, genetic liability to BD, number of hospitalizations, and lifetime experiences of psychotic symptoms were not significantly associated with cortical thickness changes; the association with mood episodes was not examined.[46]

We observed thinner bilateral frontotemporal cortices in BD type II at follow-up, consistent with the baseline examination.[18] The neuroanatomical distribution of these reductions also mirrors the recent work from the ENIGMA Bipolar Disorder consortium,[6] which is the largest study of cortical thickness in BD to date. Whether thinner cortices in BD is the result of abnormal corticogenesis or emerges early in the illness course remains to be clarified. Studies of cortical thickness in non-affected family members of individuals with BD suggest that the illness' genetic risk is associated with subtle thickness abnormalities in frontotemporal cortices.[47,48] However, a twin study did not find an association between genetic liability for BD and reduced cortical thickness, but rather that unique environmental factors related to BD were associated with cortical thinning in right frontal, limbic, and occipital cortices.[49] Further longitudinal studies are therefore needed to fully clarify the temporal profile of cortical thinning in BD.

We found reduced cortical thickness in BD type II primarily in bilateral dorsolateral, ventromedial, and dorsomedial prefrontal cortices, right perigenual anterior cingulate cortex, and left temporal cortices. Current neural models of mood regulation highlight the importance of neural circuits involving lateral and medial prefrontal cortices, temporal cortices, and subcortical structures.[7,9] Functional neuroimaging studies suggest that medial prefrontal

cortices are involved in automatic subprocesses of mood regulation, whereas lateral prefrontal cortices are recruited for voluntary regulation.[7,9] Previous studies found that patients with BD show abnormal activity in medial prefrontal cortices during emotion processing, emotion regulation and reward anticipation and processing.[50] Furthermore, studies found that modulation of dorsolateral prefrontal cortical activity improved cognitive control and emotion regulation in MDD.[51] Moreover, activity in the ventral perigenual anterior cingulate and ventral parts of medial prefrontal cortices are linked to reward processing in control subjects, and to anhedonia and amotivation in depressed individuals.[52] Parts of the medial prefrontal cortex, including the subgenual anterior cingulate cortex, have been linked to experience and regulation of dysphoric emotion in healthy subjects, and show abnormal activity patterns in subjects with depression.[9,52] Furthermore, activity in this region was found to predict treatment outcome in unipolar depression.[53] We therefore emphasize the observed effect of recurrent depressive episodes on accelerated thinning in ventromedial prefrontal cortices, including the left subgenual anterior cingulate cortex, in the present study, although this finding did not survive correction for comparisons across the brain. Also, patients with many depressive episodes had significant accelerated thinning in a left temporal region including the superior temporal sulcus and visual areas in the inferior temporal cortex, which provide sensory input to ventromedial and ventrolateral prefrontal networks, forming circuits that support emotional processing.[52] Anterior and lateral temporal regions are activated during reappraisal, [54]and lesions of these areas can lead to emotional disturbances.[55] Altogether, these findings indicate that reduced frontotemporal cortical thickness is one potential neurobiological substrate for mood dysregulation in BD type II.

We found an effect of depressive episodes on cortical thinning in the left temporal difference cluster, which remained significant after adjustment for hypomanic episodes, while an effect of hypomanic episodes was no longer significant after adjustment for depressive

episodes. Despite these findings indicating that only depressive episodes were associated with increased left temporal cortical thinning, we also ran explorative whole-brain analyses for the effects of hypomanic episodes on cortical thickness change. The results indicated that patients with few, but not patients with many hypomanic episodes had increased thinning rate in bilateral temporal cortex clusters and in a right ventromedial prefrontal cortex cluster relative to HCs. This finding was unexpected since previous longitudinal studies found that episodes of mania or hypomania in BD type I were associated with temporal GM loss[36] and frontal cortical thinning.[11] We therefore examined whether patients with few hypomanic episodes had more depressive episodes than patients with many hypomanic episodes, yet found no difference (Supporting Table S3). However, we cannot rule out the possibility that the depressive episodes were more severe in the former patient subgroup and could underlie the increased cortical thinning. The results of these exploratory analyses should therefore be considered cautiously and further longitudinal studies are needed to clarify the effects of hypomanic episodes and episode severity on cortical thinning.

Some limitations of the current study warrant notice. First, we included a modest number of participants, which limits the power to detect subtle structural changes over time. In particular, the association between ventromedial prefrontal thinning and larger number of depressive episodes did not remain significant after corrections for multiple testing and further longitudinal studies are needed to confirm this finding. Second, the attrition rate was ~20% in both groups. We cannot rule out that missing subjects may differ from participants who completed follow-up examinations; however, we replicated group differences in cortical thickness at baseline in the longitudinal subsample, similar to previously published results from the total sample. Third, the number of mood episodes between baseline and follow-up examinations was based on self-report and assessed retrospectively, and was thus prone to recall bias and inaccuracy. We therefore dichotomized this covariate in the main analyses.

However, we also ran analyses for absolute number of mood episodes, but found no significant effects on cortical thickness change. Fourth, normal structural cortical changes may differ between young (where frontal cortex maturation is still taking place) and middle-aged individuals,[56] suggesting that the biological interpretation of the cortical thickness phenotype is not invariant to the age of the sample. However, since the patients and controls in the present study were adults with a similar age distribution, this should not have a major impact on the observed group differences. Fifth, there has been a concern that a cluster-forming threshold of <.05 might be too low. We therefore reran the analyses using a cluster-forming threshold of <.01, and found that the main group differences remained significant. Sixth, a large majority of the patients was medicated at both examinations and further longitudinal studies are therefore required to elucidate the effects of psychotropic drugs on cortical thickness. Seventh, we did not include comparison groups of patients with BD type I or MDD, which limits the specificity and generalizability of our findings. Further studies are thus needed to elucidate whether the observed effects on cortical thinning are specific for BD type II or an effect of mood episode-related mechanisms regardless of underlying disorder. Finally, future studies could adopt designs with better control over possible confounding variables, such as duration and severity of mood episodes and medication use, e.g., with cortical thickness examinations during a depressive episode and then after remission. However, based on the findings of the present study, one depressive episode might not cause detectable cortical thickness changes.

In conclusion, the present study provides additional evidence for reduced cortical thickness in regions implicated in mood regulation in BD type II, and the first preliminary longitudinal evidence linking depressive episodes to increased cortical thinning in mood disorders.

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FINANCIAL DISCLOSURES

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FIGURE LEGENDS

Figure 1. *P* value maps for effects surviving cluster-wise correction for multiple comparisons at the .05 level. **(A)** Regions showing thinner cortices (blue-cyan color) in patients with bipolar disorder (BD) type II compared to healthy controls (HCs) at follow-up. **(B)** Regions showing significant longitudinal cortical thinning (blue-cyan color) in HCs. **(C)** Regions showing significant longitudinal cortical thinning (blue-cyan color) in subjects with BD type II. **(D)** Increased rate of cortical thinning (blue-cyan color) in BD type II compared to HCs from baseline to follow-up. **(E)** Boxplot showing rate of cortical thickness change (mm/year) within the left temporal difference cluster (indicated by arrow in (D)) for HCs, patients who experienced few depressive episodes between baseline and follow-up (≤2) and patients who had many depressive episodes (≥3). The horizontal lines in the boxes indicate median values. Asterisks indicate significant differences in mean rate of cortical change (**P*<.05; ***P*<.001).

Figure 2. (A) Corrected *P* value maps showing regions with increased rate of cortical thinning in mm/year (blue-cyan color) in patients who experienced many depressive episodes between baseline and follow-up compared to patients who experienced few depressive episodes. **(B)** Corrected and **(C)** uncorrected *P* value maps showing regions with increased rate of cortical thinning in mm/year (blue-cyan color) in patients who experienced many depressive episodes between baseline and follow-up compared to healthy controls. Corrected maps show effects surviving cluster-wise correction for multiple comparisons at the .05 level.

Figure 3. (A) Corrected *P* value maps showing regions with increased rate of cortical thinning in mm/year (blue-cyan color) in patients who experienced few hypomanic episodes between baseline and follow-up compared to HCs. **(B)** Corrected *P* value maps showing regions with increased rate of cortical thinning in mm/year (red-yellow color) in patients who experienced few hypomanic episodes between baseline and follow-up compared to patients who experienced many hypomanic episodes between baseline and follow-up. Corrected maps

show effects surviving cluster-wise correction for multiple comparisons at the .05 level. **(C)** Boxplot showing rate of cortical thickness change (mm/year) within a right temporal difference cluster (indicated by arrow in (B)) for HCs, patients who experienced few hypomanic, and patients who had many hypomanic episodes. The horizontal lines in the boxes indicate median values. Asterisks indicate significant ($P<.05$) differences in mean rate of cortical change.

TABLES

TABLE 1. Demographic and Clinical Characteristics of Individuals With Bipolar Disorder type II and Healthy Controls in the Cross-Sectional Study at Follow-up

	Bipolar Disorder Type II (n=36)	**Healthy controls (n=35)**	***P* value**
Age, years	36.4 ± 7.5	35.0 ± 9.4	.491
Females	26 (72.2)	19 (54.3)	.117
Educational level			.032
0–10 years	2 (5.6)	0 (0)	
11–13 years	11 (30.6)	3 (8.6)	
14–17 years	11 (30.6)	13 (37.1)	
17+ years	12 (33.3)	19 (54.3)	
Handedness, right	35 (97.2)	35 (100)	.321
MADRS score	9.7 ± 7.1	1.2 ± 1.6	<.001
YMRS score[a]	2.6 ± 2.4	0.3 ± 0.7	<.001
Illness duration, years	18.1 ± 6.8		
Social phobia	9 (31,0)		
Panic disorder	13 (44.8)		
General anxiety disorder	2 (6.9)		
Family history of BD[b]	5 (13.9)		
Medication			
Unmedicated[c]	10 (27.8)		
Mood stabilizers[d]	21 (58.3)		
Antidepressants[e]	10 (27.8)		
Oxazepam	3 (8.3)		
Antipsychotics[f]	5 (13.9)		
Methylphenidate	2 (5.6)		
Zopiclone	1 (2.8)		
Alcohol abuse	3 (8.3)	0 (0)	.083
Substance abuse	0 (0)	0 (0)	-
Cigarette smoking	6 (16.7)	4 (11.4)	.229
BMI	25.7 ± 4.5	23.3 ± 2.9	.011

MADRS; Montgomery-Asberg Depression Rating Scale. YMRS; Young Mania Rating Scale. BD; Bipolar disorder.
Continuous variables are reported as mean ± SD, whereas categorical variables are reported as n (%).
[a]Missing for one subjects with bipolar disorder type II.
[b]Family history of bipolar disorder in first-degree relatives. Data was missing for one individual with bipolar disorder type II.
[c]No psychotropic medication at least one month prior to examination.
[d]Mood stabilizers at T2 were lamotrigine (n=18), lithium (n=2), carbamazepine (n=1), and topiramate (n=1).
[e]Antidepressants were citalopram (n=1), escitalopram (n=3), bupropion (n=4), sertraline (n=1), venlafaxine (n=1), mirtazapine (n=2), and mianserine (n=1).
[f]Antipsychotic agents were quetiapine (n=4) and perfenazin (n=1).

Table 2. Demographic and Clinical Characteristics of Individuals With Bipolar Disorder Type II and Healthy Controls of the Longitudinal Sample

	Bipolar Disorder Type II (n=29)		**Healthy Controls (n=33)**		***P* value**	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
Age, years	33.3 ± 6.8	35.5 ± 7.0	32.5 ± 9.4	35.0 ± 9.5	.714	.785
Females		20 (69.0)		18 (54.5)		.245
Educational level					.820	.088
0–10 years	2 (6.9)	1 (3.4)	2 (6.1)	0 (0)		
11–13 years	6 (20.7)	9 (31.0)	5 (15.2)	3 (9.1)		
14–17 years	12 (41.4)	9 (31.0)	12 (36.4)	13 (39.4)		
17+ years	9 (31.0)	10 (34.5)	14 (42.4)	17 (51,5)		
Handedness, right		28 (96.6)		33 (100)		.468
MADRS score	10.4 ± 7.3	9.7 ± 7.0	1.1 ± 2.1	1.3 ± 1.7	<.001	<.001
YMRS score[a]	2.7 ± 2.6	2.3 ± 2.1	.3 ± .9	.3 ± .7	<.001	<.001
Illness duration, years		17.5 ± 6.9				
Social phobia		9 (31.0)				
Panic disorder		13 (44.8)				
General anxiety disorder		2 (6.9)				
Family history of BD[b]		3 (10.3)				
Years between scans		2.3 ± .47		2.4 ± .13		.047
Medication						
Unmedicated[c]	9 (31.0)	9 (31.0)				
Mood stabilizers[d]	12 (41.4)	15 (51.7)				
Antidepressants[e]	10 (34.5)	8 (27.6)				
Benzodiazepines[f]	3 (10.3)	3 (10.3)				
Antipsychotics[g]	3 (10.3)	4 (13.8)				
Methylphenidate	0 (0)	1 (3.4)				
Zopiclone	0 (0)	1 (3.4)				
No psychotropic drugs during follow-up		3 (10.3)				
No. of depressive episodes during follow-up[h]		3.0 ± 2.8				
No. of hypomanic episodes during follow-up[h]		5.0 ± 5.7				
Psychiatric hospitalization during follow-up		5 (17.2)				
Suicide attempt during follow-up		0 (0)				
ECT during follow-up		0 (0)				
Alcohol abuse	0 (0)	2 (6.9)	0 (0)	0 (0)		.215
Substance abuse	0 (0)	0 (0)	0 (0)	0 (0)		-
Cigarette smoking		6 (20.7)		4 (12.1)		.493
BMI	25.6 ± 4.4	26.0 ± 4.6	23.5 ± 3.3	23.5 ± 2.9	.038	.015

MADRS; Montgomery-Asberg Depression Rating Scale. YMRS; Young Mania Rating Scale. BD; Bipolar disorder.
Continuous variables are reported as mean ± SD, whereas categorical variables are reported as n (%).
[a]Missing for one subject with bipolar disorder type II at follow-up.
[b]Family history of bipolar disorder in first-degree relatives.
[c]No psychotropic medication at least one month prior to examination.
[d]Mood stabilizers at baseline were lamotrigine (n=11), lithium (n=1), and valproate (n=1), whereas mood stabilizers at follow-up were lamotrigine (n=13), lithium (n=1), carbamazepine (n=1), and topiramate (n=1).
[e]Antidepressants at baseline were citalopram (n=1), escitalopram (n=5), bupropion (n=1), sertraline (n=1), mirtazapine (n=2), and fluoxetine (n=1), whereas antidepressants at follow-up were citalopram (n=1), escitalopram (n=2), bupropion (n=3), sertraline (n=1), venlafaxine (n=1), mirtazapine (n=2), and mianserine (n=1).
[f]Benzodiazepines at baseline were oxazepam (n=2) and nitrazepam (n=1), whereas benzodiazepines at follow-up was oxazepam (n=3).
[g]Antipsychotic agents at baseline were quetiapine (n=2) and flupentixol (n=1), and quetiapine (n=4) at follow-up.
[h]One patient could not provide valid information on number of depressive and hypomanic episodes.

Table 3. Clusters Showing Significant Differences in Cortical Thickness in Patients with Bipolar Disorder type II Relative to Healthy Controls in **A)** Cross-Sectional and **B)** Longitudinal Analyses

A) Cross-sectional analyses (n=71)			
Cluster	**No. of Vertices in Cluster**	**Cluster *P* Value**	**Regions Within Cluster[a]**
Left Frontal Cluster	19262	<.0001	Superior frontal gyrus, rostral middle frontal gyrus, pars opercularis of the inferior frontal gyrus, lateral orbitofrontal cortex, frontal pole
Left Temporal Cluster	9174	<.0001	Superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, temporal pole
Right Frontal Cluster	16303	<.0001	Superior frontal gyrus, rostral middle frontal gyrus, rostral anterior cingulate cortex
B) Longitudinal analyses (n=62)			
BD Type II vs. HCs			
Left Temporal Cluster	8000	<.0001	Middle temporal gyrus, inferior temporal gyrus, banks of the superior temporal sulcus, lateral occipital cortex, fusiform gyrus
Patients With Many vs. Patients With few Depressive Episodes			
Left Temporal Cluster	5600	.0013	Superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, fusiform gyrus
Patients With Many Depressive Episodes vs. HCs			
Left Temporal Cluster	17690	<.0001	Superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, fusiform gyrus, lateral occipital cortex, parahippocampal gyrus, inferior parietal cortex, banks of the superior temporal sulcus
Left Parietal Cluster	4626	.0353	Postcentral gyrus, supramarginal gyrus

BD; Bipolar disorder. HCs; Healthy controls.

[a]The cortical regions of the Desikan-Killiany atlas[31] were employed.