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Longitudinal changes in resting-state functional connectivity as markers of vulnerability or resilience in first-degree relatives of patients with bipolar disorder

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Abstract

Background. There is a significant contribution of genetic factors to the etiology of bipolar disorder (BD). Unaffected first-degree relatives of patients (UR) with BD are at increased risk of developing mental disorders and may manifest cognitive impairments and alterations in brain functional and connective dynamics, akin to their affected relatives.

Methods. In this prospective longitudinal study, resting-state functional connectivity was used to explore stable and progressive markers of vulnerability i.e. abnormalities shared between UR and BD compared to healthy controls (HC) and resilience i.e. features unique to UR compared to HC and BD in full or partial remission (UR $n = 72$, mean age = 28.0 ± 7.2 years; HC $n = 64$, mean age = 30.0 ± 9.7 years; BD patients $n = 91$, mean age = 30.6 ± 7.7 years). Out of these, 34 UR, 48 BD, and 38 HC were investigated again following a mean time of 1.3 ± 0.4 years.

Results. At baseline, the UR showed lower connectivity values within the default mode network (DMN), frontoparietal network, and the salience network (SN) compared to HC. This connectivity pattern in UR remained stable over the follow-up period and was not present in BD, suggesting a resilience trait. The UR further demonstrated less negative connectivity between the DMN and SN compared to HC, abnormality that remained stable over time and was also present in BD, suggesting a vulnerability marker.

Conclusion. Our findings indicate the coexistence of both vulnerability-related abnormalities in resting-state connectivity, as well as adaptive changes possibly promoting resilience to psychopathology in individual at familial risk.

Introduction

Bipolar disorder (BD) is a heritable mood disorder characterized by recurring episodes of depression and (hypo)mania affecting around 1–3% of the global population (Johansson, Kuja-Halkola, Cannon, Hultman, & Hedman, 2019; Merikangas et al., 2011). Individuals with BD often display significant psychosocial disability and are at risk of losing 10–20 years of life due to an increased prevalence of cardiovascular disease and suicidality (McIntyre et al., 2020). In addition to clinical symptoms, cognitive dysfunction is another dimension of BD that contributes to functional deficits (Lewandowski, Sperry, Malloy, & Forester, 2014). There is therefore increased motivation to understand the underlying brain mechanisms as a means to develop novel and targeted interventions.

Functional magnetic resonance imaging (fMRI) studies have identified neural alterations in BD, notably in tasks related to emotional processing, reward anticipation, and working memory (Mesbah et al., 2023). There are also abnormalities in resting-state functional connectivity

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(RSFC), which characterizes the inherent functional organization of the brain during rest, in the absence of specific task engagement (van den Heuvel & Hulshoff Pol, 2010). Resting-state connectivity is organized into resting-state networks (RSNs) that support both higher-order cognition and primary somatic and sensory processing (Beckmann, DeLuca, Devlin, & Smith, 2005; Yeo et al., 2011). Patients with BD predominantly exhibit reduced RSFC in several RSNs, notably the task-negative default mode network (DMN), the frontoparietal network (FPN) involved in cognitive control, and the salience network (SN) involved in salience detection (Claeys, Mantingh, Morrens, Yalin, & Stokes, 2022; Gong et al., 2021; Yoon, Kim, Kim, & Lyoo, 2021; Zovetti et al., 2020). Interestingly, genome-wide association studies support a polygenic risk structure of BD (Mullins et al., 2021) and higher genetic risk scores were shown to be associated with lower RSFC of the SN and higher RSFC of the FPN (Jiang et al., 2023).

First-degree unaffected relatives (UR) of patients with BD also present with decreased socio-economic functioning compared to the general population (Sletved, Ziersen, Andersen, Vinberg, & Kessing, 2023), and face an increased risk of developing BD as well as a range of other psychiatric conditions (Chen et al., 2019). This indicates a significant influence of genetic factors on the etiology of BD (Chen et al., 2019) with heritability rates estimated to be at least 60% in first-degree relatives (Johansson et al., 2019). This population offers a unique opportunity to disentangle brain alterations related to familial vulnerability to BD from those that are associated with the avoidance of psychopathology, hereafter referred to as resilience.

Cognitive and neuroimaging studies of UR have identified abnormalities akin to those seen in BD suggesting that such alternations are an expression of familial vulnerability (Frangou, 2019; Miskowiak et al., 2017; Piguet, Fodoulian, Aubry, Vuilleumier, & Houenou, 2015). For instance, patients with BD and UR showed similar abnormalities in tasks of working memory, and executive functions (Piguet et al., 2015), interference control and facial affect recognition (Frangou, 2019) and decreased RSFC between the fronto-occipital and DMN (Meda et al., 2012) and within the striatal-thalamo-cortical network (Lui et al., 2015). In addition, based on graph theory methods examining global and regional brain network topology, Doucet, Bassett, Yao, Glahn, and Frangou (2017) showed reductions in the cohesiveness of the sensorimotor network were also present in UR.

By contrast, features that differentiate UR from both patients and HC have been interpreted as markers of resilience, i.e. compensatory mechanisms associated with avoidance or delay of psychopathology despite genetic predisposition (Frangou, 2019; Wiggins et al., 2017). Such markers include hyperactivation of the dorsolateral and ventrolateral prefrontal cortex (PFC) during interference control (Pompei, Dima, Rubia, Kumari, & Frangou, 2011), enhanced connectivity between the ventrolateral and dorsolateral PFC during working memory tasks (Dima, Roberts, & Frangou, 2016), and increased within-network integration of core DMN regions (Doucet et al., 2017). It is noteworthy, however, that the few RSFC studies in BD and their UR were all cross-sectional and the clinical outcomes were not followed up to determine potential clinical outcomes.

The current study aims to bridge a gap in the existing literature by conducting prospective investigations on a distinctive cohort of UR over an average follow-up period of 1.3 years. We aimed to identify RSFC markers in UR compared to HC and patients with BD that either remain stable over time or progress and test whether such connectivity features are associated with vulnerability, resilience, or progression to an overt expression of psychopathology. Based on previous literature, we anticipate these connectivity abnormalities to be evident within the DMN, FPE, and SN networks.

Material and methods

Study design and participants

The study sample comprised 91 patients newly diagnosed with bipolar disorder (BD), 70 unaffected first-degree relatives (UR), and 64 healthy controls (HC) (Table 1) enrolled in the ongoing longitudinal Bipolar Illness Onset (BIO) study (Kessing et al., 2017). Patients who were diagnosed with their first manic episode or BD within the preceding two years were recruited from the Copenhagen Affective Disorder Clinic. The initial BD diagnosis (type I or II), was established through a comprehensive clinical evaluation performed by highly specialized psychiatrists at the clinic. Subsequently, participants' diagnostic status was confirmed using the semi-structured Schedules for Clinical Assessment in Neuropsychiatry (SCAN) interview (Wing et al., 1990) employing International Classification of Diseases (ICD-10) criteria (World Health Organization, 1996). The interview was conducted by a team of five research team members, all of whom had received formal training in SCAN administration. Patients received treatment as chosen by their psychiatrist, independent of their participation in the study. Patients were eligible if they were aged 15–70 years and were in full or partial remission, defined by a score of $\leqslant$14 on the Hamilton Depression Rating Scale (HDRS) (Hamilton, 1967) and the Young Mania Rating Scale (YMRS) (Young, Biggs, Ziegler, & Meyer, 1978). UR were identified based on information and consent from the patients with BD and encompassed both siblings ($n = 67$) and offspring ($n = 3$) of the patients. The UR had no personal history of mental disorders ascertained through the SCAN assessment. HC were recruited from Copenhagen University Hospital Rigshospital's Blood Bank and had no personal or first-degree familial history of mental disorders. General exclusion criteria for all individuals were a history of severe brain injury, substance abuse disorder (ICD-10 F10–F19), current severe somatic illness, and contraindication to MRI (e.g. pregnancy, claustrophobia, or metal implants). Due to limited resources, about half of the participants (BD $n = 48$, UR $n = 32$, HC $n = 38$) were randomly selected to attend a second assessment 1.3 years on average following enrolment. Two UR and one HC had a depressive episode during the follow-up period; as this sample was too small, they were excluded from the longitudinal analysis which focused on unaffected individuals only. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The study received approval from the local ethics committee in the Capital Region of Denmark (H-7-2014-007).

Sociodemographic clinical and cognitive assessment

At baseline, sociodemographic information was collected on participants' age, sex, and years of education. The clinical assessment comprised the severity of psychopathology based on the HDRS and YMRS, current smoking status [yes/no], level of social function was evaluated with the Functioning Assessment Short Test

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Table 1. Demographic and clinical information for patients diagnosed with bipolar disorder, their high-risk first-degree healthy relatives, and healthy controls at the baseline assessment

	BD	UR	HC	UR v. HC *p*-value	BD v. HC *p*-value
n baseline (follow-up)	91 (48)	70 (32)	64 (38)		
Age, years	30.25 [25.41–35.58]	27.14 [22.99–31.20]	26.15 [23.68–34.71]	0.64	0.17
Sex, female, *n* (%)	64 (70%)	36 (51%)	39 (61%)	0.26	0.20
Verbal IQ	112 [108–117]	112 [106–114]	113 [109–116]	0.03	0.98
Current smokers, *n* (%)	28 (31%)	10 (14%)	13 (20%)	0.29	0.15
Follow-up time, weeks	64 [52–84]	59 [52–69]	63 [50–89]	0.38	0.87
Education (years)	15[12.4–17.7]	15[13–17]	16 [14.5–17.5]	0.15	0.07
Clinical data					
HDRS	5 [2–8]	1 [0–2]	1 [0–2]	0.48	<0.001
YMRS	2 [0–4]	0 [0–1]	0 [0–0.75]	0.45	<0.001
FAST	15 [7–25]	2 [0–4]	0 [0–2]	0.003	<0.001
Quality of life, EQ-5D index	0.86 [0.79–1.00]	1 [0.89–1.00]	1 [1–1]	0.20	<0.001
No. prior depressive episodes	7 [3–12]				
No. prior hypomanic episodes	6 [2–12]				
No. prior manic episodes	0 [0–1]				
No. prior mixed episodes	0 [0–0]				
No. hospitalizations	0 [0–1]				
Illness duration[a]	5 [2.50–13.00]				
Untreated BD[b]	3.5 [1–12]				
Age at diagnosis	28 [23–34]				
Age at onset[c]	20 [16–25]				
Bipolar type II, *n* (%)	60 (66%)				
Comorbid anxiety, *n* (%)[d]	10 (11%)				
Current medication					
Lithium, *n* (%)	42 (46%)				
Anticonvulsants, *n* (%)	34 (37%)				
Antidepressants, *n* (%)	22 (24%)				
Antipsychotics, *n* (%)	24 (26%)				
No medication, *n* (%)	20 (22%)				

Continuous variables are presented as medians [interquartile range]. Categorical variables are presented as *n* (%).
Abbreviations: BD, bipolar disorder patients; UR, unaffected relatives; HC, healthy controls; HAMD, Hamilton Depression Rating Scale; YMRS, Young Mania Rating Scale; FAST, Functioning Assessment Short Test.
*Significant *p* values.
[a]Defined as time between first depressive, (hypo)manic, manic, or mixed episode and time of study participation.
[b]Defined as time between first (hypo)manic or mixed episode and time of diagnosis of BD.
[c]Patients' age at time of first hypomanic, manic, or mixed episode.
[d]ICD-10 F40 - F42.

(FAST) (Rosa et al., 2007), and quality of life with the European Quality of Life 5-Domain (EuroQol, 1990). An estimate of verbal IQ was obtained using the Danish version of the National Adult Reading Test (DART) (Nelson, 1982). The participants underwent the same clinical and cognitive assessment at follow-up with all groups showing stable subsyndromal symptoms and cognitive function, and an improvement in functioning in BD patients (Kjærstad, Søhol, Vinberg, Kessing, & Miskowiak, 2023).

Resting state-fMRI acquisition protocol

Neuroimaging data were acquired at the Copenhagen University Hospital, Rigshospitalet using a 3 Tesla Siemens Prisma scanner and a 64-channel head-neck coil. During the rs-fMRI sequence, participants were asked to keep their eyes closed. Blood oxygen level-dependent (BOLD) signal was acquired using a T2*-weighted gradient echo spiral echo-planar (EPI) sequence with an echo time (TE) of 30 ms, repetition time (TR)

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of 2s, and flip angle of 90°. A total of 217 volumes were acquired, each consisting of 32 slices with a slice thickness of 3 mm with 25% gaps in-between, and a field of view (FOV) of 230 × 230 mm using a 64 × 64 grid. The BOLD images were registered to T1-weighted structural images (TR = 1900 ms; TE = 2.58 ms; flip angle = 9°; distance factor = 50%; FOV = 230 × 230 mm; slice thickness = 0.9 mm). A standard B0 field map sequence was also acquired with the same FOV and resolution as the rs-fMRI sequence (TR = 400 ms; TE = 7.38 ms; flip angle = 60°) and used for geometric distortions correction of the BOLD images. The image quality was ascertained by visual inspection of all individual images.

Statistical analysis

Sociodemographic and clinical data

Sociodemographic and clinical variables were compared at baseline between UR and HC, and BD and HC participants using Statistical Packages for the Social Sciences (SPSS) v28 (IBM Corporation, Armonk, NY) with α-level of significance of $p < 0.05$ (two-tailed). Data normality distribution for each variable was determined with Shapiro–Wilk's test (Shapiro & Wilk, 1965). We used independent samples t test for normally distributed data, non-parametric Mann–Whitney U tests for non-normally distributed data, and Pearson's Chi-square ($\chi 2$) for categorical data (sex) to investigate differences in demographic and clinical characteristics between groups.

MRI data pre-processing and resting state network analysis

MRI data were pre-processed and analyzed using FSL (FMRIB's Software Library v6.0.4; (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). Pre-processing involved motion correction using rigid-body transformations (MCFLIRT, FSL), high-pass temporal filtering cut-off of 100 s period, non-brain tissue removal, linear registration to the individual T1-weighted image, and spatial smoothing using a 5 mm full-width-half-maximum gaussian kernel. Participants with head motion that exceeded an average relative framewise movement of 0.25 as obtained by MCFLIRT were subject to exclusion ($n = 0$). Then, to further denoise the functional data, we used single-session independent component analysis (ICA) to decompose the rs-fMRI signal into spatially independent components.

Denoising was carried out by using FIX classifier, a tool that automatically classifies components as signals *v.* artifacts (i.e. head movement, respiratory, cardiac, or scanner noise) (Griffanti et al., 2017, 2015; Salimi-Khorshidi, Douaud, Beckmann, Griffanti, & Smith, 2015). The classifier used was trained with resting-state data based on an identical MRI acquisition protocol from the cohort (Fortea et al., 2023). The denoised resting-state data obtained from FIX were manually inspected to verify the accuracy of the pre-trained classifier. FIX identified the RSN in the functional dataset and regressed the noise. Finally, we applied non-linear registration to align the cleaned single-subject data to the standard MNI (Montreal Neurologic Institute) space at 2 mm isotropic voxel size.

Within network connectivity analysis

We conducted group-level ICA using multivariate exploratory linear optimized decomposition into independent components (MELODIC) with multi-session temporal concatenation to identify the common RSNs across the three groups at baseline. We set the ICA dimensionality to 20 components; a common degree of clustering previously applied to rs-fMRI data (Beckmann et al., 2005; Smith et al., 2009), and we further validated the resulting components in comparison to publicly available RSNs (Yeo et al., 2011). For this, we used automatic spatial correlation analysis (FSL's fslcc function) to correlate our components with the selected RSNs from Yeo et al. (2011) based on our a priori hypothesis (DMN, FPN, and SN) and chose the component with the highest correlation coefficient to represent each network. We used a two-step multivariate regression analysis (dual regression) (Beckmann, Mackay, Filippini, & Smith, 2009) to estimate the subject-specific contribution to the identified group RSNs. Next, we used non-parametric permutation inference (FSL randomize) with 5000 permutations (Winkler, Ridgway, Webster, Smith, & Nichols, 2014) to investigate within-RSN connectivity differences between either UR and HC or between patients with BD and HC using independent sample t tests for each of the three selected RSNs at baseline. We implemented threshold-free cluster enhancement (TFCE) (Smith & Nichols, 2009) correction for multiple comparisons to asses cluster significance based on a minimum cluster size of $k > 25$ voxels and $p < 0.008$ ($\alpha = 0.05/6$ to correct for the three studied networks and HC *v.* BD, and UR *v.* BD comparisons).

Longitudinal region-of-interest analysis

Following the identification of regional abnormalities in UR or BD compared to HC in the voxel-wise baseline analysis within the DMN, FPN, and SN described above, we extracted average loadings representing individual contributions to the group RSNs from the networks showing significant group differences. These loadings were extracted across all participants (UR, BD, HC) at baseline for visualization purposes, as well as at follow-up for the UR and BD groups. The longitudinal data in these groups was used to assess whether baseline values remained stable over time or progressed. For this, we implemented a within-subject linear mixed models in SPSS with subject ID and family ID as random effects, time as repeated factor with unstructured covariance type, and follow-up time in years as covariate. Notably, longitudinal interaction effects between UR and HC, or BD and HC were not assessed on this data showing an effect of group at baseline in order to avoid overestimation bias. We interpreted a non-significant effect of time ($p > 0.008$) on the extracted RSFC loadings as longitudinally stable RSFC. Conversely, significant effects of time ($p < 0.008$) were interpreted as progressive changes based on qualitative comparison with the HC and BD groups.

Longitudinal whole-brain analysis

We further implemented two longitudinal whole-brain voxel-wise GLM models including either the UR and HC or BD and HC participants with rs-fMRI data at both baseline and follow-up. We assessed group-by-time interaction effects based on cluster significance corrected for multiple comparisons at $p < 0.008$ ($\alpha = 0.05/6$ as above).

Between-network RSFC analysis

Between-RSN RSFC was calculated using a partial correlation between each pair of RSNs for all 3 groups using the FSLNETs package (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLNets). The

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partial correlation method aims to estimate more accurately the 'direct' connections between networks than the full correlation method (Smith et al., 2013). We assessed significance corrected for multiple comparisons at $p < 0.008$. Similar to the within-network connectivity analysis, we estimated the trajectory of the aberrant between-network connectivity values in UR or BD identified above using analogue longitudinal mixed models in SPSS assessing the effect of time for each group separately. We lastly performed a longitudinal analysis in the subsamples with longitudinal rs-fMRI data assessing group-by-time interaction effects.

Post-hoc analyses

We tested the effect of current use of psychotropic medication within the patient group (medicated $n = 71$ *v.* unmedicated $n = 20$) both in within- and between-network RSFC. Upon significant findings, the connectivity analyses where patients showed significant differences compared to HC were rerun adjusting for mood symptoms (HDRS and YMRS). In addition, we compared at baseline the subgroups randomly selected to participate in the follow-up assessment (34 UR, 48 BD, and 38 HC) with the subgroups with baseline data only (36 UR, 43 BD, and 26 HC) on demographic and clinical variables, and within- and between-network RSFC using the extracted average loadings from the three investigated networks as above.

Results

Demographic and clinical characteristics

At baseline, 70 UR, 91 patients with BD, and 64 HC were included in the analysis. There were no group differences in age, sex, years of education, smoking status, and verbal IQ (Table 1). Depressive and manic symptoms were similar in UR and HC. Despite being in partial or full remission, BD patients still exhibited higher HDRS and YMRS scores relative to HC. The functioning scores (FAST total) were lower in both UR and BD compared to HC.

From the initial sample investigated at baseline, 34 UR, 48 BD, and 38 HC attended a similar investigation at 1.3 years in average (S.D. = 0.4 years) follow-up. Within each of the three groups, participants with only baseline data did not differ significantly from participants with both baseline and follow-up assessment in terms of demographic and clinical variables (i.e. age, sex, IQ, HDRS, YMRS: BD ps > 0.12; UR ps > 0.12; HC ps > 0.06; with the exception of subsyndromal manic symptoms, which were higher at baseline in UR with both assessments *v.* those with only baseline assessments $p = 0.02$).

Within-network RSFC analysis

We selected the three network components showing the highest correlation with the DMN, FPN, SN components from Yeo et al. (2011) (r values: DMN 0.48; FPN 0.38; SN 0.38) to represent our respective group networks (see online Supplementary figure). At baseline the UR group showed significantly lower within-RSN connectivity compared to HC in the DMN (parietal operculum), FPN (eight significant clusters in widespread cortical regions), and the SN (three significant clusters in the parietal cortex and thalamus) (Fig. 1, Table 2). The average loadings across the regions showing aberrant values in UR within respective RSN remained relatively stable over time based on non-significant effect of time at $p > 0.008$ (DMN $p = 0.314$, FPN $p = 0.016$, SN $p = 0.026$). These RSFC values did not differ significantly at baseline between the subgroup with follow-up assessment and the subgroup with baseline only investigation (DMN $p = 0.521$, FPN $p = 0.679$, SN $p = 0.446$).

There were no significant differences in RSFC between the BD and HC in any of the three tested networks. Use of psychotropic medication had no significant impact on the average connectivity estimates across the networks ($p \geqslant 0.277$).

The longitudinal whole-brain voxel-wise analyses within the subgroups with both baseline and follow-up data revealed no significant regions where over-time changes in within-RSN RSFC were significantly different between either UR and HC or between BD and HC.

Between-network RSFC analysis

There was a negative connectivity between the DMN to SN across the groups. In both the UR and BD, the connectivity values were significantly less negative compared to HC (UR *v.* HC $p < 0.001$, BD *v.* HC $p = 0.005$ & $p = 0.008$ adjusted for HDRS and YMRS scores, Fig. 1). There was no significant difference between the connectivity values of UR and BD ($p > 0.05$). Use of psychotropic medication in patients had no significant impact on the connectivity between the DMN and SN ($p = 0.353$). The trajectory for the DMN to SN connectivity remained stable over time for both the UR and BD (effect of time: UR $p = 0.453$, BD $p = 0.367$). The baseline RSFC values did not differ significantly between the subgroups with follow-up assessment and respective subgroups with baseline only investigation (UR $p = 0.672$, BD $p = 0.068$).

At baseline, the RSFC between the DMN and FPN, and between the FPN and SN was not found significantly different between UR and HC, nor between BD and HC ($p > 0.05$).

The longitudinal analysis within the subgroups with both baseline and follow-up data revealed no pair of networks showing significantly different over-time changes in between-network RSFC ($p > 0.05$).

Discussion

This longitudinal study of resting state functional connectivity (RSFC) in patients with BD, UR, and matched healthy controls (HC) aimed to identify markers of risk and resilience in UR over an average follow-up time of 1.3 years. Abnormalities shared between UR and BD compared to HC were identified as markers of risk, whereas features unique to UR compared to HC and BD as resilience traits. Based on a priori hypothesis, we investigated the connectivity of three RSNs, namely the DMN, the FPN, and the SN. At baseline, we found lower connectivity in UR compared to HC within all investigated RSNs, and this finding was stable over the follow-up period. We further found a longitudinally stable less negative RSFC between the DMN and SN networks in both UR and BD compared to HC. In the subsample with longitudinal data, we found no significant group-by-time interaction effects in within and between-network RSFC for either UR *v.* HC, or BD *v.* HC. Consequently, none of the observed connectivity abnormalities in UR progressed significantly over the follow-up period.

Identification of stable RSFC abnormalities in UR that could signify either vulnerability or resilience traits in BD

The within-network analysis identified at baseline a regional pattern of lower RSFC in UR *v.* HC within the DMN, FPN, and the SN networks. Since this RSFC pattern was unique to the UR

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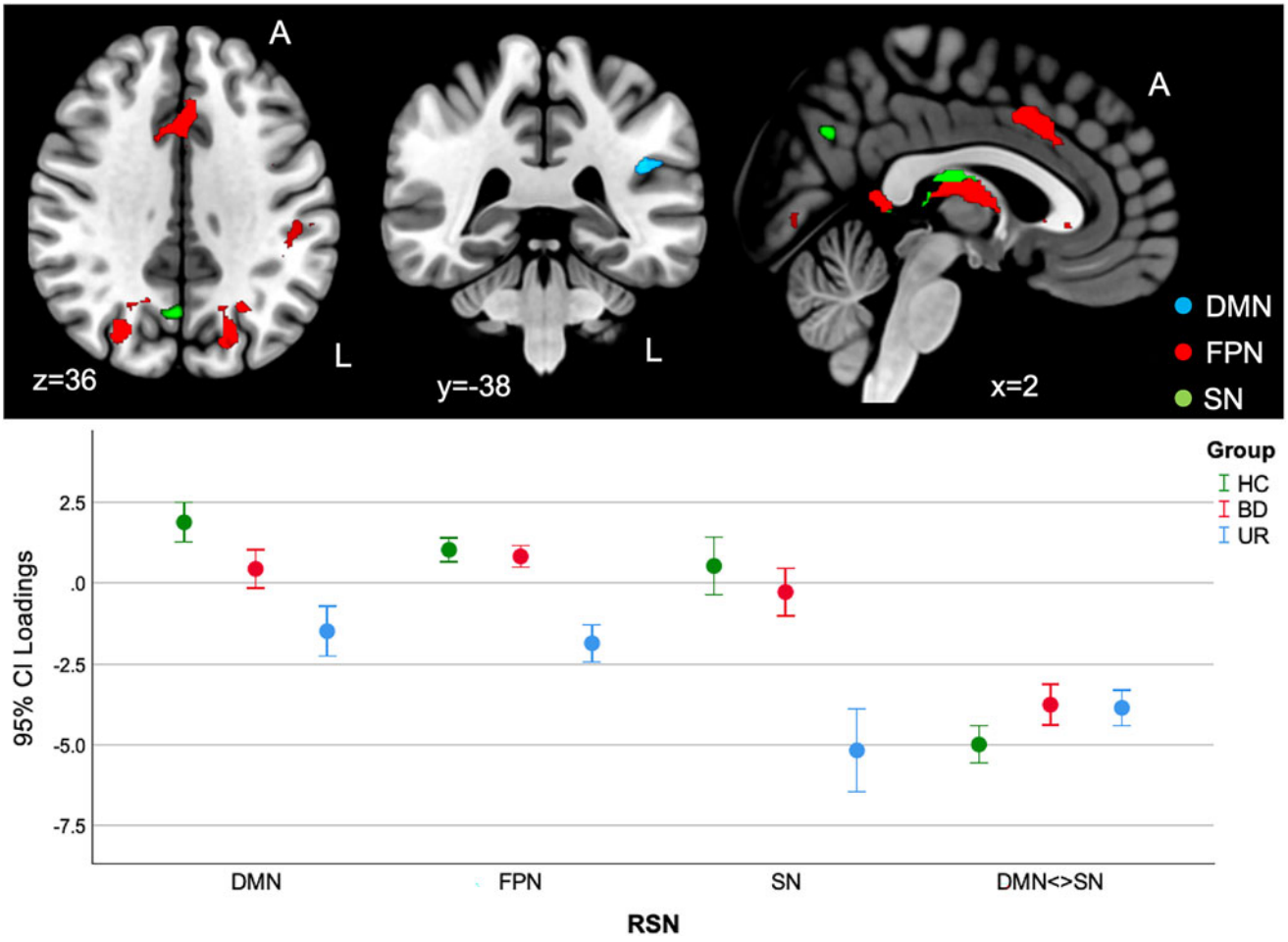

Figure 1. Resting state functional connectivity analysis at baseline. Top: regions where unaffected relatives (UR) of patients with bipolar disorder (BD) show significantly lower resting state functional connectivity compared to healthy controls (HC) within the default mode network (DMN), the right fronto-parietal network (FPN), and the salience network (SN). Bottom: average loadings with 95% confidence interval (CI) within respective network and between DMN and SN across the three groups. Abbreviations: RSN – resting state network, L – left hemisphere, A – anterior.

group (i.e. was not present in BD), we interpret it as markers of resilience based on the proposed criteria (Frangou, 2019; Wiggins et al., 2017). The averaged RSFC loadings in UR remained stable over the follow-up period across the RSNs. The hypoconnectivity in UR within the task-negative DMN, the task-positive FPN, and the salience detection SN may reflect an altered communication in both internal self-monitoring processing and external cognitive processing. In particular, the lower RSFC in the right lateral orbitofrontal cortex, a central hub within the FPN linked to inhibitory and attentional control (Hampshire, Chamberlain, Monti, Duncan, & Owen, 2010), might contribute to disinhibited behavior (Clauss, Avery, & Blackford, 2015).

In contrast, the between-network analysis at baseline revealed a less negative RSFC between the DMN and the SN in both UR and BD compared to HC, connectivity dysfunction that remained stable over the follow-up period and that could be classified as a potential vulnerability trait in UR. This finding aligns with the results from the synthesis of resting-state studies in individuals with BD as reviewed by Yoon et al. (2021). In the model proposed by Menon (2011), the SN plays a critical role in initiating network switching by engaging the FPN that mediates attentional, working memory, and higher order cognitive processes and disengaging the DMN when salient stimuli are detected. Excessive coupling between DMN and SN may be linked to a diminished capacity to disengage from internal mental processes. This is particularly evident in individuals with depression, where persistent rumination may interfere with cognitive resource allocation (Zhou et al., 2020).

A less negative RSFC between the DMN and the SN could potentially serve as a predictive biomarker that forecasts the likelihood of developing BD. Detection of this network connectivity dysfunction in UR may also be targeted by early intervention strategies such as cognitive-behavioral therapy, transcranial magnetic, or direct current neuromodulation approaches aimed at preventing or delaying the onset of the disorder.

In conclusion, we demonstrate the coexistence of abnormalities in first-degree UR that may be related to either risk or resilience to psychopathology suggesting a disorder risk model where risk and resilience mechanisms are not antithetical, instead inherited familial vulnerabilities may be compensated by a distinct acquired protective mechanism. It is plausible that such a protective mechanism e.g. a cognitive strategy to stabilize mood, may become more efficient over time thereby reducing the total risk. This hypothesis could account for the decline in the frequency of BD onset following its peak around the age of 22 (Manchia et al., 2017).

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Table 2. Regions showing significantly lower resting state functional connectivity in healthy relatives of patients with bipolar disorder compared to matched healthy controls at baseline

Region	Cluster size	Cluster sig.	*MNI Coordinates*		
Healthy relatives < healthy controls	voxels	*p*-value	X	Y	Z
Default mode network (DMN)					
Parietal operculum	28	0.01	−42	−38	26
Fronto parietal network (FPN)					
Lateral OFC	10 092	<0.001	−38	18	−12
Lateral occipital	1197	<0.001	28	−62	30
Anterior cingulate	397	0.001	−8	20	26
Insula	84	0.005	32	−20	12
Posterior cingulate	82	0.008	−4	−12	38
Middle temporal	71	0.001	48	−36	−4
Middle frontal gyrus	55	0.007	−32	−2	66
Frontal pole	40	0.001	−34	42	8
Salience network (SN)					
Thalamus	208	0.001	−6	−32	10
Precuneus	73	0.006	2	−64	34
Posterior cingulate	40	0.006	6	−36	0

Claster significance was assessed at $p < 0.008$.

Complementing our findings, a few extant RSFC studies in first-degree relatives of BD patients have identified distinct connectivity abnormalities in this group and interpreted them as either vulnerability or resilience markers in relation to the proband patient group. Patients with psychotic BD and their relatives showed decreased RSFC between fronto-occipital and DMN (Meda et al., 2012) and within striatal-thalamo-cortical network (Lui et al., 2015). The psychotic feature of the BD diagnosis in probands and distinct methodological approach may have contributed to the reason our data did not replicate these previous findings. UR of patients with BD were also found to exhibit unique connectivity features associated with resilience compared to patients and HC such as hyperactivation of dorsolateral and ventrolateral PFC during interference control (Pompei et al., 2011) and enhanced connectivity between the ventrolateral and dorsolateral PFC during working memory tasks (Dima et al., 2016). Interestingly, a study of global and regional brain network topology based on RSFC data in BD patients, their relatives and HC (Doucet et al., 2017), also reports coexistence of connectivity patterns related to vulnerability in relatives (reductions in the cohesiveness of the sensorimotor network) as well as resilience in the same group (increased within-network integration of core DMN regions). Adaptive brain responses that could promote resilience or delay the onset of mood disorders may be conceptualized in terms of increased neural reserve, the ability of brain networks to resist psychopathology by means of recruitment of additional neural resources, or increased plasticity (Frangou, 2019). Another study of RSFC in relatives of patients with BD has further shown increased connectivity in the ventrolateral prefrontal cortex, subregion of the left executive control network compared to HC (Singh et al., 2014). However, since the study lacked a patient control group no distinction between vulnerability or resilience was possible, underlining the importance of inclusion of both patient and HC groups when investigating traits of risk or resilience.

The hyperconnectivity between the DMN and SN is in line with previous reports in BD as reviewed by Yoon et al. (2021). However, contrary to our expectations, our BD sample ($n = 91$) in full or partial remission displayed no significant differences in within-network RSFC compared to HC ($n = 64$) across the investigated RSNs. The significant effect of group observed in the comparison between the smaller UR sample ($n = 72$) and the corresponding HC sample supports the interpretation that the negative findings in BD patients are genuine rather than a result of inadequate statistical power. This negative finding is also corroborated by a systematic review of rs-fMRI studies in BD by Syan et al. (2018) where the stability of the DMN, FPN, and SN was suggested to reflect the state of remission. In line with this, Wang et al. (2020) have further identified different connectivity patterns both within and between RSNs when contrasting the acute and remitted states of BD. There is emerging evidence indicating that medication exposure may mitigate the differences between BD and HC (Hafeman, Chang, Garrett, Sanders, & Phillips, 2012). In particular, lithium has been found to normalize gray matter volume, especially in areas subserving emotion processing and mood regulation (ibid.), and antidepressants have been shown to reverse functional deficits by increasing cortical activation, decreasing limbic activation, and increasing corticolimbic connectivity (Anand et al., 2005; Davidson, Irwin, Anderle, & Kalin, 2003; Sheline et al., 2001).

Strengths of our study included the large baseline sample size of UR and the inclusion of both matched HC and patients with BD in full or partial remission that allowed for the distinction between traits of vulnerability or resilience in UR. Additionally, we had both longitudinal imaging and clinical data. Since rs-fMRI data generally shows high within-subjects reproducibility estimates (Song, Panych, & Chen, 2016), we were able to assess the trajectories of the identified RSFC abnormalities over the follow-up period. Limitations include a relatively short follow-up time (average of 1.3 years). Longer follow-up would have increased the likelihood of having a larger UR group with onset of mood disorder at follow-up which could have been analyzed separately. Another limitation is the smaller sample size at follow-up compared to baseline. Due to limited financial resources, the investigation was concluded after 48 out of 91 patients, 34 out of 70 UR, and 38 out of 64 HC were randomly offered a second MRI investigation. Importantly, there were no differences in demographic and most clinical factors between participants with only baseline investigation compared to participants with longitudinal data. Finally, we did not account for the use of non-psychotropic medications in the entire sample.

In conclusion, our study identified specific features in the resting-state functional connectivity of first-degree relatives of patients with bipolar disorder that could be classified as either vulnerability or resilience traits to psychopathology. Importantly, these features demonstrated stability over the follow-up period. The coexistence of such characteristics in brain connectivity dynamics suggests a disorder risk model where inherited familial vulnerabilities may be compensated by a distinct acquired protective mechanism. Awareness of such mechanisms that rely on individuals' cognitive abilities to self-stabilize mood, may encourage those at risk to actively cultivate and refine their cognitive strategies. Future studies with longer

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follow-up assessments should investigate whether the identified markers of resilience result in lasting protection in high-risk relatives or whether they are absent in relatives with onset of psychopathology.

Competing interests. GMK has the past three years received payment as speaker of Sage Therapeutics, H. Lundbeck, and Angelini and has served as consultant for Sanos, Gilgamesh, Onsero and Pangea. MV has received consultancy fees from Lundbeck and Janssen Cilag for the past three years. LVK has within the preceding three years been a consultant for Lundbeck and Teva. KWM has received consultancy fees from Lundbeck, Janssen-Cilag, Gedeon Richter, and Angelini in the past three years. The remaining authors declare no conflicts of interest.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0033291724000898.

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