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The Bipolar Illness Onset study - research protocol for the BIO cohort study

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The Bipolar Illness Onset study - research protocol for the BIO cohort study

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4 **Introduction:** Bipolar disorder is an often disabling mental illness with a lifetime prevalence of 1-
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6 2%, a high risk of recurrence of manic and depressive episodes, a lifelong elevated risk of suicide
7
8 and a substantial heritability. The course of illness is frequently characterised by progressive
9
10 shortening of inter-episode intervals with each recurrence and increasing cognitive dysfunction in a
11
12 subset of individuals with this condition. Clinically, diagnostic boundaries between bipolar disorder
13
14 and other psychiatric disorders such as unipolar depression are unclear although pharmacological
15
16 and psychological treatment differs substantially. Patients with bipolar disorder are often
17
18 misdiagnosed and the mean delay between onset and diagnosis is 5-10 years. Although the risk of
19
20 relapse of depression and mania is high it is for most patients impossible to predict and
21
22 consequently prevent upcoming episodes in an individual tailored way. The identification of
23
24 objective biomarkers can both inform bipolar disorder diagnosis and provide biological targets for
25
26 the development of new and personalized treatments. Accurate diagnosis of bipolar disorder in its
27
28 early stages could help to prevent the long-term detrimental effects of the illness.
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31
32 The present Bipolar Onset study (BIO) study aims to identify 1) a composite blood-based biomarker
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34 2) a composite electronic smartphone-based biomarker and 3) a neurocognitive and neuroimaging
35
36 based signature for bipolar disorder.
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39 **Methods and analysis:** The study will include 300 patients with newly diagnosed/first episode
40
41 bipolar disorder, 200 of their healthy siblings or offspring, and 100 healthy individuals without a
42
43 family history of affective disorder. All participants will be followed longitudinally with repeated
44
45 blood samples and other biological tissues, self-monitored and automatically generated smartphone
46
47 data, neuropsychological tests and a subset of the cohort with neuroimaging during a 5-10 year
48
49 study period.
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52 **Ethics and dissemination:** The study has been approved by the Local Ethical Committee (H-7-
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54 2014-007) and the data agency, Capital Region of Copenhagen (RHP-2015-023).
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Introduction

Biomarkers in bipolar disorders

Bipolar disorder is a disabling mental illness with a lifetime prevalence of 1-2%, a high risk of recurrence of manic and depressive episodes, a lifelong elevated risk of suicide and a substantial heritability of 60-80% (1). Bipolar disorder is often conceptualised as a progressive disorder with increasing risk of recurrence for every new affective episode (2-5) and with increasing cognitive disabilities during the course of illness (6-9). Clinically, diagnostic boundaries between bipolar disorder and other psychiatric disorders such as unipolar disorder are unclear although some pharmacological and psychological treatment strategies differ substantially. Patients with bipolar disorder are often misdiagnosed as having from unipolar disorder, transient psychosis, reaction to stress/adjustment disorder or psychoactive substance abuse (10) and the mean delay between onset and diagnosis is 5-10 years (11). Although the risk of relapse of depression and mania is high it is for most patients impossible to predict and consequently prevent upcoming episodes in an individual tailored way. The identification of objective biomarkers as measures of pathophysiologic processes can both inform bipolar disorder diagnosis and provide biological targets for the development of new and personalized treatments (12). Accurate diagnosis of bipolar disorder in its early stages could help to prevent the long-term detrimental effects of the illness.

Recently, promising results have been presented regarding a diagnostic test for unipolar depression comprising levels of nine biomarkers in peripheral blood (13). Although the nature of bipolar disorder seems more biological driven than the nature of major depression with a higher heritability there has been no or few attempts to identify a similar composite biomarker for bipolar disorder.

Onset of illness and staging in bipolar disorder

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4 Although the course of illness is heterogeneous there is a body of evidence for clinical progression
5
6 on average of unipolar and bipolar disorders as increasing number of affective episodes seem
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8 associated with 1) increasing risk of recurrence 2) increasing duration of episodes 3) increasing
9
10 symptomatic severity of episodes 4) decreasing threshold for developing episodes 5) increasing risk
11
12 of developing dementia (9). It is likely that this clinical progression with deteriorating effects of
13
14 affective episodes and duration of illness is associated with neurobiological changes over the course
15
16 of illness. Unfortunately, results of all longitudinal studies on the biology of bipolar disorder are
17
18 hitherto hampered by three major limitations: 1) Only few studies have recruited patients with
19
20 bipolar illness from onset of the illness and most of these have used first onset mania as inclusion
21
22 criteria thereby excluding patients with a hypomanic episode (bipolar disorder, type II). As the
23
24 biology of bipolar disorder - based on cross-sectional studies - seem to change over the course of
25
26 illness from first episode to first relapse and recurrent relapses to an unremitting or rapid cycling
27
28 course (14-18), this is a major limitation in our knowledge internationally, 2) the number of patients
29
30 included in prior studies are less than 200, which is a limitation taking the heterogeneity of bipolar
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32 disorder into account (e.g. bipolar disorder type I and II may have different biology, etc.), and 3) the
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34 prospective follow-up period is less than a few years in all studies.
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40 In the BIO study we will establish a large cohort comprising of three sub cohorts that will be
41
42 followed long-term with systematic diagnostic, blood-based biomarkers, smartphone data, cognitive
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44 and brain imaging assessment. The three sub cohort will consist of: 1) patients with newly
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46 diagnosed/first episode bipolar disorder and 2) their healthy first-generation siblings and offspring
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48 3) healthy individuals without a family history of affective disorder.
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53 Overall aims:
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4 1. To identify a composite blood-based biomarker measure as well as a composite electronic
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6 smartphone-based biomarker from onset of bipolar disorder, during progression and in later
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8 stages
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11 2. To investigate if the composite blood-based biomarker measure and electronic smartphone-
12
13 based biomarker identified among patients with bipolar disorder predicts onset of illness
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15 (depression or mania) among these patients healthy first-generation siblings and offspring
16
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- 18 3. By applying an integrated systems approach, to identify patterns of “cerebral signatures”
19
20 across neuro circuitry and cognitive levels and to validate the composite blood-based
21
22 biomarker and electronic smartphone-based biomarkers against these bio-signatures
23
- 24 4. To investigate long-term developmental trajectories in neurocognitive function and brain
25
26 imaging from the high risk state to onset of bipolar disorder following first relapse and
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28 recurrent relapses and in the late stage with an unremitting, multi-episode or rapid cycling
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30 course.
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35 Methods and analysis

36 Overall methods

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38 The BIO study is a long-term cohort study started April 2015 and planned to include 300 newly
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40 diagnosed/first episode bipolar patients from the Copenhagen Affective Disorder Clinic, 200 of
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42 these patients’ healthy first-generation relatives and 100 healthy individuals *without* a first-
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44 generation family history of affective disorders.
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49 The Copenhagen Affective Disorder Clinic

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51 The Copenhagen Affective Disorder Clinic is a mood disorder clinic that provides treatment service
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53 for patients with newly diagnosed/first episode bipolar disorder (19). The Copenhagen Affective
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4 Disorder Clinic receives patients from the entire Capital Region of Denmark covering a catchment
5 area of 1.6 million people and all psychiatric centres in the region. All patients referred to the Clinic
6 as newly diagnosed/first episode patients, i.e., onset of first manic or hypomanic episode or when
7 the diagnosis of bipolar disorder is made for the first time, will routinely be asked for inclusion in
8 the BIO study.
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17 Recruitment of the three cohorts:
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20 1. 300 patients referred to the Copenhagen Affective Disorder Clinic as newly diagnosed/first
21 episode bipolar patients, i.e., onset of first manic or hypomanic episode or when the
22 diagnosis of bipolar disorder is made for the first time. The Clinic receives more than 100
23 newly diagnosed/first episode bipolar patients each year and we expect that nearly all will
24 accept participation in the BIO study as this implies an extensive clinical evaluation.
25
26
- 27 2. 200 first-generation relatives (siblings and children aged 15 to 40 years) to the recruited
28 newly diagnosed/first episode bipolar patients.
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- 31 3. 100 age- and gender-matched healthy individuals *without* a first-generation family history of
32 affective disorders recruited among blood donors from the Blood Bank at Rigshospitalet,
33 Copenhagen, as in prior studies.
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44 Diagnostic assessments at inclusion
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46 The initial diagnostic assessment will be done using the Structured Clinical Interview for DSM-IV-
47 TR Axis I Disorders (SCID (20)) categorizing patients in bipolar disorder type I or type II as part of
48 daily praxis by the experienced specialists in psychiatry during the patients two year stay in the
49 Copenhagen Affective Disorder Clinic. This clinical diagnosis of bipolar disorder will be confirmed
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4 in a semi-structured research based interview using the Schedules for Clinical Assessment in
5
6 Neuropsychiatry (SCAN) (21).
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10 11 12 13 Follow-up

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15 Besides the assessments at inclusion, patients will be assessed during remitted, depressive and
16
17 manic phases. Healthy control individuals will be assessed initially and every year during the first
18
19 four years and after this, every second year for five years.
20

21
22 As part of daily clinical praxis in Copenhagen Affective Disorder Clinic and as part of the BIO
23
24 study all patients will get access to a smartphone app for electronic continuous monitoring of illness
25
26 activity during a 5-year follow-up period (see sub-study BIO-2). Additionally, research assistants
27
28 will contact all participants regularly to identify upcoming episodes / onset of illness and to ensure
29
30 continued participation in the BIO study.
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33 It is estimated that patients will develop four to five affective episodes on average during the
34
35 follow-up period. It estimated that 20-30% of the healthy first-generation relatives will develop
36
37 onset of affective illness compared to 2% among the healthy individuals *without* a first-generation
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39 family history of affective disorders. Finally, linkage to Danish nation-wide register based data will
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41 be included for all individuals on psychiatric hospitalisations, prescribed medication and
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43 socioeconomic variables during the five years follow-up.
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48 49 Investigations

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51 We will include state of the art methods within clinical assessment as well as biomarkers including
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53 a range of new methods:
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4 1. Clinical assessments using the Hamilton Depression Scale-17 items (HAMD-17) (22), the
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6 Young Mania Rating Scale (YMRS) (23), the functional assessment short test (FAST) (a
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8 measure of psychosocial function) (24)
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- 10 2. Questionnaires including the Hypomania Check list (25), Standardized Assessment of
11
12 Personality - Abbreviated Scale (SAPAS) (26), the WHO (Five) well-being index (27) and
13
14 the Verona Satisfaction Scale-Affective Disorder (VSS-A) (28).
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- 16 3. Standardised fasting blood tests for a large number of potential biomarkers (see later
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18 section: BIO-1, Biological tests).
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- 20 4. Spot urine samples for oxidative generated damage to DNA and RNA
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- 22 5. Hair cortisol as a valid and reliable index of long-term systemic cortisol levels (29)
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- 24 6. Digital retinal imaging of the retinal venular caliber is a new, noninvasive method to
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26 visualize microcirculation in the eye, and seems to reflect vascular conditions in the brain as
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28 well as early decrease in neuropsychological functioning (30).
29
- 30 7. Daily electronic smartphone based self-monitoring of depressive and manic symptoms
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- 33 8. Daily electronic smartphone-based automatically generated data (e.g. data on phone usage,
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35 social activity and physical activity)
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- 38 9. Neuropsychological assessment (only during full or partial remission)
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- 40 10. Structural magnetic resonance imaging (MRI) focusing on prefrontal cortex and
41
42 hippocampus as well as functional MRI (fMRI) (only during full or partial remission).
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48 The BIO study includes four separate but interacting sub-studies (BIO-1, BIO-2, BIO-3, BIO-4), as
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50 illustrated in Table 1 with specified aims, background and theoretical basis, and methods, as
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52 described in the following.
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BIO-1: peripheral blood-based biomarker in bipolar disorder

Aims

To identify a composite blood-based biomarker that 1) discriminates bipolar disorder from healthy control individuals 2) discriminates between manic, depressive and remitted states 3) predicts emerging affective episodes, and 4) to validate the composite blood-based biomarker against the Smartphone-based biomarker, and the neurocognitive and brain imaging signature, and 5) to investigate the change in individual biomarkers as well as the composite blood-based biomarker following onset of first manic episode, during successive relapses and in the end late stages of the illness (15).

Background and theoretical basis

In a series of meta-analyses we concluded that although a number of candidate peripheral biomarkers related to neuroplasticity, inflammation, oxidative stress and gene expression seem promising, findings are limited by poor study designs, small cross sectional samples, lack of adjustment for important confounders related to most peripheral biomarkers and poor laboratory methodology (31-34). Because of high inter-individual variation in peripheral biomarkers, assessment of intra-individual alterations from onset of illness through different affective phases and into the late illness stage is necessary to identify clinically relevant and valid biomarkers, necessitating a longitudinal study design (32;35).

We have in two longitudinal studies with repeated assessments of patients with bipolar disorder during affective states (manic/mixed, depressive and euthymic) and healthy control individuals found that brain derived neurotrophic factor (BDNF) (36), increased oxidative DNA and RNA

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4 damage (37;38) and decreased mRNA expression of the PTGDS gene encoding the prostaglandin D
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6 synthase enzyme (39) are markers related to the illness trait in bipolar disorder. The level of the
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8 cytokines IL-6 and IL-18 was related to manic episodes only and the activity of GSK-3beta varied
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10 with affective states (40) suggesting that these may be state markers (41). These results have
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12 contributed to the research area of biomarkers in bipolar disorder, moving the area closer towards
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14 identifying clinically applicable biomarkers.

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17 Nevertheless, it is unlikely, that one single biomarker will provide a useful diagnostic tool; instead a
18
19 composite of several relevant biomarkers appears a more viable approach (42). Recently promising
20
21 results have been presented regarding a diagnostic test in unipolar depression comprising serum
22
23 levels of nine individual biomarkers in peripheral blood (13). Similarly, preliminary studies have
24
25 suggested composite biomarkers for bipolar disorder (43-45). We identified a composite biomarker
26
27 consisting of gene expression from 19 candidate genes for bipolar disorder that accurately
28
29 discriminated bipolar patients from healthy control individuals (43). Thus, such approaches highly
30
31 increase the chances of obtaining a high specificity and sensitivity of the composite blood-based
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33 biomarker (43-45).
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37 In order to establish relevant markers of risk and markers related to illness stage, it is necessary to
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39 include assessment before onset of illness and during first- and recurrent relapses and in the late
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41 stages of the illness according to the staging model of bipolar disorder (46). Further, the study of
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43 early onset individuals is necessary to evaluate biomarker levels without influence from medication,
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45 which may otherwise limit the validity of identified biomarkers.
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49 50 Methods

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52 BIO-1 will include repeated clinical assessments and corresponding samples of blood and other
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54 tissues among the 300 newly diagnosed/first episode bipolar patients, the 200 first-generation
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4 relatives and the 100 healthy individuals *without* a first-generation family history of affective
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6 disorder.

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8 We will estimate a composite blood-based biomarker based on a number of individual markers
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10 including brain-derived neurotrophic factor (BDNF), neutrophin-3 (NT-3), 5 different cytokines,
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12 gene expression, additional thirty candidate genes and other potential biomarkers (see Biological
13
14 tests), and identify the composite biomarker that correlates best with affective states and Hamilton
15
16 Depression Rating Scale and Young Mania Rating Scale scores of depression and mania,
17
18 respectively. A final blood-based biomarker will be chosen based on its ability to 1) discriminate
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20 patients with bipolar disorder from healthy control individuals and to 2) discriminate between
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22 manic, depressive and remitted states in bipolar disorder.
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28 Laboratory procedures

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30 We will obtain careful standardization of blood sampling and laboratory analysis by obtaining
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32 blood samples in a fasting state and in a one hour interval in the morning. At the same day as blood
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34 sampling, smoking status, alcohol use, BMI and menstrual cycle, etc., will be assessed. Blood
35
36 sampling and all phases of laboratory processing for plasma and DNA/RNA analyses will be done
37
38 at the Department of Clinical Biochemistry, Rigshospitalet using standard operational methods
39
40 conducted by a team of technicians blinded with respect to participant status. All plasma samples
41
42 will be stored at – 80 C. The BIO study will include a total of 2400 blood samples: 300 patients x 5
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44 + 200 healthy first-generation relatives x 3 + 100 healthy participants without a first-generation
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46 family history x 3.
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53 Biological tests

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4 We will use a multianalyte panel including a large number of potential biomarkers such as plasma
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6 levels of Neutrophins3, GSK-3, β -amyloid A β 40 and A β 42, inflammatory markers, high sensitive
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8 C-reactive protein, lipoproteins (VLDL, LDL, HDL) and specific apolipoproteins (e.g. apoE,
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10 ApoA-I, apoA-II, and apoM) as potential markers of low-grade inflammation particularly salient in
11
12 the early stages of bipolar disorder. Total RNA, microRNA, genomic DNA and histones are
13
14 isolated from peripheral blood mononuclear cells. Gene expression and alternative slicing of RNA
15
16 transcripts are analysed using array reverse transcription quantitative real-time PCR (RT-qPCR) and
17
18 next generation sequencing. Epigenetic modifications of the DNA (e.g. methylation) are measured
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20 using antibody based methods or bisulphite treatments in combination with next generation
21
22 sequencing. The genomic positions of histones with specific modifications are detected using
23
24 immune precipitation and sequencing (CHIP-Seq). The degree of histone modifications is measured
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26 by semi-quantitative antibody based detection.
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30 Measurements of DNA and RNA damage by oxidation are obtained from spot urine samples and
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32 analyzed using ultra performance liquid chromatographic (UPLC) and mass spectrometry.
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35 Hair cortisol will be included as a valid and reliable index of long-term systemic cortisol levels
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37 (29).
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40 41 Statistical analyses

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43 Data represent repeated measures within and between individuals and will be analysed using a
44
45 combination of generalized linear mixed models, integrated data analysis, and penalized regression
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47 approaches to facilitate the combined feature selection and prediction of the available high-
48
49 dimensional data. Integrative data analysis ensures that we are able to identify an improved
50
51 composite blood-based biomarker since data from different molecular levels are combined in a
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53 simultaneous analysis that closer resembles the biological system (47;48). Further, we will use cross
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4 validation or alternatively split sample designs in the development and validation of the composite
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6 biomarker.
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10 Statistical power

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12 The study has a power of 80% to detect a minimum increment of 6.5 percentage points in sensitivity
13 if we assume that the existing diagnostic tools have a sensitivity of 70% to diagnose bipolar
14 disorder for a patient that has the disorder (see (49)). Thus, if the composite biomarker score
15 increases the sensitivity by a minimum of 6.5 percentage points then we have a power of 80% to
16 detect that increase based on 300 patients with bipolar disorder using a one-sided exact binomial
17 test (for fixed specificity).
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28 **BIO-2: Smartphone-based electronic biomarker in bipolar disorder**

29 Aims

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31 To identify a composite smartphone-based electronic biomarker that 1) discriminates patients with
32 bipolar disorder from healthy control individuals 2) discriminates between manic, depressive and
33 remitted states 3) predicts emerging affective episodes, and 4) to investigate the change in the
34 composite smartphone-based biomarker following onset of first manic episode, during successive
35 relapses and in the end stages of the illness as according to the staging system by Berk et al (15).
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46 Background and theoretical basis

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48 Recently, electronic self-monitoring of the severity of depressive and manic symptoms using text
49 messages has been suggested as an easy and inexpensive way to identify early signs of affective
50 episodes, providing opportunities for mental health care providers to intervene shortly after
51 prodromal symptoms first appear (50). We have, in the MONARCA project developed and tested a
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4 smartphone-based electronic monitoring system including daily subjective self-assessments of
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6 illness activity in bipolar disorder as well as a bi-directional feedback loop between the patient and
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8 clinicians (the MONARCA system (51-55)). Using the MONARCA system, fine-grained electronic
9
10 data were collected during everyday life in naturalistic settings in patients with bipolar disorder.
11
12 The MONARCA system was reported highly usable and useful by patients with bipolar disorder
13
14 with a high self-assessment adherence (87-95%), and the patients reported that the MONARCA
15
16 system helped them to better manage their disease (51;52). Further, the severity of depressive and
17
18 manic symptoms was found to correlate with automatically generated smartphone data including 1)
19
20 physical activity as reflected by the number of changes in cell tower ID per day (56), 2) social
21
22 activity as reflected by the number of incoming and outgoing calls per day, the duration of
23
24 incoming and outgoing calls per day, and the number of outgoing text messages per day (57), and 3)
25
26 voice features collected during phone calls (58). Although these findings are encouraging there is a
27
28 need to integrate self-monitored smartphone data with automatically generated smartphone data on
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30 social and physical activity, speech and sleep into one composite smartphone generated electronic
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32 biomarker measure. This composite measure should be modeled to 1) discriminate patients with
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34 bipolar disorder from healthy control individuals 2) have a high correlation with depressive and
35
36 manic symptoms 3) discriminate between euthymic, manic and depressive states 4) early predict
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38 emerging affective episodes for the individual patient to increase the possibility for early
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40 intervention.
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48 Methods:

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50 All newly diagnosed/first episode patients will have access to a smartphone based system
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52 (Monsenso that is a developed from the MONARCA system) for continuous self-monitoring as well
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54 as fine-grained automatically monitoring of behavioural activity and early identification of
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4 emerging affective episodes during the first two years and following relapse of episodes during a 5-
5
6 year follow-up period.
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9 Data analyses: In contrast to data in BIO-1, data in BIO-2 represent big data collected on a *daily* basis
10 within individuals. We will use hierarchical Bayesian predictive models that can handle big data through
11 sampling and visualization techniques that summarize data.
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14 15 16 17 **BIO-3: neurocognitive and brain imaging signatures in bipolar disorder**

18 19 Aims

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21 1) To identify an integrated brain-based biomarker of bipolar disorder including neurocognitive and
22 neuroimaging measures tapping into ‘hot’ (i.e. emotion-laden) and ‘cold’ (non-emotional)
23 cognition, 2) to examine whether the degree of abnormality in these measures predicts illness
24 onset in the high-risk group and/or relapse in the patient group, 3) to identify developmental
25 trajectories in ‘hot’ and ‘cold’ cognitive dysfunction and to identify structural and functional MRI
26 correlates in bipolar disorder via longitudinal assessments of high-risk individuals to remission after
27 onset of first manic episode and following successive relapses, and 4) to identify associations
28 between aberrant ‘hot’ and ‘cold’ neurocognitive function, structural and functional brain changes
29 and the composite blood-based and Smartphone-based biomarkers. Such ‘integrated systems
30 approach’ involving identification of patterns of biomarkers (bio-signatures) across these multiple
31 levels of investigation is considered imperative for deeper understanding of the dimensions of
32 underlying pathophysiological processes in bipolar disorder (12).
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50 51 Background and theoretical basis

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53 Results from a number of meta-analyses of a large number of cross sectional studies of patients with
54 bipolar disorder in remission suggest trait-related ‘cold’ cognitive deficits in attention/processing
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4 speed, memory, and executive function compared to healthy controls (59-62) that correlate with
5
6 everyday functioning (63). Cross-sectional comparison of patients at different illness stages
7
8 revealed more pronounced cognitive deficits during late compared with early stages in line with the
9
10 staging hypothesis of bipolar disorder (64). However, there are only a few longitudinal studies of
11
12 neuropsychological functioning with small sample sizes (12 studies including a total of 152 bipolar
13
14 patients (65)). A meta-analysis of these studies found no support for a progressive nature of
15
16 cognitive deficits (65). However, results from these studies are hampered by a number of limitations
17
18 including small sample sizes, short follow-up (mean follow-up period of 4.6 years) and high
19
20 attrition rates (up to 45%) (65). Consequently, it is unclear whether cognitive function assessed with
21
22 neuropsychological tests deteriorate with the number and duration of illness episodes in bipolar
23
24 disorder although epidemiological studies consistently revealed increased risk of developing
25
26 dementia long-term (6;8;66-68) and there are some evidence for increasing risk of dementia with
27
28 the number of episodes (7) (see also (9)).

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33 Deficits in 'hot' cognition are closely linked to emotional disturbances (69) and difficulties in socio-
34
35 emotional behaviour and interpersonal relations in bipolar disorder (70). 'Hot' cognition
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37 abnormalities in bipolar disorder have been observed within three domains; (i) emotional
38
39 processing, (ii) reward processing and (iii) emotion regulation (reviews in (71;72)).

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42 Results from a large number of cross sectional structural imaging studies suggest that patients also
43
44 show increased lateral ventricular volumes and greater prevalence of white matter hyper-intensities
45
46 (73). While these findings are rather unspecific, studies also suggest that treatment with lithium
47
48 increases the grey matter volume of prefrontal cortex, amygdala and hippocampus (73). In addition,
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50 a number of functional imaging studies suggest that bipolar disorder is associated with
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52 abnormalities within fronto-limbic-subcortical structures (35).
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4 As long-term, integrative studies are lacking, it is unclear how neurocognitive and brain imaging
5 abnormalities correlate with the staging of bipolar disorder, illness progression and treatment
6 (35;74) or with changes in biological markers such as neurotropic, inflammatory and oxidative
7 stress markers (75;76).
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14 Methods

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16 Using a comprehensive neurocognitive test battery, we will assess all participants from the three
17 groups (patients with bipolar disorder, first-generation relatives, and healthy individuals *without* a
18 family history of affective disorders). Patients will be followed from first onset of affective disorder
19 and during successive periods of remission or at an annual basis (bipolar patients with no relapse,
20 first-generation relatives with no onset, and healthy controls. Among these, a subgroup of 60
21 patients, 60 healthy relatives (UR) and 30 healthy without a family history of affective disorders
22 (HC) will be undergo functional and structural MRI at these time points
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35 Neurocognitive testing

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37 Within ‘cold’ cognition verbal learning/memory and executive function have been highlighted as
38 the most suitable candidates for biomarkers of bipolar disorder (77;78). ‘Cold’ cognition will
39 therefore be assessed with neurocognitive tests probing verbal memory, attention and executive
40 function including the Rey Auditory Verbal Learning Test (RAVLT; (79;80)) and the WAIS-III
41 letter-number sequencing, RBANS digit span, n-back working memory, verbal fluency and Trail
42 Making Test B. Verbal intelligence will be estimated with the Danish Adult Reading Test (DART).
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‘Hot’ cognition will be assessed with a comprehensive battery of computerized neurocognitive tests
outside the scanner probing (i) emotional processing (ii) reward processing and (iii) emotion
regulation. These include the facial expression recognition and faces dot-probe tasks from the

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4 Emotional Test Battery (ETB) (P1Vital, Oxford), and an ecologically valid social scenarios test
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6 developed by our group (81). During fMRI we will also administer the following experimental
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8 paradigms: an emotional face processing task using face stimuli from the Nimstim
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10 (<http://www.macbrain.org/resources.htm>), a monetary reward processing task (82), and (iii) a
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12 negative affective picture task using validated stimuli sets from the International Affective Picture
13
14 System (IAPS) developed in collaboration with researchers at the Universities of Chicago. In
15
16 addition, we will explore the neuronal basis for ‘cold’ cognition (executive function) using n-back
17
18 working memory and picture encoding tasks programmed in house (83). Finally, self-report
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20 measures (BIS/BAS, and the CERQ (84)) are used to assess reward responsiveness and habitual
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22 emotional regulation strategies.
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30 Structural and functional MRI

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32 Structural MRI: using T1-weighted images acquired at a 3T Siemens scanner at the Copenhagen
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34 University Hospital, Rigshospitalet, we will focus on lateral ventricular volumes, grey matter
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36 volume of prefrontal cortex, amygdala and hippocampus, relative to whole brain volume.
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38 Specifically, segmentation and analysis of subcortical and regional cortical volume, shape and grey
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40 matter density will be conducted FMRIB Software Library (FSL) tools, including the FMRIB's
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42 Integrated Registration and Segmentation Tool (FIRST), the FSL-VBM tool and FSL vertex (shape)
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44 analysis (<http://fsl.fmrib.ox.ac.uk/>).
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48 Functional MRI (fMRI): T2-weighted images will be acquired to investigate white matter hyper-
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50 intensities. We will also use fMRI to investigate neuronal underpinnings of ‘hot’ and ‘cold’
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52 cognition with the previously described experimental paradigms. Functional MRI data processing
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54 will be carried out with the FMRI Expert Analysis Tool, part of FMRIB's Software Library
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(www.fmrib.ox.ac.uk/fsl). We will examine mean percent BOLD signal change within predefined hippocampal and amygdala Regions of Interest (ROIs) obtained in standard space with mri3dX (<http://www.idoimaging.com/program/160>). In addition, whole-brain exploratory analysis will be conducted to explore neural activity differences in other cortical regions. For this group analysis, individual contrasts of interest will be included in separate general linear models with nonparametric permutation inference (n = 5000) using the ‘randomize’ algorithm implemented in FSL (85).

Statistical power

The above sample size for participants undergoing fMRI assessments is determined based on our previous fMRI studies. In particular, inclusion of about 17-22 participants per treatment/diagnostic group (matched for age and gender) had a power of >0.8 to detect differences between groups in neural and cognitive response to emotional faces (e.g. (86;87) and Miskowiak et al, under review) at an alpha-level of $p < 0.05$ for cross-sectional designs. For longitudinal designs, we were able to demonstrate differences between groups in *the change* in task-related neural activity and in hippocampal structure with a sample of about 40 participants per group (88-90). Given this, our inclusion of 60 participants per group is expected to ensure adequate statistical power for both the cross-sectional and longitudinal parts of the fMRI study.

BIO-4: at risk or prodromal phase for bipolar disorder

Aims

To test whether 1) the composite blood-based biomarker, 2) the composite electronic smartphone-based biomarker and 3) the neurocognitive signature for bipolar disorder predict onset of illness

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4 (depression or mania) among a healthy (i.e., non-syndromal level) high risk population of first
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6 degree siblings and offspring to the patients with newly diagnosed/first episode mania/bipolar
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8 disorder, included in BIO-1, BIO-2 and BIO-3.
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10 Background and theoretical basis: First-generation relatives to patients with bipolar disorder have a
11
12 nine-fold increased risk of developing bipolar disorder and a two to three-fold increased risk of
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14 developing unipolar disorder (1). Although there is a great need for early detection and primary
15
16 prevention of onset of illness among relatives to patients with bipolar disorder recent prior attempts
17
18 have not been successful due to retrospective designs, poor characterisation of high risk individuals
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20 and small sample sizes (91). This is the first time a composite blood-based, a composite
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22 smartphone-based and a composite neurocognitive biomarker identified among patients with bipolar
23
24 disorder will be investigated among their healthy relatives as a predictor measure of onset of illness
25
26 (18). This approach increases the chances of obtaining a high specificity and sensitivity of the
27
28 composite biomarkers.
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35 Methods

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37 This BIO-4 includes first-generation relatives (siblings and offspring aged 15 to 40 years) to the
38
39 recruited patients with first manic or bipolar diagnosis. We expect that at least 200 individuals will
40
41 be asymptomatic or present mild symptoms or prodromal patterns to affective disorders and will be
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43 included in the study. Biological tissues will be drawn (as part of BIO-1) and neurocognitive
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45 function will be assessed on all individuals and brain imaging will be done on 60 individuals with
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47 longitudinal assessments.
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54 Feasibility

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4 The BIO study is fully feasible as patients are recruited as part of daily health care for patients
5 referred to the Copenhagen Affective Disorder Clinic.
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10 Ethical considerations

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12 The BIO study has been approved by the Local Ethical Committee (H-7-2014-007) and the data
13 agency, Capital Region of Copenhagen (RHP-2015-023). The study complies with the Declaration
14 of Helsinki principles (Seoul, October 2008).
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20 Dissemination

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22 Study results will be presented in peer reviewed journals and at international conferences in
23 accordance with relevant reporting guidelines (92;93).
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30 Discussion

31 Summary

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33 It is expected that the BIO cohort will provide valid biological, electronic, neurocognitive and
34 neuroimaging data and for the first time longitudinally identify changes in biomarkers during
35 different stages of bipolar illness, i.e., at risk stage, following onset, during first relapse and
36 recurrent relapses and in the late stages of the illness as according to Berk et al (15).
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50 Limitations

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52 Some limitations of the BIO study should be noted beforehand. First, the rather extensive initial
53 assessment study procedure may potentially result in selection of participants that are intrinsically
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4 positive towards clinical research and readily willing to cooperate. Nevertheless, we expect that
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6 such selection will be decreased as it is likely that the vast majority of the more than 100 newly
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8 diagnosed/first episode bipolar patients referred to the Copenhagen Affective Disorder Clinic each
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10 year will accept participation in the BIO study as this implies an extensive clinical evaluation.
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12 Second, attrition may increase during long-term follow-up and patients who stay in the study may
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14 adhere more to treatment in general. Such selection is inherent in clinical longitudinal research, and
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16 the large number of participants that will be included will increase external validity. Third, potential
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18 confounding effects of psychotropic medication may influence comparisons between patients with
19
20 bipolar disorder and healthy control individuals as well as comparisons within patients as the vast
21
22 majority of patients will get medication that may change during the course of illness. Lithium,
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24 mood stabilizers and antipsychotics may have effects on the collected biological, smartphone-based,
25
26 neuropsychological and brain imaging data. Effects of medication on biological measures are not
27
28 clear (94) although analyses from systematic reviews and meta-analyses involving only bipolar
29
30 disorder patients have not found clear effects of medication on cytokines (31;32), BDNF (34), gene
31
32 expression (33) nor has subsequent individual studies on cytokines (41;95), BDNF (36;95), gene
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34 expression (43) or DNA and RNA damage (37;38). Effects of medication on electronic smartphone
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36 generated data as well as on neuropsychological and brain imaging data are poorly investigated and
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38 warrants further studies (96).
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46 Fourth, due to the large number of biological and statistical tests included in the BIO study, chance
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48 findings may occur in relation to the individual biological test. However, the aim of the BIO study
49
50 is to identify a composite biomarker measure related to bipolar illness, depression and mania using
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52 cross validation or alternatively split sample designs in the development and validation of the
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54 composite biomarker.
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Finally, the BIO study does not include (randomised) interventions limiting casual interpretations of the results. Nevertheless, with the BIO prospective, repeated measures design it is possible to identify valid associations between the composite measures (of biological, electrical, neuropsychological and brain imaging data) and depressive and manic symptoms and states.

Strengths

The BIO study is the first study aiming to identify 1) a composite blood-based biomarker, 2) a composite electronic smartphone-based biomarker and 3) a neurocognitive signature for bipolar disorder as well as to measure the same biomarkers in newly diagnosed/first episode bipolar patients and their healthy first-generation relatives. It is possible to recruit newly diagnosed/first episode patients with mania/bipolar as all such patients from the entire Capital Region of Denmark are referred to the Copenhagen Affective Disorder Clinic and routinely asked for inclusion in the BIO study. Long-term attrition is supposed to be low as all patients will be followed by the Copenhagen Affective Disorder Clinic for the first two years and subsequently in other treatment settings in the Capital Region of Denmark. Including longitudinal assessment of healthy individuals is of paramount importance to control for the effect of timing and ageing (97), but among all studies on biomarkers in bipolar disorder, this has been done only in two studies from our group (37;41). The study will be performed by an experienced international research group, combining expertise from all areas of the study.

Conclusion

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4 The BIO study is a large long-term cohort study on biomarkers in bipolar disorder and we expect
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6 that the findings for the first time will be representative of biomarkers in bipolar disorder in general
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8 as no prior study on newly diagnosed/first episode bipolar disorder has been conducted. It is
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10 expected that the BIO cohort will provide valid biological, electronically, neuropsychological and
11
12 brain imaging longitudinal data.
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21 Authors' contribution

22 LVK designed the study together with MV, KM, KWM and MFJ. LVK drafted the study protocol
23 and the manuscript. All authors contributed to development of the study protocol and to editing the
24 manuscript and read and approved the final version.

25 KM, LBN, RFS, CE, BKP, HEP, RSM, FK, WFG, and MV contributed specifically to BIO-1 on
26 peripheral blood-based biomarkers. MFJ, OW, JB, MF and OM contributed specifically to BIO-2
27 on smartphone-based electronic biomarkers. KWM, GMK and MP contributed specifically to BIO-
28 3 on neurocognitive and brain imaging signatures. MV contributed specifically to BIO-4 on at risk
29 or prodromal phase of bipolar disorder (in addition to BIO-1).
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33 Funding

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35 Danish Council for Independent Research, Medical Sciences (DFF – 4183-00570), Weimans Fund,
36 Markedmodningsfonden (the Market Development Fund, (2015-310), Gangstedfonden (A29594),
37 Helsefonden (16-B-0063), Innovation Fund Denmark (the Innovation Fund, Denmark, 5164-
38 00001B), Copenhagen Center for Health Technology (CACHET), EU H2020 ITN (EU project
39 722561), Augustinusfonden (16-0083), Lundbeck foundation (R215-2015-4121).
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42 Competing interests

43 LVK has within the preceding three years been a consultant for Lundbeck, AstraZeneca and
44 Sunovion. KWM has received consultancy fees in the past three years from Lundbeck and Allergan.
45 MFJ has been a consultant for Eli Lilly and Lundbeck. MV has within the preceding three years
46 been a consultant for Astra Zeneca and Servier. FK has been a speaker for Ache, Daiichi Sankyo
47 and Janssen. MP is a consultant for Roche Pharmaceuticals.

48 RSM: Advisory Boards: Lundbeck, Pfizer, AstraZeneca, Elli-Lilly, Janssen, Ortho Purdue, Johnson
49 & Johnson, Moksha8, Sunovion, Mitsubishi, Takeda, Forest, Otsuka, Bristol-Myers Squibb, Shire,
50 Speakers Fees: Lundbeck, Pfizer, AstraZeneca, Elli-Lilly, JanssenOrtho, Purdue, Johnson &
51 Johnson, Moksha8, Sunovion, Mitsubishi, Takeda, Forest, Otsuka, Bristol-Myers Squibb, Shire,
52 Research Grants: Lundbeck, JanssenOrtho, Shire, Purdue, AstraZeneca, Pfizer, Otsuka, Allergan.
53 HEP has received a research Grant from Boeringer Ingelheim. GMK has received honoraria as
54 Field Editor of the International Journal of Neuropsychopharmacology and as scientific advisor for
55 H Lundbeck A/S. JEB and MF are co-founders and shareholders in Monsenso.
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4 LBN, RFS and WFG declare no competing interests.
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6 The validity of the research cannot be influenced by any of these potential secondary interest (such
7 as financial gain or personal relationship).
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10 Patient consent
11 Obtained

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13 Ethical approval
14 The study has been approved by the Local Ethical Committee (H-7-2014-007) and the data agency,
15 Capital Region of Copenhagen (RHP-2015-023).
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18 Study registration
19 The BIO study has been registered at clinicaltrials.gov with Trial Registration Number:
20 NCT02888262.
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Table 1. Overview of the longitudinal assessments during risk periods and following onset of bipolar disorder in the four sub-studies of the BIO study.

Course	Healthy first-gen. relatives	First episode	Remission	First relapse	Remission	Second relapse	Etc.
BIO-1	x	x	x	x	x	x	x
BIO-2	x	x	x	x	x	x	x
BIO-3	x		x		x		x
BIO-4	x	x	x	x	x	x	x

BIO-1: Peripheral blood-based biomarker in bipolar disorder

BIO-2: Smartphone-based electronic biomarker in bipolar disorder

BIO-3: Neurocognitive and brain imaging signatures in bipolar disorder

BIO-4: At risk or prodromal phase for bipolar disorder

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The Bipolar Illness Onset study - research protocol for the BIO cohort study

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Manuscripts

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The Bipolar Illness Onset study - research protocol for the BIO cohort study

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4 **Introduction:** Bipolar disorder is an often disabling mental illness with a lifetime prevalence of 1-
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6 2%, a high risk of recurrence of manic and depressive episodes, a lifelong elevated risk of suicide
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8 and a substantial heritability. The course of illness is frequently characterised by progressive
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10 shortening of inter-episode intervals with each recurrence and increasing cognitive dysfunction in a
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12 subset of individuals with this condition. Clinically, diagnostic boundaries between bipolar disorder
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14 and other psychiatric disorders such as unipolar depression are unclear although pharmacological
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16 and psychological treatment differs substantially. Patients with bipolar disorder are often
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18 misdiagnosed and the mean delay between onset and diagnosis is 5-10 years. Although the risk of
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20 relapse of depression and mania is high it is for most patients impossible to predict and
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22 consequently prevent upcoming episodes in an individual tailored way. The identification of
23
24 objective biomarkers can both inform bipolar disorder diagnosis and provide biological targets for
25
26 the development of new and personalized treatments. Accurate diagnosis of bipolar disorder in its
27
28 early stages could help to prevent the long-term detrimental effects of the illness.
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32 The present Bipolar Onset study (BIO) study aims to identify 1) a composite blood-based biomarker
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34 2) a composite electronic smartphone-based biomarker and 3) a neurocognitive and neuroimaging
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36 based signature for bipolar disorder.
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39 **Methods and analysis:** The study will include 300 patients with newly diagnosed/first episode
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41 bipolar disorder, 200 of their healthy siblings or offspring, and 100 healthy individuals without a
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43 family history of affective disorder. All participants will be followed longitudinally with repeated
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45 blood samples and other biological tissues, self-monitored and automatically generated smartphone
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47 data, neuropsychological tests and a subset of the cohort with neuroimaging during a 5-10 year
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49 study period.
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52 **Ethics and dissemination:** The study has been approved by the Local Ethical Committee (H-7-
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54 2014-007) and the data agency, Capital Region of Copenhagen (RHP-2015-023).
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Trial Registration Number: NCT02888262.

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Introduction

Biomarkers in bipolar disorders

Bipolar disorder is a disabling mental illness with a lifetime prevalence of 1-2%, a high risk of recurrence of manic and depressive episodes, a lifelong elevated risk of suicide and a substantial heritability of 60-80% (1). Bipolar disorder is often conceptualised as a progressive disorder with increasing risk of recurrence for every new affective episode (2-5) and with increasing cognitive disabilities during the course of illness (6-9). Clinically, diagnostic boundaries between bipolar disorder and other psychiatric disorders such as unipolar disorder are unclear although some pharmacological and psychological treatment strategies differ substantially. Patients with bipolar disorder are often misdiagnosed as having from unipolar disorder, transient psychosis, reaction to stress/adjustment disorder or psychoactive substance abuse (10) and the mean delay between onset and diagnosis is 5-10 years (11). Although the risk of relapse of depression and mania is high it is for most patients impossible to predict and consequently prevent upcoming episodes in an individual tailored way. The identification of objective biomarkers as measures of pathophysiologic processes can both inform bipolar disorder diagnosis and provide biological targets for the development of new and personalized treatments (12). Accurate diagnosis of bipolar disorder in its early stages could help to prevent the long-term detrimental effects of the illness.

Recently, promising results have been presented regarding a diagnostic test for unipolar depression comprising levels of nine biomarkers in peripheral blood (13). Although the nature of bipolar disorder seems more biological driven than the nature of major depression with a higher heritability there has been no or few attempts to identify a similar composite biomarker for bipolar disorder.

Onset of illness and staging in bipolar disorder

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4 Although the course of illness is heterogeneous there is a body of evidence for clinical progression
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6 on average of unipolar and bipolar disorders as increasing number of affective episodes seem
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8 associated with 1) increasing risk of recurrence 2) increasing duration of episodes 3) increasing
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10 symptomatic severity of episodes 4) decreasing threshold for developing episodes 5) increasing risk
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12 of developing dementia (9). It is likely that this clinical progression with deteriorating effects of
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14 affective episodes and duration of illness is associated with neurobiological changes over the course
15
16 of illness. Unfortunately, results of all longitudinal studies on the biology of bipolar disorder are
17
18 hitherto hampered by three major limitations: 1) Only few studies have recruited patients with
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20 bipolar illness from onset of the illness and most of these have used first onset mania as inclusion
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22 criteria thereby excluding patients with a hypomanic episode (bipolar disorder, type II). As the
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24 biology of bipolar disorder - based on cross-sectional studies - seem to change over the course of
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26 illness from first episode to first relapse and recurrent relapses to an unremitting or rapid cycling
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28 course (14-18), this is a major limitation in our knowledge internationally, 2) the number of patients
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30 included in prior studies are less than 200, which is a limitation taking the heterogeneity of bipolar
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32 disorder into account (e.g. bipolar disorder type I and II may have different biology, etc.), and 3) the
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34 prospective follow-up period is less than a few years in all studies.
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40 In the BIO study we will establish a large cohort comprising of three sub cohorts that will be
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42 followed long-term with systematic diagnostic, blood-based biomarkers, smartphone data, cognitive
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44 and brain imaging assessment. The three sub cohort will consist of: 1) patients with newly
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46 diagnosed/first episode bipolar disorder and 2) their healthy first-generation siblings and offspring
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48 3) healthy individuals without a family history of affective disorder.
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53 Overall aims:
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4 1. To identify a composite blood-based biomarker measure as well as a composite electronic
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6 smartphone-based biomarker from onset of bipolar disorder, during progression and in later
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8 stages
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11 2. To investigate if the composite blood-based biomarker measure and electronic smartphone-
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13 based biomarker identified among patients with bipolar disorder predicts onset of illness
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15 (depression or mania) among these patients healthy first-generation siblings and offspring
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- 18 3. By applying an integrated systems approach, to identify patterns of “cerebral signatures”
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20 across neuro circuitry and cognitive levels and to validate the composite blood-based
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22 biomarker and electronic smartphone-based biomarkers against these bio-signatures
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- 24 4. To investigate long-term developmental trajectories in neurocognitive function and brain
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26 imaging from the high risk state to onset of bipolar disorder following first relapse and
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28 recurrent relapses and in the late stage with an unremitting, multi-episode or rapid cycling
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30 course.
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33 5. To investigate whether the course of illness is progressive on average in bipolar disorder and
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35 to identify corresponding changes in biomarkers during the course of illness within BIO-1 to
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37 BIO-4.
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42 Methods and analysis

43 Overall methods

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46 The BIO study is a long-term cohort study started April 2015 and planned to include 300 newly
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48 diagnosed/first episode bipolar patients from the Copenhagen Affective Disorder Clinic, 200 of
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50 these patients’ healthy first-generation relatives and 100 healthy individuals *without* a first-
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52 generation family history of affective disorders.
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4 The Copenhagen Affective Disorder Clinic

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6 The Copenhagen Affective Disorder Clinic is a mood disorder clinic that provides treatment service
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8 for patients with newly diagnosed/first episode bipolar disorder (19). The Copenhagen Affective
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10 Disorder Clinic receives patients from the entire Capital Region of Denmark covering a catchment
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12 area of 1.6 million people and all psychiatric centres in the region. All patients referred to the Clinic
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14 as newly diagnosed/first episode patients, i.e., onset of first manic or hypomanic episode or when
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16 the ICD-10 diagnosis of bipolar disorder is made for the first time, will routinely be asked for
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18 inclusion in the BIO study.
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24 Recruitment of the three cohorts:

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26 1. 300 patients referred to the Copenhagen Affective Disorder Clinic (aged 15-70 years) as
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28 newly diagnosed/first episode bipolar patients, i.e., onset of first manic or hypomanic
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30 episode or when the diagnosis of bipolar disorder is made for the first time. The Clinic
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32 receives more than 100 newly diagnosed/first episode bipolar patients each year and we
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34 expect that nearly all will accept participation in the BIO study as this implies an extensive
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36 clinical evaluation.
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39 2. 200 first-generation relatives (siblings and children aged 15 to 40 years) to the recruited
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41 newly diagnosed/first episode bipolar patients.
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44 3. 100 age- and gender-matched healthy individuals *without* a first-generation family history of
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46 affective disorders recruited among blood donors from the Blood Bank at Rigshospitalet,
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48 Copenhagen, as in prior studies.
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53 Diagnostic assessments at inclusion
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4 The initial diagnostic assessment will be done using the Structured Clinical Interview for DSM-IV-
5 TR Axis I Disorders (SCID (20)) categorizing patients in bipolar disorder type I or type II as part of
6 daily praxis by the experienced specialists in psychiatry during the patients two year stay in the
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The initial diagnostic assessment will be done using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID (20)) categorizing patients in bipolar disorder type I or type II as part of daily praxis by the experienced specialists in psychiatry during the patients two year stay in the Copenhagen Affective Disorder Clinic. This clinical diagnosis of bipolar disorder will be confirmed in a semi-structured research based interview using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) providing an ICD-10 diagnosis (21).

Follow-up

Besides the assessments at inclusion, patients will be assessed during remitted, depressive and manic/mixed phases. Patients and healthy control individuals will be face to face assessed initially and at least every year during the first four years and after this, every second year for five years. As part of daily clinical praxis in Copenhagen Affective Disorder Clinic and as part of the BIO-2 sub-study all patients will get access to a smartphone app for electronic continuous monitoring of illness activity during a 5-year follow-up period (see sub-study BIO-2). Additionally, research assistants will contact all participants every third months to identify upcoming episodes / onset of illness and to ensure continued participation in the BIO study. At each assessment the present, clinical state (remission, manic, hypomanic, depressive, mixed episode) of all participants will be established according to ICD-10. The severity of depressive and manic symptoms (if present) will be assessed using the 17-item Hamilton Depression Rating Scale (HAMD-17) (22) and the Young Mania Rating Scale (YMRS) (23) with a time period of three days applied. Remission is defined as score of ≤ 7 on the HAMD-17 and the YMRS. In this way upcoming episodes and onset of illness (for healthy individuals) will be assessed with great certainty.

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4 Based on prior findings (2;3;19), it is estimated that patients will develop four to five affective
5
6 episodes on average during the follow-up period, i.e., relapse or recurrence, defined as above. It
7
8 estimated that 20-30% of the healthy first-generation relatives will develop onset of affective illness
9
10 compared to 2-5% among the healthy individuals *without* a first-generation family history of
11
12 affective disorders (24;25). Finally, linkage to Danish nation-wide register based data will be
13
14 included for all individuals on psychiatric hospitalisations, prescribed medication and
15
16 socioeconomic variables during the five years follow-up.
17
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20
21

22 Investigations

23
24 We will include state of the art methods within clinical assessment as well as biomarkers including
25
26 a range of new methods:
27

- 28 1. Clinical assessments using the Hamilton Depression Scale-17 items (HAMD-17) (22), the
29 Young Mania Rating Scale (YMRS) (23), the Functional Assessment Short Test (FAST) (a
30 measure of psychosocial function) (26)
31
32
- 33 2. Questionnaires including the Hypomania Check list (27), Standardized Assessment of
34 Personality - Abbreviated Scale (SAPAS) (28), the WHO (Five) well-being index (29) and
35 the Verona Satisfaction Scale-Affective Disorder (VSS-A) (30).
36
37
- 38 3. Standardised fasting blood tests for a large number of potential biomarkers (see later
39 section: BIO-1, Biological tests).
40
41
- 42 4. Spot urine samples for oxidative generated damage to DNA and RNA
43
44
- 45 5. Hair cortisol as a valid and reliable index of long-term systemic cortisol levels (31)
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47
- 48 6. Combined heart rate and movement sensor mounted at the thorax (Actiheart; (32;33)).
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- 51 7. Daily electronic smartphone based self-monitoring of depressive and manic symptoms
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- 4 8. Daily electronic smartphone-based automatically generated data (e.g. data on phone usage,
- 5 social activity and physical activity)
- 6
- 7
- 8
- 9 9. Neuropsychological assessment (only during full or partial remission)
- 10
- 11 10. Structural magnetic resonance imaging (MRI) focusing on prefrontal cortex and
- 12 hippocampus as well as functional MRI (fMRI) (only during full or partial remission).
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Clinical assessments will be performed by six PhD students (Master degree as a Medical Doctor or psychologist). They will be certified in a PhD training course in the SCAN interview and will be trained in using rating scales (HAM-D-21, YMRS, FAST). Inter-rater sessions supervised by senior clinicians from the Copenhagen Affective Disorder Clinic and senior researchers in the BIO study will be conducted among the researchers at regular time points during the entire study period.

The BIO study includes four separate but interacting sub-studies (BIO-1, BIO-2, BIO-3, BIO-4), as illustrated in Table 1 with specified aims, background and theoretical basis, and methods, as described in the following. Background and reasons for selection of putative biomarkers is an iterative process presented in each sub-study. Nevertheless, as the area of potential biomarkers is constantly evolving and as the sub-studies cover four different areas, the research protocol does not include systematic reviews of the literature.

BIO-1: peripheral blood-based biomarker in bipolar disorder

Aims

To identify a composite blood-based biomarker that 1) discriminates bipolar disorder from healthy control individuals 2) discriminates between manic, depressive and remitted states 3) predicts

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4 emerging affective episodes, and 4) to validate the composite blood-based biomarker against the
5
6 Smartphone-based biomarker, and the neurocognitive and brain imaging signature, and 5) to
7
8 investigate the change in individual biomarkers as well as the composite blood-based biomarker
9
10 following onset of first manic episode, during successive relapses and in the end late stages of the
11
12 illness (15).
13

14 15 16 17 Background and theoretical basis

18
19 In a series of meta-analyses we concluded that although a number of candidate peripheral
20
21 biomarkers related to neuroplasticity, inflammation, oxidative stress and gene expression seem
22
23 promising, findings are limited by poor study designs, small cross sectional samples, lack of
24
25 adjustment for important confounders related to most peripheral biomarkers and poor laboratory
26
27 methodology (34-37). Because of high inter-individual variation in peripheral biomarkers,
28
29 assessment of intra-individual alterations from onset of illness through different affective phases
30
31 and into the late illness stage is necessary to identify clinically relevant and valid biomarkers,
32
33 necessitating a longitudinal study design (35;38).
34
35
36

37 We have in two longitudinal studies with repeated assessments of patients with bipolar disorder
38
39 during affective states (manic/mixed, depressive and euthymic) and healthy control individuals
40
41 found that brain derived neurotrophic factor (BDNF) (39), increased oxidative DNA and RNA
42
43 damage (40;41) and decreased mRNA expression of the PTGDS gene encoding the prostaglandin D
44
45 synthase enzyme (42) are markers related to the illness trait in bipolar disorder. The level of the
46
47 cytokines IL-6 and IL-18 was related to manic episodes only and the activity of GSK-3beta varied
48
49 with affective states (43) suggesting that these may be state markers (44). These results have
50
51 contributed to the research area of biomarkers in bipolar disorder, moving the area closer towards
52
53 identifying clinically applicable biomarkers.
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4 Nevertheless, it is unlikely, that one single biomarker will provide a useful diagnostic tool; instead a
5
6 composite of several relevant biomarkers appears a more viable approach (45). Recently promising
7
8 results have been presented regarding a diagnostic test in unipolar depression comprising serum
9
10 levels of nine individual biomarkers in peripheral blood (13). Similarly, preliminary studies have
11
12 suggested composite biomarkers for bipolar disorder (46-48). We identified a composite biomarker
13
14 consisting of gene expression from 19 candidate genes for bipolar disorder that accurately
15
16 discriminated bipolar patients from healthy control individuals (46). Thus, such approaches highly
17
18 increase the chances of obtaining a high specificity and sensitivity of the composite blood-based
19
20 biomarker (46-48).
21
22

23
24 In order to establish relevant markers of risk and markers related to illness stage, it is necessary to
25
26 include assessment before onset of illness and during first- and recurrent relapses and in the late
27
28 stages of the illness according to the staging model of bipolar disorder (49). Further, the study of
29
30 early onset individuals is necessary to evaluate biomarker levels without influence from medication,
31
32 which may otherwise limit the validity of identified biomarkers.
33
34
35
36

37 Methods

38
39 BIO-1 will include repeated clinical assessments and corresponding samples of blood and other
40
41 tissues among the 300 newly diagnosed/first episode bipolar patients, the 200 first-generation
42
43 relatives and the 100 healthy individuals *without* a first-generation family history of affective
44
45 disorder.
46
47

48 We will estimate a composite blood-based biomarker based on a number of individual markers
49
50 including brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), 5 different cytokines,
51
52 gene expression, additional thirty candidate genes and other potential biomarkers (see Biological
53
54 tests), and identify the composite biomarker that correlates best with affective states and Hamilton
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4 Depression Rating Scale and Young Mania Rating Scale scores of depression and mania,
5
6 respectively. A final blood-based biomarker will be chosen based on its ability to 1) discriminate
7
8 patients with bipolar disorder from healthy control individuals and to 2) discriminate between
9
10 manic, depressive and remitted states in bipolar disorder.
11
12

13 14 15 Laboratory procedures

16
17 We will obtain careful standardization of blood sampling and laboratory analysis by obtaining
18
19 blood samples in a fasting state and in a one hour interval in the morning. At the same day as blood
20
21 sampling, smoking status, alcohol use, BMI and menstrual cycle, etc., will be assessed. Blood
22
23 sampling and all phases of laboratory processing for plasma and DNA/RNA analyses will be done
24
25 at the Department of Clinical Biochemistry, Rigshospitalet using standard operational methods
26
27 conducted by a team of technicians blinded with respect to participant status. All plasma samples
28
29 will be stored at – 80 C. The BIO study will include a total of 2400 blood samples: 300 patients x 5
30
31 + 200 healthy first-generation relatives x 3 + 100 healthy participants without a first-generation
32
33 family history x 3.
34
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40 Biological tests

41
42 We will use a multianalyte panel including a large number of potential biomarkers such as plasma
43
44 levels of Neutrophins3, GSK-3, β -amyloid A β 40 and A β 42, BDNF, inflammatory markers, high
45
46 sensitive C-reactive protein, lipoproteins (VLDL, LDL, HDL) and specific apolipoproteins (e.g.
47
48 apoE, ApoA-I, apoA-II, and apoM) as potential markers of low-grade inflammation particularly
49
50 salient in the early stages of bipolar disorder. Total RNA, microRNA, genomic DNA and histones
51
52 are isolated from peripheral blood mononuclear cells. Gene expression and alternative slicing of
53
54 RNA transcripts are analysed using array reverse transcription quantitative real-time PCR (RT-
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4 qPCR) and next generation sequencing. Epigenetic modifications of the DNA (e.g. methylation) are
5
6 measured using antibody based methods or bisulphite treatments in combination with next
7
8 generation sequencing. The genomic positions of histones with specific modifications are detected
9
10 using immune precipitation and sequencing (CHIP-Seq). The degree of histone modifications is
11
12 measured by semi-quantitative antibody based detection.

13
14 Measurements of DNA and RNA damage by oxidation are obtained from spot urine samples and
15
16 analyzed using ultra performance liquid chromatographic (UPLC) and mass spectrometry.
17

18
19 Hair cortisol will be included as a valid and reliable index of long-term systemic cortisol levels
20
21 (31).
22

23
24 We will report on these individual biological tests including comparing patients, first degree
25
26 relatives and healthy control individuals, when appropriate. The BIO study sample of 600
27
28 participants is rather small for genetic analyses discriminating patients with bipolar disorder from
29
30 healthy controls but some genetic analyses, including the CACNA1C gene, can be conducted in
31
32 cooperation with national and international genetic network groups.
33
34

35 36 37 Statistical analyses

38
39 Data represent repeated measures within and between individuals and will be analysed using a
40
41 combination of generalized linear mixed models, integrated data analysis, and penalized regression
42
43 approaches to facilitate the combined feature selection and prediction of the available high-
44
45 dimensional data. Integrative data analysis ensures that we are able to identify an improved
46
47 composite blood-based biomarker since data from different molecular levels are combined in a
48
49 simultaneous analysis that closer resembles the biological system (50;51). Further, we will use cross
50
51 validation or alternatively split sample designs in the development and validation of the composite
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4 biomarker. Finally, if possible we will evaluate the biomarker(s) in external non-Danish data sets in
5
6 collaboration with other international researchers.
7

8
9 A general principle that pertains to statistical analyses of all four sub-studies is the “intention to
10
11 treat” principle. Accordingly, participants who during follow-up get a diagnosis with a higher
12
13 diagnostic validity than bipolar disorder (i.e. a lower ICD-10 diagnostic number, DF00, DF10 and
14
15 DF20) that may substantially influence the biomarker measures are included in the analyses until
16
17 onset of symptoms from the disorder but excluded from subsequent analyses. These disorders
18
19 include significant neurological disorders such as dementia, stroke, brain tumour, multiple sclerosis,
20
21 Parkinson’s disease, as well as disorders due to significant psychoactive substance use and
22
23 schizophrenia“.
24
25

26
27 Furthermore, in all four sub-studies, the problem of missing data will be alleviated by the use of mixed
28
29 effect model (for longitudinal measurements) and multiple imputations using chained equations
30
31 when applicable. If possible, joint modeling will be considered, depending on the missing
32
33 mechanism observed.
34
35
36

37 Statistical power

38
39 The study has a power of 80% to detect a minimum increment of 6.5 percentage points in sensitivity
40
41 if we assume that the existing diagnostic tools have a sensitivity of 70% to diagnose bipolar
42
43 disorder for a patient that has the disorder (see (52)). Thus, if the composite biomarker score
44
45 increases the sensitivity by a minimum of 6.5 percentage points then we have a power of 80% to
46
47 detect that increase based on 300 patients with bipolar disorder using a one-sided exact binomial
48
49 test (for fixed specificity).
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55 **BIO-2: Smartphone-based electronic biomarker in bipolar disorder**

Aims

To identify a composite smartphone-based electronic biomarker that 1) discriminates patients with bipolar disorder from healthy control individuals 2) discriminates between manic, depressive and remitted states 3) predicts emerging affective episodes, and 4) to investigate the change in the composite smartphone-based biomarker following onset of first manic episode, during successive relapses and in the end stages of the illness as according to the staging system by Berk et al (15).

Background and theoretical basis

Recently, electronic self-monitoring of the severity of depressive and manic symptoms using text messages has been suggested as an easy and inexpensive way to identify early signs of affective episodes, providing opportunities for mental health care providers to intervene shortly after prodromal symptoms first appear (53). We have, in the MONARCA project developed and tested a smartphone-based electronic monitoring system including daily subjective self-assessments of illness activity in bipolar disorder as well as a bi-directional feedback loop between the patient and clinicians (the MONARCA system (54-58)). Using the MONARCA system, fine-grained electronic data were collected during everyday life in naturalistic settings in patients with bipolar disorder. The MONARCA system was reported highly usable and useful by patients with bipolar disorder with a high self-assessment adherence (87-95%), and the patients reported that the MONARCA system helped them to better manage their disease (54;55). Further, the severity of depressive and manic symptoms was found to correlate with automatically generated smartphone data including 1) physical activity as reflected by the number of changes in cell tower ID per day (59), 2) social activity as reflected by the number of incoming and outgoing calls per day, the duration of incoming and outgoing calls per day, and the number of outgoing text messages per day (60), and 3) voice features collected during phone calls (61). Although these findings are encouraging there is a

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3
4 need to integrate self-monitored smartphone data with automatically generated smartphone data on
5
6 social and physical activity, speech and sleep into one composite smartphone generated electronic
7
8 biomarker measure. This composite measure should be modeled to 1) discriminate patients with
9
10 bipolar disorder from healthy control individuals 2) have a high correlation with depressive and
11
12 manic symptoms 3) discriminate between euthymic, manic and depressive states 4) early predict
13
14 emerging affective episodes for the individual patient to increase the possibility for early
15
16 intervention.

17
18
19 To validate smartphone based measures of physical activity and sleep, a subset of patients will wear
20
21 a combined heart rate and movement sensor mounted at the thorax that has been shown to correlate
22
23 with mood symptoms and affective states and differentiate between patients and controls (Actiheart;
24
25 (32;33)) like other wearable actigraphs (62-64).

26 27 28 29 30 31 Methods:

32
33 All newly diagnosed/first episode patients will have access to a smartphone based system
34
35 (Monsenso that is a developed from the MONARCA system) for continuous self-monitoring as well
36
37 as fine-grained automatically monitoring of behavioural activity and early identification of
38
39 emerging affective episodes during the first two years and following relapse of episodes during a 5-
40
41 year follow-up period.

42
43 Data analyses: In contrast to data in BIO-1, data in BIO-2 represent big data collected on a *daily* basis
44
45 within individuals. We will use hierarchical Bayesian predictive models that can handle big data through
46
47 sampling and visualization techniques that summarize data.
48
49

50 51 52 **BIO-3: neurocognitive and brain imaging signatures in bipolar disorder**

53 54 55 Aims

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4 1) To identify an integrated brain-based biomarker of bipolar disorder including neurocognitive and
5
6 neuroimaging measures tapping into ‘hot’ (i.e. emotion-laden) and ‘cold’ (non-emotional)
7
8 cognition, 2) to examine whether the degree of abnormality in these measures predicts illness
9
10 onset in the high-risk group and/or relapse in the patient group, 3) to identify developmental
11
12 trajectories in ‘hot’ and ‘cold’ cognitive dysfunction and to identify structural and functional MRI
13
14 correlates in bipolar disorder via longitudinal assessments of high-risk individuals to remission after
15
16 onset of first manic episode and following successive relapses, and 4) to identify associations
17
18 between aberrant ‘hot’ and ‘cold’ neurocognitive function, structural and functional brain changes
19
20 and the composite blood-based and Smartphone-based biomarkers. Such ‘integrated systems
21
22 approach’ involving identification of patterns of biomarkers (bio-signatures) across these multiple
23
24 levels of investigation is considered imperative for deeper understanding of the dimensions of
25
26 underlying pathophysiological processes in bipolar disorder (12).
27
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33 Background and theoretical basis

34
35 Results from a number of meta-analyses of a large number of cross sectional studies of patients with
36
37 bipolar disorder in remission suggest trait-related ‘cold’ cognitive deficits in attention/processing
38
39 speed, memory, and executive function compared to healthy controls (65-68) that correlate with
40
41 everyday functioning (69). Cross-sectional comparison of patients at different illness stages
42
43 revealed more pronounced cognitive deficits during late compared with early stages in line with the
44
45 staging hypothesis of bipolar disorder (70). However, there are only a few longitudinal studies of
46
47 neuropsychological functioning with small sample sizes (12 studies including a total of 152 bipolar
48
49 patients (71)). A meta-analysis of these studies found no support for a progressive nature of
50
51 cognitive deficits (71). However, results from these studies are hampered by a number of limitations
52
53 including small sample sizes, short follow-up (mean follow-up period of 4.6 years) and high
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4 attrition rates (up to 45%) (71). Consequently, it is unclear whether cognitive function assessed with
5
6 neuropsychological tests deteriorate with the number and duration of illness episodes in bipolar
7
8 disorder although epidemiological studies consistently revealed increased risk of developing
9
10 dementia long-term (6;8;72-74) and there is some evidence for increasing risk of dementia with
11
12 the number of episodes (7) (see also (9)).

13
14 Deficits in 'hot' cognition are closely linked to emotional disturbances (75) and difficulties in socio-
15
16 emotional behaviour and interpersonal relations in bipolar disorder (76). 'Hot' cognition
17
18 abnormalities in bipolar disorder have been observed within three domains; (i) emotional
19
20 processing, (ii) reward processing and (iii) emotion regulation (reviews in (77;78)).

21
22 Results from a large number of cross sectional structural imaging studies suggest that patients also
23
24 show increased lateral ventricular volumes and greater prevalence of white matter hyper-intensities
25
26 (79). While these findings are rather unspecific, studies also suggest that treatment with lithium
27
28 increases the grey matter volume of prefrontal cortex, amygdala and hippocampus (79). In addition,
29
30 a number of functional imaging studies suggest that bipolar disorder is associated with
31
32 abnormalities within fronto-limbic-subcortical structures (38).

33
34 As long-term, integrative studies are lacking, it is unclear how neurocognitive and brain imaging
35
36 abnormalities correlate with the staging of bipolar disorder, illness progression and treatment
37
38 (38;80) or with changes in biological markers such as neurotropic, inflammatory and oxidative
39
40 stress markers (81;82).
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48 Methods

49
50 Using a comprehensive neurocognitive test battery, we will assess all participants from the three
51
52 groups (patients with bipolar disorder, first-generation relatives, and healthy individuals *without* a
53
54 family history of affective disorders). Patients will be followed from first onset of affective disorder
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4 and during successive periods of remission or at an annual basis (bipolar patients with no relapse,
5
6 first-generation relatives with no onset, and healthy controls. Among these, a subgroup of 60
7
8 patients, 60 healthy relatives (UR) and 30 healthy without a family history of affective disorders
9
10 (HC) will be undergo functional and structural MRI at these time points
11
12
13

14 Neurocognitive testing

15
16
17 Within ‘cold’ cognition verbal learning/memory and executive function have been highlighted as
18
19 the most suitable candidates for biomarkers of bipolar disorder (83;84). ‘Cold’ cognition will
20
21 therefore be assessed with neurocognitive tests probing verbal memory, attention and executive
22
23 function including the Rey Auditory Verbal Learning Test (RAVLT; (85;86)) and the WAIS-III
24
25 letter-number sequencing, RBANS digit span, n-back working memory, verbal fluency and Trail
26
27 Making Test B. Verbal intelligence will be estimated with the Danish Adult Reading Test (DART).
28
29 ‘Hot’ cognition will be assessed with a comprehensive battery of computerized neurocognitive tests
30
31 outside the scanner probing (i) emotional processing (ii) reward processing and (iii) emotion
32
33 regulation. These include the facial expression recognition and faces dot-probe tasks from the
34
35 Emotional Test Battery (ETB) (P1Vital, Oxford), and an ecologically valid social scenarios test
36
37 developed by our group (87). During fMRI we will also administer the following experimental
38
39 paradigms: an emotional face processing task using face stimuli from the Nimstim
40
41 (<http://www.macbrain.org/resources.htm>), a monetary reward processing task (88), and (iii) a
42
43 negative affective picture task using validated stimuli sets from the International Affective Picture
44
45 System (IAPS) developed in collaboration with researchers at the Universities of Chicago. In
46
47 addition, we will explore the neuronal basis for ‘cold’ cognition (executive function) using n-back
48
49 working memory and picture encoding tasks programmed in house (89). Finally, self-report
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4 measures (BIS/BAS, and the CERQ (90)) are used to assess reward responsiveness and habitual
5
6 emotional regulation strategies.
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9

10 11 12 13 Structural and functional MRI

14
15 Structural MRI: using T1-weighted images acquired at a 3T Siemens scanner at the Copenhagen
16
17 University Hospital, Rigshospitalet, we will focus on lateral ventricular volumes, grey matter
18
19 volume of prefrontal cortex, amygdala and hippocampus, relative to whole brain volume.
20

21
22 Specifically, segmentation and analysis of subcortical and regional cortical volume, shape and grey
23
24 matter density will be conducted FMRIB Software Library (FSL) tools, including the FMRIB's
25
26 Integrated Registration and Segmentation Tool (FIRST), the FSL-VBM tool and FSL vertex (shape)
27
28 analysis (<http://fsl.fmrib.ox.ac.uk/>).
29

30
31 Functional MRI (fMRI): T2-weighted images will be acquired to investigate white matter hyper-
32
33 intensities. We will also use fMRI to investigate neuronal underpinnings of 'hot' and 'cold'
34
35 cognition with the previously described experimental paradigms. Functional MRI data processing
36
37 will be carried out with the FMRI Expert Analysis Tool, part of FMRIB's Software Library
38
39 (www.fmrib.ox.ac.uk/fsl). We will examine mean percent BOLD signal change within predefined
40
41 hippocampal and amygdala Regions of Interest (ROIs) obtained in standard space with mri3dX
42
43 (<http://www.idoimaging.com/program/160>). In addition, whole-brain exploratory analysis will be
44
45 conducted to explore neural activity differences in other cortical regions. For this group analysis,
46
47 individual contrasts of interest will be included in separate general linear models with
48
49 nonparametric permutation inference (n = 5000) using the 'randomize' algorithm implemented in
50
51 FSL (91).
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Statistical power

The above sample size for participants undergoing fMRI assessments is determined based on our previous fMRI studies. In particular, inclusion of about 17-22 participants per treatment/diagnostic group (matched for age and gender) had a power of >0.8 to detect differences between groups in neural and cognitive response to emotional faces (e.g. (92;93) and Miskowiak et al, under review) at an alpha-level of $p < 0.05$ for cross-sectional designs. For longitudinal designs, we were able to demonstrate differences between groups in *the change* in task-related neural activity and in hippocampal structure with a sample of about 40 participants per group (94-96). Given this, our inclusion of 60 participants per group is expected to ensure adequate statistical power for both the cross-sectional and longitudinal parts of the fMRI study.

BIO-4: at risk or prodromal phase for bipolar disorder

Aims

To test whether 1) the composite blood-based biomarker, 2) the composite electronic smartphone-based biomarker and 3) the neurocognitive signature for bipolar disorder predict onset of illness (depression or mania) among a healthy (i.e., non-syndromal level) high risk population of first degree siblings and offspring to the patients with newly diagnosed/first episode mania/bipolar disorder, included in BIO-1, BIO-2 and BIO-3.

Background and theoretical basis: First-generation relatives to patients with bipolar disorder have a nine-fold increased risk of developing bipolar disorder and a two to three-fold increased risk of developing unipolar disorder (1). Although there is a great need for early detection and primary prevention of onset of illness among relatives to patients with bipolar disorder recent prior attempts have not been successful due to retrospective designs, poor characterisation of high risk individuals

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4 and small sample sizes (97). This is the first time a composite blood-based, a composite
5
6 smartphone-based and a composite neurocognitive biomarker identified among patients with bipolar
7
8 disorder will be investigated among their healthy relatives as a predictor measure of onset of illness
9
10 (18). This approach increases the chances of obtaining a high specificity and sensitivity of the
11
12 composite biomarkers.
13

14 15 16 17 Methods

18
19 This BIO-4 includes first-generation relatives (siblings and offspring aged 15 to 40 years) to the
20
21 recruited patients with first manic or bipolar diagnosis. All recruited patients will be asked about the
22
23 lifetime psychiatric history of first-degree relatives (their biological parents, siblings and offspring)
24
25 based on the Brief Screening for Family Psychiatric History questionnaire described by Weissmann
26
27 and colleagues (98). We expect that at least 200 first-generation relatives will be asymptomatic or
28
29 present mild symptoms or prodromal patterns to affective disorders and will be included in the study.
30
31 Biological tissues will be drawn (as part of BIO-1) and neurocognitive function will be assessed on
32
33 all individuals and brain imaging will be done on 60 individuals with longitudinal assessments.
34
35

36 37 Statistical analyses

38
39 BIO4 will use a combination of penalized regression techniques, and random forests to infer the
40
41 importance of the original and combined markers and to compare the similarity of prediction error
42
43 from the models with and without combining the markers.
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52 Feasibility

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54 The BIO study is fully feasible as patients are recruited as part of daily health care for patients
55
56 referred to the Copenhagen Affective Disorder Clinic.
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Ethical considerations

The BIO study has been approved by the Local Ethical Committee (H-7-2014-007) and the data agency, Capital Region of Copenhagen (RHP-2015-023). According to these specifications, adolescents aged 15-18 years will be invited only if parents have given consent. Data will be saved, encrypted and assessed according to the regulations from the Capital Region of Denmark. The study complies with the Declaration of Helsinki principles (Seoul, October 2008).

Dissemination

Study results will be presented in peer reviewed journals and at international conferences in accordance with relevant reporting guidelines (99;100).

Discussion

Summary

It is expected that the BIO cohort will provide valid biological, electronic, neurocognitive and neuroimaging data and for the first time longitudinally identify changes in biomarkers during different stages of bipolar illness, i.e., at risk stage, following onset, during first relapse and recurrent relapses and in the late stages of the illness as according to Berk et al (15).

Limitations

Some limitations of the BIO study should be noted beforehand. First, the rather extensive initial assessment study procedure may potentially result in selection of participants that are intrinsically

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4 positive towards clinical research and readily willing to cooperate. Nevertheless, we expect that
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6 such selection will be decreased as it is likely that the vast majority of the more than 100 newly
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8 diagnosed/first episode bipolar patients referred to the Copenhagen Affective Disorder Clinic each
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10 year will accept participation in the BIO study as this implies an extensive clinical evaluation.
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12 Second, attrition may increase during long-term follow-up and patients who stay in the study may
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14 adhere more to treatment in general. Such selection is inherent in clinical longitudinal research, and
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16 the large number of participants that will be included will increase external validity. Third, potential
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18 confounding effects of psychotropic medication may influence comparisons between patients with
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20 bipolar disorder and healthy control individuals as well as comparisons within patients as the vast
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22 majority of patients will get medication that may change during the course of illness. Lithium,
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24 mood stabilizers and antipsychotics may have effects on the collected biological, smartphone-based,
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26 neuropsychological and brain imaging data. Effects of medication on biological measures are not
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28 clear (101) although analyses from systematic reviews and meta-analyses involving only bipolar
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30 disorder patients have not found clear effects of medication on cytokines (34;35), BDNF (37), gene
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32 expression (36) nor has subsequent individual studies on cytokines (44;102), BDNF (39;102), gene
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34 expression (46) or DNA and RNA damage (40;41). Effects of medication on electronic smartphone
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36 generated data as well as on neuropsychological and brain imaging data are poorly investigated and
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38 warrants further studies (103).
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46 Fourth, due to the large number of biological and statistical tests included in the BIO study, chance
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48 findings may occur in relation to the individual biological test. However, the aim of the BIO study
49
50 is to identify a composite biomarker measure related to bipolar illness, depression and mania using
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52 cross validation or alternatively split sample designs in the development and validation of the
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54 composite biomarker.
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9 Finally, the BIO study does not include (randomised) interventions limiting casual interpretations of
10 the results. Nevertheless, with the BIO prospective, repeated measures design it is possible to
11 identify valid associations between the composite measures (of biological, electrical,
12 neuropsychological and brain imaging data) and depressive and manic symptoms and states.
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19 Strengths

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21 The BIO study is the first study aiming to identify 1) a composite blood-based biomarker, 2) a
22 composite electronic smartphone-based biomarker and 3) a neurocognitive signature for bipolar
23 disorder as well as to measure the same biomarkers in newly diagnosed/first episode bipolar
24 patients and their healthy first-generation relatives. It is possible to recruit newly diagnosed/first
25 episode patients with mania/bipolar as all such patients from the entire Capital Region of Denmark
26 are referred to the Copenhagen Affective Disorder Clinic and routinely asked for inclusion in the
27 BIO study. Long-term attrition is supposed to be low as all patients will be followed by the
28 Copenhagen Affective Disorder Clinic for the first two years and subsequently in other treatment
29 settings in the Capital Region of Denmark. Including longitudinal assessment of healthy individuals
30 is of paramount importance to control for the effect of timing and ageing (104), but among all
31 studies on biomarkers in bipolar disorder, this has been done only in two studies from our group
32 (40;44). The study will be performed by an experienced international research group, combining
33 expertise from all areas of the study.
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53 Conclusion

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4 The BIO study is a large long-term cohort study on biomarkers in bipolar disorder and we expect
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6 that the findings for the first time will be representative of biomarkers in bipolar disorder in general
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8 as no prior study on newly diagnosed/first episode bipolar disorder has been conducted. It is
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10 expected that the BIO cohort will provide valid biological, electronically, neuropsychological and
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12 brain imaging longitudinal data.
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21 Authors' contribution

22 LVK designed the study together with MV, KM, KWM and MFJ. LVK drafted the study protocol
23 and the manuscript. All authors contributed to development of the study protocol and to editing the
24 manuscript and read and approved the final version.

25 KM, LBN, RFS, CE, BKP, HEP, RSM, FK, WFG, and MV contributed specifically to BIO-1 on
26 peripheral blood-based biomarkers. MFJ, OW, JB, MF and OM contributed specifically to BIO-2
27 on smartphone-based electronic biomarkers. KWM, GMK and MP contributed specifically to BIO-
28 3 on neurocognitive and brain imaging signatures. MV contributed specifically to BIO-4 on at risk
29 or prodromal phase of bipolar disorder (in addition to BIO-1).
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35 Danish Council for Independent Research, Medical Sciences (DFF – 4183-00570), Weimans Fund,
36 Markedmodningsfonden (the Market Development Fund, (2015-310), Gangstedfonden (A29594),
37 Helsefonden (16-B-0063), Innovation Fund Denmark (the Innovation Fund, Denmark, 5164-
38 00001B), Copenhagen Center for Health Technology (CACHET), EU H2020 ITN (EU project
39 722561), Augustinusfonden (16-0083), Lundbeck foundation (R215-2015-4121).
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42 Competing interests

43 LVK has within the preceding three years been a consultant for Lundbeck, AstraZeneca and
44 Sunovion. KWM has received consultancy fees in the past three years from Lundbeck and Allergan.
45 MFJ has been a consultant for Eli Lilly and Lundbeck. MV has within the preceding three years
46 been a consultant for Astra Zeneca and Servier. FK has been a speaker for Ache, Daiichi Sankyo
47 and Janssen. MP is a consultant for Roche Pharmaceuticals.

48 RSM: Advisory Boards: Lundbeck, Pfizer, AstraZeneca, Elli-Lilly, Janssen, Ortho Purdue, Johnson
49 & Johnson, Moksha8, Sunovion, Mitsubishi, Takeda, Forest, Otsuka, Bristol-Myers Squibb, Shire,
50 Speakers Fees: Lundbeck, Pfizer, AstraZeneca, Elli-Lilly, JanssenOrtho, Purdue, Johnson &
51 Johnson, Moksha8, Sunovion, Mitsubishi, Takeda, Forest, Otsuka, Bristol-Myers Squibb, Shire,
52 Research Grants: Lundbeck, JanssenOrtho, Shire, Purdue, AstraZeneca, Pfizer, Otsuka, Allergan.
53 HEP has received a research Grant from Boeringer Ingelheim. GMK has received honoraria as
54 Field Editor of the International Journal of Neuropsychopharmacology and as scientific advisor for
55 H Lundbeck A/S. JEB and MF are co-founders and shareholders in Monsenso.
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4 LBN, RFS and WFG declare no competing interests.
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6 The validity of the research cannot be influenced by any of these potential secondary interest (such
7 as financial gain or personal relationship).
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10 Patient consent
11 Obtained

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13 Ethical approval
14 The study has been approved by the Local Ethical Committee (H-7-2014-007) and the data agency,
15 Capital Region of Copenhagen (RHP-2015-023).
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18 Study registration
19 The BIO study has been registered at clinicaltrials.gov with Trial Registration Number:
20 NCT02888262.
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Table 1. Overview of the longitudinal assessments during risk periods and following onset of bipolar disorder in the four sub-studies of the BIO study.

Course	Healthy first-gen. relatives	First episode	Remission	First relapse	Remission	Second relapse	Etc.
BIO-1	x	x	x	x	x	x	x
BIO-2	x	x	x	x	x	x	x
BIO-3	x		x		x		x
BIO-4	x	x	x	x	x	x	x

BIO-1: Peripheral blood-based biomarker in bipolar disorder

BIO-2: Smartphone-based electronic biomarker in bipolar disorder

BIO-3: Neurocognitive and brain imaging signatures in bipolar disorder

BIO-4: At risk or prodromal phase for bipolar disorder

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The Bipolar Illness Onset study - research protocol for the BIO cohort study

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Manuscripts

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The Bipolar Illness Onset study - research protocol for the BIO cohort study

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4 **Introduction:** Bipolar disorder is an often disabling mental illness with a lifetime prevalence of 1-
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6 2%, a high risk of recurrence of manic and depressive episodes, a lifelong elevated risk of suicide
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8 and a substantial heritability. The course of illness is frequently characterised by progressive
9
10 shortening of inter-episode intervals with each recurrence and increasing cognitive dysfunction in a
11
12 subset of individuals with this condition. Clinically, diagnostic boundaries between bipolar disorder
13
14 and other psychiatric disorders such as unipolar depression are unclear although pharmacological
15
16 and psychological treatment differs substantially. Patients with bipolar disorder are often
17
18 misdiagnosed and the mean delay between onset and diagnosis is 5-10 years. Although the risk of
19
20 relapse of depression and mania is high it is for most patients impossible to predict and
21
22 consequently prevent upcoming episodes in an individual tailored way. The identification of
23
24 objective biomarkers can both inform bipolar disorder diagnosis and provide biological targets for
25
26 the development of new and personalized treatments. Accurate diagnosis of bipolar disorder in its
27
28 early stages could help to prevent the long-term detrimental effects of the illness.

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32 The present Bipolar Onset study (BIO) study aims to identify 1) a composite blood-based biomarker
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34 2) a composite electronic smartphone-based biomarker and 3) a neurocognitive and neuroimaging
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36 based signature for bipolar disorder.
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39 **Methods and analysis:** The study will include 300 patients with newly diagnosed/first episode
40
41 bipolar disorder, 200 of their healthy siblings or offspring, and 100 healthy individuals without a
42
43 family history of affective disorder. All participants will be followed longitudinally with repeated
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45 blood samples and other biological tissues, self-monitored and automatically generated smartphone
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47 data, neuropsychological tests and a subset of the cohort with neuroimaging during a 5-10 year
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49 study period.
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52 **Ethics and dissemination:** The study has been approved by the Local Ethical Committee (H-7-
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54 2014-007) and the data agency, Capital Region of Copenhagen (RHP-2015-023) and the findings
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4 will be widely disseminated at international conferences and meetings including conferences for the
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6 International Society for Bipolar Disorders (ISBD) and the World Federation of Societies for
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8 Biological Psychiatry (WFSBP) and in scientific peer reviewed papers.
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11 **Trial Registration Number:** NCT02888262.
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Strengths

- The BIO study is the first study aiming to identify 1) a composite blood-based biomarker, 2) a composite electronic smartphone-based biomarker and 3) a neurocognitive signature for bipolar disorder
- The same biomarkers will be measured longitudinally in newly diagnosed/first episode bipolar patients and their healthy first-generation relatives
- The BIO study will be performed by an experienced international research group, combining expertise from all areas of the study

Limitations

- Extensive initial assessment study procedures may result in selection of participants that are intrinsically positive towards clinical research
- Confounding effects of psychotropic medication may influence results
- Lack of randomized interventions may limit causal interpretations of the results

Introduction

Biomarkers in bipolar disorders

Bipolar disorder is a disabling mental illness with a lifetime prevalence of 1-2%, a high risk of recurrence of manic and depressive episodes, a lifelong elevated risk of suicide and a substantial heritability of 60-80% (1). Bipolar disorder is often conceptualised as a progressive disorder with increasing risk of recurrence for every new affective episode (2-5) and with increasing cognitive disabilities during the course of illness (6-9). Clinically, diagnostic boundaries between bipolar disorder and other psychiatric disorders such as unipolar disorder are unclear although some pharmacological and psychological treatment strategies differ substantially. Patients with bipolar disorder are often misdiagnosed as having from unipolar disorder, transient psychosis, reaction to stress/adjustment disorder or psychoactive substance abuse (10) and the mean delay between onset and diagnosis is 5-10 years (11). Although the risk of relapse of depression and mania is high it is for most patients impossible to predict and consequently prevent upcoming episodes in an individual tailored way. The identification of objective biomarkers as measures of pathophysiologic processes can both inform bipolar disorder diagnosis and provide biological targets for the development of new and personalized treatments (12). Accurate diagnosis of bipolar disorder in its early stages could help to prevent the long-term detrimental effects of the illness.

Recently, promising results have been presented regarding a diagnostic test for unipolar depression comprising levels of nine biomarkers in peripheral blood (13). Although the nature of bipolar disorder seems more biological driven than the nature of major depression with a higher heritability there has been no or few attempts to identify a similar composite biomarker for bipolar disorder.

Onset of illness and staging in bipolar disorder

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4 Although the course of illness is heterogeneous there is a body of evidence for clinical progression
5 on average of unipolar and bipolar disorders as increasing number of affective episodes seem
6 associated with 1) increasing risk of recurrence 2) increasing duration of episodes 3) increasing
7 symptomatic severity of episodes 4) decreasing threshold for developing episodes 5) increasing risk
8 of developing dementia (9). It is likely that this clinical progression with deteriorating effects of
9 affective episodes and duration of illness is associated with neurobiological changes over the course
10 of illness. Unfortunately, results of all longitudinal studies on the biology of bipolar disorder are
11 hitherto hampered by three major limitations: 1) Only few studies have recruited patients with
12 bipolar illness from onset of the illness and most of these have used first onset mania as inclusion
13 criteria thereby excluding patients with a hypomanic episode (bipolar disorder, type II). As the
14 biology of bipolar disorder - based on cross-sectional studies - seem to change over the course of
15 illness from first episode to first relapse and recurrent relapses to an unremitting or rapid cycling
16 course (14-18), this is a major limitation in our knowledge internationally, 2) the number of patients
17 included in prior studies are less than 200, which is a limitation taking the heterogeneity of bipolar
18 disorder into account (e.g. bipolar disorder type I and II may have different biology, etc.), and 3) the
19 prospective follow-up period is less than a few years in all studies.

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40 In the BIO study we will establish a large cohort comprising of three sub cohorts that will be
41 followed long-term with systematic diagnostic, blood-based biomarkers, smartphone data, cognitive
42 and brain imaging assessment. The three sub cohort will consist of: 1) patients with newly
43 diagnosed/first episode bipolar disorder and 2) their healthy first-generation siblings and offspring
44 3) healthy individuals without a family history of affective disorder.

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53 Overall aims:
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4 1. To identify a composite blood-based biomarker measure as well as a composite electronic
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6 smartphone-based biomarker from onset of bipolar disorder, during progression and in later
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8 stages
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11 2. To investigate if the composite blood-based biomarker measure and electronic smartphone-
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13 based biomarker identified among patients with bipolar disorder predicts onset of illness
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15 (depression or mania) among these patients healthy first-generation siblings and offspring
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- 18 3. By applying an integrated systems approach, to identify patterns of “cerebral signatures”
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20 across neuro circuitry and cognitive levels and to validate the composite blood-based
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22 biomarker and electronic smartphone-based biomarkers against these bio-signatures
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- 24 4. To investigate long-term developmental trajectories in neurocognitive function and brain
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26 imaging from the high risk state to onset of bipolar disorder following first relapse and
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28 recurrent relapses and in the late stage with an unremitting, multi-episode or rapid cycling
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30 course.
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33 5. To investigate whether the course of illness is progressive on average in bipolar disorder and
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35 to identify corresponding changes in biomarkers during the course of illness within BIO-1 to
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37 BIO-4.
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42 Methods and analysis

43 Overall methods

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46 The BIO study is a long-term cohort study started April 2015 and planned to include 300 newly
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48 diagnosed/first episode bipolar patients from the Copenhagen Affective Disorder Clinic, 200 of
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50 these patients’ healthy first-generation relatives and 100 healthy individuals *without* a first-
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52 generation family history of affective disorders.
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The Copenhagen Affective Disorder Clinic

The Copenhagen Affective Disorder Clinic is a mood disorder clinic that provides treatment service for patients with newly diagnosed/first episode bipolar disorder (19). The Copenhagen Affective Disorder Clinic receives patients from the entire Capital Region of Denmark covering a catchment area of 1.6 million people and all psychiatric centres in the region. All patients referred to the Clinic as newly diagnosed/first episode patients, i.e., onset of first manic or hypomanic episode or when the ICD-10 diagnosis of bipolar disorder is made for the first time, will routinely be asked for inclusion in the BIO study. Nearly all patients treated in the Clinic have a diagnosis of bipolar disorder type I or type II whereas patients with bipolar disorder-not otherwise specified (NOS) or patients with cyclothymia not are treated in the Clinic and consequently not included in the BIO study.

Recruitment of the three cohorts:

1. 300 patients referred to the Copenhagen Affective Disorder Clinic (aged 15-70 years) as newly diagnosed/first episode bipolar patients, i.e., onset of first manic or hypomanic episode or when the diagnosis of bipolar disorder is made for the first time. The Clinic receives more than 100 newly diagnosed/first episode bipolar patients each year and we expect that nearly all will accept participation in the BIO study as this implies an extensive clinical evaluation.
2. 200 first-generation relatives (siblings and children aged 15 to 40 years) to the recruited newly diagnosed/first episode bipolar patients.
3. 100 age- and gender-matched healthy individuals *without* a first-generation family history of affective disorders recruited among blood donors from the Blood Bank at Rigshospitalet, Copenhagen, as in prior studies.

Diagnostic assessments at inclusion

The initial diagnostic assessment will be done using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID (20)) categorizing patients in bipolar disorder type I or type II as part of daily praxis by the experienced specialists in psychiatry during the patients two year stay in the Copenhagen Affective Disorder Clinic. This clinical diagnosis of bipolar disorder will be confirmed in a semi-structured research based interview using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) providing an ICD-10 diagnosis (21). There will be no attempt to balance the prevalence of bipolar subtypes in the BIO study.

Follow-up

Besides the assessments at inclusion, patients will be assessed during remitted, depressive and manic/mixed phases. Patients and healthy control individuals will be face to face assessed initially and at least every year during the first four years and after this, every second year for five years.

As part of daily clinical praxis in Copenhagen Affective Disorder Clinic and as part of the BIO-2 sub-study all patients will get access to a smartphone app for electronic continuous monitoring of illness activity during a 5-year follow-up period (see sub-study BIO-2). Additionally, research assistants will contact all participants every third months to identify upcoming episodes / onset of illness and to ensure continued participation in the BIO study. At each assessment the present, clinical state (remission, manic, hypomanic, depressive, mixed episode) of all participants will be established according to ICD-10. The severity of depressive and manic symptoms (if present) will be assessed using the 17-item Hamilton Depression Rating Scale (HAM-D-17) (22) and the Young Mania Rating Scale (YMRS) (23) with a time period of three days applied. Remission is defined as

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4 score of ≤ 7 on the HAMD-17 and the YMRS. In this way upcoming episodes and onset of illness
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6 (for healthy individuals) will be assessed with great certainty.
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9 Based on prior findings (2;3;19), it is estimated that patients will develop four to five affective
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11 episodes on average during the follow-up period, i.e., relapse or recurrence, defined as above. It
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13 estimated that 20-30% of the healthy first-generation relatives will develop onset of affective illness
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15 compared to 2-5% among the healthy individuals *without* a first-generation family history of
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17 affective disorders (24;25). Finally, linkage to Danish nation-wide register based data will be
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19 included for all individuals on psychiatric hospitalisations, prescribed medication and
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21 socioeconomic variables during the five years follow-up.
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24 25 26 Investigations

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28 We will include state of the art methods within clinical assessment as well as biomarkers including
29
30 a range of new methods:
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33 1. Clinical assessments using the Hamilton Depression Scale-17 items (HAMD-17) (22), the
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35 Young Mania Rating Scale (YMRS) (23), the Functional Assessment Short Test (FAST) (a
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37 measure of psychosocial function) (26)
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40 2. Questionnaires including the Hypomania Check list (27), Standardized Assessment of
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42 Personality - Abbreviated Scale (SAPAS) (28), the WHO (Five) well-being index (29) and
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44 the Verona Satisfaction Scale-Affective Disorder (VSS-A) (30).
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47 3. Standardised fasting blood tests for a large number of potential biomarkers (see later
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49 section: BIO-1, Biological tests).
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52 4. Spot urine samples for oxidative generated damage to DNA and RNA
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55 5. Hair cortisol as a valid and reliable index of long-term systemic cortisol levels (31)
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58 6. Combined heart rate and movement sensor mounted at the thorax (Actiheart; (32;33)).
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- 4 7. Daily electronic smartphone based self-monitoring of depressive and manic symptoms
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- 6 8. Daily electronic smartphone-based automatically generated data (e.g. data on phone usage,
- 7 social activity and physical activity)
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- 11 9. Neuropsychological assessment (only during full or partial remission)
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- 13 10. Structural magnetic resonance imaging (MRI) focusing on prefrontal cortex and
- 14 hippocampus as well as functional MRI (fMRI) (only during full or partial remission).
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20 Clinical assessments will be performed by six PhD students (Master degree as a Medical Doctor or
21 psychologist). They will be certified in a PhD training course in the SCAN interview and will be
22 trained in using rating scales (HAM-D-YMRS, FAST). Inter-rater sessions supervised by senior
23 clinicians from the Copenhagen Affective Disorder Clinic and senior researchers in the BIO study
24 will be conducted among the researchers at regular time points during the entire study period.
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33 The BIO study includes four separate but interacting sub-studies (BIO-1, BIO-2, BIO-3, BIO-4), as
34 illustrated in Table 1 with specified aims, background and theoretical basis, and methods, as
35 described in the following. Background and reasons for selection of putative biomarkers is an
36 iterative process presented in each sub-study. Nevertheless, as the area of potential biomarkers is
37 constantly evolving and as the sub-studies cover four different areas, the research protocol does not
38 include systematic reviews of the literature.
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49 **BIO-1: peripheral blood-based biomarker in bipolar disorder**

50 Aims

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4 To identify a composite blood-based biomarker that 1) discriminates bipolar disorder from healthy
5 control individuals 2) discriminates between manic, depressive and remitted states 3) predicts
6 emerging affective episodes, and 4) to validate the composite blood-based biomarker against the
7 Smartphone-based biomarker, and the neurocognitive and brain imaging signature, and 5) to
8 investigate the change in individual biomarkers as well as the composite blood-based biomarker
9 following onset of first manic episode, during successive relapses and in the end late stages of the
10 illness (15).
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20 21 Background and theoretical basis

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23 In a series of meta-analyses we concluded that although a number of candidate peripheral
24 biomarkers related to neuroplasticity, inflammation, oxidative stress and gene expression seem
25 promising, findings are limited by poor study designs, small cross sectional samples, lack of
26 adjustment for important confounders related to most peripheral biomarkers and poor laboratory
27 methodology (34-37). Because of high inter-individual variation in peripheral biomarkers,
28 assessment of intra-individual alterations from onset of illness through different affective phases
29 and into the late illness stage is necessary to identify clinically relevant and valid biomarkers,
30 necessitating a longitudinal study design (35;38).
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42 We have in two longitudinal studies with repeated assessments of patients with bipolar disorder
43 during affective states (manic/mixed, depressive and euthymic) and healthy control individuals
44 found that brain derived neurotrophic factor (BDNF) (39), increased oxidative DNA and RNA
45 damage (40;41) and decreased mRNA expression of the PTGDS gene encoding the prostaglandin D
46 synthase enzyme (42) are markers related to the illness trait in bipolar disorder. The level of the
47 cytokines IL-6 and IL-18 was related to manic episodes only and the activity of GSK-3beta varied
48 with affective states (43) suggesting that these may be state markers (44). These results have
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4 contributed to the research area of biomarkers in bipolar disorder, moving the area closer towards
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6 identifying clinically applicable biomarkers.
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9 Nevertheless, it is unlikely, that one single biomarker will provide a useful diagnostic tool; instead a
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11 composite of several relevant biomarkers appears a more viable approach (45). Recently promising
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13 results have been presented regarding a diagnostic test in unipolar depression comprising serum
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15 levels of nine individual biomarkers in peripheral blood (13). Similarly, preliminary studies have
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17 suggested composite biomarkers for bipolar disorder (46-48). We identified a composite biomarker
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19 consisting of gene expression from 19 candidate genes for bipolar disorder that accurately
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21 discriminated bipolar patients from healthy control individuals (46). Thus, such approaches highly
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23 increase the chances of obtaining a high specificity and sensitivity of the composite blood-based
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25 biomarker (46-48).
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29 In order to establish relevant markers of risk and markers related to illness stage, it is necessary to
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31 include assessment before onset of illness and during first- and recurrent relapses and in the late
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33 stages of the illness according to the staging model of bipolar disorder (49). Further, the study of
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35 early onset individuals is necessary to evaluate biomarker levels without influence from medication,
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37 which may otherwise limit the validity of identified biomarkers.
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41 Methods

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43 BIO-1 will include repeated clinical assessments and corresponding samples of blood and other
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45 tissues among the 300 newly diagnosed/first episode bipolar patients, the 200 first-generation
46
47 relatives and the 100 healthy individuals *without* a first-generation family history of affective
48
49 disorder.
50
51

52
53 We will estimate a composite blood-based biomarker based on a number of individual markers
54
55 including brain-derived neurotrophic factor (BDNF), neutrophin-3 (NT-3), 5 different cytokines,
56
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3
4 gene expression, additional thirty candidate genes and other potential biomarkers (see Biological
5 tests), and identify the composite biomarker that correlates best with affective states and Hamilton
6
7
8
9 Depression Rating Scale and Young Mania Rating Scale scores of depression and mania,
10
11 respectively. A final blood-based biomarker will be chosen based on its ability to 1) discriminate
12
13 patients with bipolar disorder from healthy control individuals and to 2) discriminate between
14
15 manic, depressive and remitted states in bipolar disorder.
16
17
18
19

20 Laboratory procedures

21
22 We will obtain careful standardization of blood sampling and laboratory analysis by obtaining
23
24 blood samples in a fasting state and in a one hour interval in the morning. At the same day as blood
25
26 sampling, smoking status, alcohol use, BMI and menstrual cycle, etc., will be assessed. Blood
27
28 sampling and all phases of laboratory processing for plasma and DNA/RNA analyses will be done
29
30 at the Department of Clinical Biochemistry, Rigshospitalet using standard operational methods
31
32 conducted by a team of technicians blinded with respect to participant status. All plasma samples
33
34 will be stored at – 80 C. The BIO study will include a total of 2400 blood samples: 300 patients x 5
35
36 + 200 healthy first-generation relatives x 3 + 100 healthy participants without a first-generation
37
38 family history x 3.
39
40
41
42
43

44 Biological tests

45
46 We will use a multianalyte panel including a large number of potential biomarkers such as plasma
47
48 levels of Neutrophins3, GSK-3, β -amyloid A β 40 and A β 42, BDNF, inflammatory markers, high
49
50 sensitive C-reactive protein, lipoproteins (VLDL, LDL, HDL) and specific apolipoproteins (e.g.
51
52 apoE, ApoA-I, apoA-II, and apoM) as potential markers of low-grade inflammation particularly
53
54 salient in the early stages of bipolar disorder. Total RNA, microRNA, genomic DNA and histones
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1
2
3
4 are isolated from peripheral blood mononuclear cells. Gene expression and alternative slicing of
5
6 RNA transcripts are analysed using array reverse transcription quantitative real-time PCR (RT-
7
8 qPCR) and next generation sequencing. Epigenetic modifications of the DNA (e.g. methylation) are
9
10 measured using antibody based methods or bisulphite treatments in combination with next
11
12 generation sequencing. The genomic positions of histones with specific modifications are detected
13
14 using immune precipitation and sequencing (CHIP-Seq). The degree of histone modifications is
15
16 measured by semi-quantitative antibody based detection.
17

18
19 Measurements of DNA and RNA damage by oxidation are obtained from spot urine samples and
20
21 analyzed using ultra performance liquid chromatographic (UPLC) and mass spectrometry.
22

23
24 Hair cortisol will be included as a valid and reliable index of long-term systemic cortisol levels
25
26 (31).
27

28
29 We will report on these individual biological tests including comparing patients, first degree
30
31 relatives and healthy control individuals, when appropriate. The BIO study sample of 600
32
33 participants is rather small for genetic analyses discriminating patients with bipolar disorder from
34
35 healthy controls but some genetic analyses, including the CACNA1C gene, can be conducted in
36
37 cooperation with national and international genetic network groups.
38

39 40 41 Statistical analyses

42
43 Data represent repeated measures within and between individuals and will be analysed using a
44
45 combination of generalized linear mixed models, integrated data analysis, and penalized regression
46
47 approaches to facilitate the combined feature selection and prediction of the available high-
48
49 dimensional data. Integrative data analysis ensures that we are able to identify an improved
50
51 composite blood-based biomarker since data from different molecular levels are combined in a
52
53 simultaneous analysis that closer resembles the biological system (50;51). Further, we will use cross
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4 validation or alternatively split sample designs in the development and validation of the composite
5
6 biomarker. Finally, if possible we will evaluate the biomarker(s) in external non-Danish data sets in
7
8 collaboration with other international researchers.
9

10 A general principle that pertains to statistical analyses of all four sub-studies is the “intention to
11
12 treat” principle. Accordingly, participants who during follow-up get a diagnosis with a higher
13
14 diagnostic validity than bipolar disorder (i.e. a lower ICD-10 diagnostic number, DF00, DF10 and
15
16 DF20) that may substantially influence the biomarker measures are included in the analyses until
17
18 onset of symptoms from the disorder but excluded from subsequent analyses. These disorders
19
20 include significant neurological disorders such as dementia, stroke, brain tumour, multiple sclerosis,
21
22 Parkinson’s disease, as well as disorders due to significant psychoactive substance use and
23
24 schizophrenia“.
25
26
27

28 Furthermore, in all four sub-studies, the problem of missing data will be alleviated by the use of mixed
29
30 effect model (for longitudinal measurements) and multiple imputations using chained equations
31
32 when applicable. If possible, joint modeling will be considered, depending on the missing
33
34 mechanism observed.
35
36
37
38

39 Statistical power

40
41 The study has a power of 80% to detect a minimum increment of 6.5 percentage points in sensitivity
42
43 if we assume that the existing diagnostic tools have a sensitivity of 70% to diagnose bipolar
44
45 disorder for a patient that has the disorder (see (52)). Thus, if the composite biomarker score
46
47 increases the sensitivity by a minimum of 6.5 percentage points then we have a power of 80% to
48
49 detect that increase based on 300 patients with bipolar disorder using a one-sided exact binomial
50
51 test (for fixed specificity).
52
53
54
55
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BIO-2: Smartphone-based electronic biomarker in bipolar disorder

Aims

To identify a composite smartphone-based electronic biomarker that 1) discriminates patients with bipolar disorder from healthy control individuals 2) discriminates between manic, depressive and remitted states 3) predicts emerging affective episodes, and 4) to investigate the change in the composite smartphone-based biomarker following onset of first manic episode, during successive relapses and in the end stages of the illness as according to the staging system by Berk et al (15).

Background and theoretical basis

Recently, electronic self-monitoring of the severity of depressive and manic symptoms using text messages has been suggested as an easy and inexpensive way to identify early signs of affective episodes, providing opportunities for mental health care providers to intervene shortly after prodromal symptoms first appear (53). We have, in the MONARCA project developed and tested a smartphone-based electronic monitoring system including daily subjective self-assessments of illness activity in bipolar disorder as well as a bi-directional feedback loop between the patient and clinicians (the MONARCA system (54-58)). Using the MONARCA system, fine-grained electronic data were collected during everyday life in naturalistic settings in patients with bipolar disorder. The MONARCA system was reported highly usable and useful by patients with bipolar disorder with a high self-assessment adherence (87-95%), and the patients reported that the MONARCA system helped them to better manage their disease (54;55). Further, the severity of depressive and manic symptoms was found to correlate with automatically generated smartphone data including 1) physical activity as reflected by the number of changes in cell tower ID per day (59), 2) social activity as reflected by the number of incoming and outgoing calls per day, the duration of incoming and outgoing calls per day, and the number of outgoing text messages per day (60), and 3)

1
2
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4 voice features collected during phone calls (61). Although these findings are encouraging there is a
5
6 need to integrate self-monitored smartphone data with automatically generated smartphone data on
7
8 social and physical activity, speech and sleep into one composite smartphone generated electronic
9
10 biomarker measure. This composite measure should be modeled to 1) discriminate patients with
11
12 bipolar disorder from healthy control individuals 2) have a high correlation with depressive and
13
14 manic symptoms 3) discriminate between euthymic, manic and depressive states 4) early predict
15
16 emerging affective episodes for the individual patient to increase the possibility for early
17
18 intervention.
19
20

21
22 To validate smartphone based measures of physical activity and sleep, a subset of patients will wear
23
24 a combined heart rate and movement sensor mounted at the thorax that has been shown to correlate
25
26 with mood symptoms and affective states and differentiate between patients and controls (Actiheart;
27
28 (32;33)) like other wearable actigraphs (62-64).
29
30

31 32 Methods:

33
34 All newly diagnosed/first episode patients will have access to a smartphone based system
35
36 (Monsenso that is a developed from the MONARCA system) for continuous self-monitoring as well
37
38 as fine-grained automatically monitoring of behavioural activity and early identification of
39
40 emerging affective episodes during the first two years and following relapse of episodes during a 5-
41
42 year follow-up period.
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44

45
46 Data analyses: In contrast to data in BIO-1, data in BIO-2 represent big data collected on a *daily* basis
47
48 within individuals. We will use hierarchical Bayesian predictive models that can handle big data through
49
50 sampling and visualization techniques that summarize data.
51
52

53 54 **BIO-3: neurocognitive and brain imaging signatures in bipolar disorder**

Aims

1) To identify an integrated brain-based biomarker of bipolar disorder including neurocognitive and neuroimaging measures tapping into ‘hot’ (i.e. emotion-laden) and ‘cold’ (non-emotional) cognition, 2) to examine whether the degree of abnormality in these measures predicts illness onset in the high-risk group and/or relapse in the patient group, 3) to identify developmental trajectories in ‘hot’ and ‘cold’ cognitive dysfunction and to identify structural and functional MRI correlates in bipolar disorder via longitudinal assessments of high-risk individuals to remission after onset of first manic episode and following successive relapses, and 4) to identify associations between aberrant ‘hot’ and ‘cold’ neurocognitive function, structural and functional brain changes and the composite blood-based and Smartphone-based biomarkers. Such ‘integrated systems approach’ involving identification of patterns of biomarkers (bio-signatures) across these multiple levels of investigation is considered imperative for deeper understanding of the dimensions of underlying pathophysiological processes in bipolar disorder (12).

Background and theoretical basis

Results from a number of meta-analyses of a large number of cross sectional studies of patients with bipolar disorder in remission suggest trait-related ‘cold’ cognitive deficits in attention/processing speed, memory, and executive function compared to healthy controls (65-68) that correlate with everyday functioning (69). Cross-sectional comparison of patients at different illness stages revealed more pronounced cognitive deficits during late compared with early stages in line with the staging hypothesis of bipolar disorder (70). However, there are only a few longitudinal studies of neuropsychological functioning with small sample sizes (12 studies including a total of 152 bipolar patients (71)). A meta-analysis of these studies found no support for a progressive nature of cognitive deficits (71). However, results from these studies are hampered by a number of limitations

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4 including small sample sizes, short follow-up (mean follow-up period of 4.6 years) and high
5
6 attrition rates (up to 45%) (71). Consequently, it is unclear whether cognitive function assessed with
7
8 neuropsychological tests deteriorate with the number and duration of illness episodes in bipolar
9
10 disorder although epidemiological studies consistently revealed increased risk of developing
11
12 dementia long-term (6;8;72-74) and there are some evidence for increasing risk of dementia with
13
14 the number of episodes (7) (see also (9)).

15
16
17 Deficits in 'hot' cognition are closely linked to emotional disturbances (75) and difficulties in socio-
18
19 emotional behaviour and interpersonal relations in bipolar disorder (76). 'Hot' cognition
20
21 abnormalities in bipolar disorder have been observed within three domains; (i) emotional
22
23 processing, (ii) reward processing and (iii) emotion regulation (reviews in (77;78)).

24
25
26 Results from a large number of cross sectional structural imaging studies suggest that patients also
27
28 show increased lateral ventricular volumes and greater prevalence of white matter hyper-intensities
29
30 (79). While these findings are rather unspecific, studies also suggest that treatment with lithium
31
32 increases the grey matter volume of prefrontal cortex, amygdala and hippocampus (79). In addition,
33
34 a number of functional imaging studies suggest that bipolar disorder is associated with
35
36 abnormalities within fronto-limbic-subcortical structures (38).

37
38
39 As long-term, integrative studies are lacking, it is unclear how neurocognitive and brain imaging
40
41 abnormalities correlate with the staging of bipolar disorder, illness progression and treatment
42
43 (38;80) or with changes in biological markers such as neurotropic, inflammatory and oxidative
44
45 stress markers (81;82).
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50 Methods

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53 Using a comprehensive neurocognitive test battery, we will assess all participants from the three
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55 groups (patients with bipolar disorder, first-generation relatives, and healthy individuals *without a*
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4 family history of affective disorders). Patients will be followed from first onset of affective disorder
5
6 and during successive periods of remission or at an annual basis (bipolar patients with no relapse,
7
8 first-generation relatives with no onset, and healthy controls. Among these, a subgroup of 60
9
10 patients, 60 healthy relatives (UR) and 30 healthy without a family history of affective disorders
11
12 (HC) will be undergo functional and structural MRI at these time points
13
14

15 16 17 Neurocognitive testing

18
19 Within ‘cold’ cognition verbal learning/memory and executive function have been highlighted as
20
21 the most suitable candidates for biomarkers of bipolar disorder (83;84). ‘Cold’ cognition will
22
23 therefore be assessed with neurocognitive tests probing verbal memory, attention and executive
24
25 function including the Rey Auditory Verbal Learning Test (RAVLT; (85;86)) and the WAIS-III
26
27 letter-number sequencing, RBANS digit span, n-back working memory, verbal fluency and Trail
28
29 Making Test B. Verbal intelligence will be estimated with the Danish Adult Reading Test (DART).
30
31 ‘Hot’ cognition will be assessed with a comprehensive battery of computerized neurocognitive tests
32
33 outside the scanner probing (i) emotional processing (ii) reward processing and (iii) emotion
34
35 regulation. These include the facial expression recognition and faces dot-probe tasks from the
36
37 Emotional Test Battery (ETB) (P1 Vital, Oxford), and an ecologically valid social scenarios test
38
39 developed by our group (87). During fMRI we will also administer the following experimental
40
41 paradigms: an emotional face processing task using face stimuli from the Nimstim
42
43 (<http://www.macbrain.org/resources.htm>), a monetary reward processing task (88), and (iii) a
44
45 negative affective picture task using validated stimuli sets from the International Affective Picture
46
47 System (IAPS) developed in collaboration with researchers at the Universities of Chicago. In
48
49 addition, we will explore the neuronal basis for ‘cold’ cognition (executive function) using n-back
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51 working memory and picture encoding tasks programmed in house (89). Finally, self-report
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4 measures (BIS/BAS, and the CERQ (90)) are used to assess reward responsiveness and habitual
5
6 emotional regulation strategies.
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10 11 12 13 Structural and functional MRI

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15 Structural MRI: using T1-weighted images acquired at a 3T Siemens scanner at the Copenhagen
16
17 University Hospital, Rigshospitalet, we will focus on lateral ventricular volumes, grey matter
18
19 volume of prefrontal cortex, amygdala and hippocampus, relative to whole brain volume.
20

21
22 Specifically, segmentation and analysis of subcortical and regional cortical volume, shape and grey
23
24 matter density will be conducted FMRIB Software Library (FSL) tools, including the FMRIB's
25
26 Integrated Registration and Segmentation Tool (FIRST), the FSL-VBM tool and FSL vertex (shape)
27
28 analysis (<http://fsl.fmrib.ox.ac.uk/>).
29

30
31 Functional MRI (fMRI): T2-weighted images will be acquired to investigate white matter hyper-
32
33 intensities. We will also use fMRI to investigate neuronal underpinnings of 'hot' and 'cold'
34
35 cognition with the previously described experimental paradigms. Functional MRI data processing
36
37 will be carried out with the FMRI Expert Analysis Tool, part of FMRIB's Software Library
38
39 (www.fmrib.ox.ac.uk/fsl). We will examine mean percent BOLD signal change within predefined
40
41 hippocampal and amygdala Regions of Interest (ROIs) obtained in standard space with mri3dX
42
43 (<http://www.idoimaging.com/program/160>). In addition, whole-brain exploratory analysis will be
44
45 conducted to explore neural activity differences in other cortical regions. For this group analysis,
46
47 individual contrasts of interest will be included in separate general linear models with
48
49 nonparametric permutation inference (n = 5000) using the 'randomize' algorithm implemented in
50
51 FSL (91).
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Statistical power

The above sample size for participants undergoing fMRI assessments is determined based on our previous fMRI studies. In particular, inclusion of about 17-22 participants per treatment/diagnostic group (matched for age and gender) had a power of >0.8 to detect differences between groups in neural and cognitive response to emotional faces (e.g. (92;93) and Miskowiak et al, under review) at an alpha-level of $p < 0.05$ for cross-sectional designs. For longitudinal designs, we were able to demonstrate differences between groups in *the change* in task-related neural activity and in hippocampal structure with a sample of about 40 participants per group (94-96). Given this, our inclusion of 60 participants per group is expected to ensure adequate statistical power for both the cross-sectional and longitudinal parts of the fMRI study.

BIO-4: at risk or prodromal phase for bipolar disorder

Aims

To test whether 1) the composite blood-based biomarker, 2) the composite electronic smartphone-based biomarker and 3) the neurocognitive signature for bipolar disorder predict onset of illness (depression or mania) among a healthy (i.e., non-syndromal level) high risk population of first degree siblings and offspring to the patients with newly diagnosed/first episode mania/bipolar disorder, included in BIO-1, BIO-2 and BIO-3.

Background and theoretical basis: First-generation relatives to patients with bipolar disorder have a nine-fold increased risk of developing bipolar disorder and a two to three-fold increased risk of developing unipolar disorder (1). Although there is a great need for early detection and primary prevention of onset of illness among relatives to patients with bipolar disorder recent prior attempts have not been successful due to retrospective designs, poor characterisation of high risk individuals

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4 and small sample sizes (97). This is the first time a composite blood-based, a composite
5
6 smartphone-based and a composite neurocognitive biomarker identified among patients with bipolar
7
8 disorder will be investigated among their healthy relatives as a predictor measure of onset of illness
9
10 (18). This approach increases the chances of obtaining a high specificity and sensitivity of the
11
12 composite biomarkers.
13

14 15 16 17 Methods

18
19 This BIO-4 includes first-generation relatives (siblings and offspring aged 15 to 40 years) to the
20
21 recruited patients with first manic or bipolar diagnosis. All recruited patients will be asked about the
22
23 lifetime psychiatric history of first-degree relatives (their biological parents, siblings and offspring)
24
25 based on the Brief Screening for Family Psychiatric History questionnaire described by Weissmann
26
27 and colleagues (98). We expect that at least 200 first-generation relatives will be asymptomatic or
28
29 present mild symptoms or prodromal patterns to affective disorders and will be included in the study.
30
31 Biological tissues will be drawn (as part of BIO-1) and neurocognitive function will be assessed on
32
33 all individuals and brain imaging will be done on 60 individuals with longitudinal assessments.
34
35

36 37 Statistical analyses

38
39 BIO4 will use a combination of penalized regression techniques, and random forests to infer the
40
41 importance of the original and combined markers and to compare the similarity of prediction error
42
43 from the models with and without combining the markers.
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52 Feasibility

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54 The BIO study is fully feasible as patients are recruited as part of daily health care for patients
55
56 referred to the Copenhagen Affective Disorder Clinic.
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Ethical considerations

The BIO study has been approved by the Local Ethical Committee (H-7-2014-007) and the data agency, Capital Region of Copenhagen (RHP-2015-023). According to these specifications, adolescents aged 15-18 years will be invited only if parents have given consent. Data will be saved, encrypted and assessed according to the regulations from the Capital Region of Denmark. The study complies with the Declaration of Helsinki principles (Seoul, October 2008).

Dissemination

Study results will be presented in peer reviewed journals and at international conferences in accordance with relevant reporting guidelines (99;100).

Discussion

Summary

It is expected that the BIO cohort will provide valid biological, electronic, neurocognitive and neuroimaging data and for the first time longitudinally identify changes in biomarkers during different stages of bipolar illness, i.e., at risk stage, following onset, during first relapse and recurrent relapses and in the late stages of the illness as according to Berk et al (15).

Limitations

Some limitations of the BIO study should be noted beforehand. First, the rather extensive initial assessment study procedure may potentially result in selection of participants that are intrinsically

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4 positive towards clinical research and readily willing to cooperate. Nevertheless, we expect that
5
6 such selection will be decreased as it is likely that the vast majority of the more than 100 newly
7
8 diagnosed/first episode bipolar patients referred to the Copenhagen Affective Disorder Clinic each
9
10 year will accept participation in the BIO study as this implies an extensive clinical evaluation.
11
12 Second, attrition may increase during long-term follow-up and patients who stay in the study may
13
14 adhere more to treatment in general. Such selection is inherent in clinical longitudinal research, and
15
16 the large number of participants that will be included will increase external validity. Third, potential
17
18 confounding effects of psychotropic medication may influence comparisons between patients with
19
20 bipolar disorder and healthy control individuals as well as comparisons within patients as the vast
21
22 majority of patients will get medication that may change during the course of illness. Lithium,
23
24 mood stabilizers and antipsychotics may have effects on the collected biological, smartphone-based,
25
26 neuropsychological and brain imaging data. Effects of medication on biological measures are not
27
28 clear (101) although analyses from systematic reviews and meta-analyses involving only bipolar
29
30 disorder patients have not found clear effects of medication on cytokines (34;35), BDNF (37), gene
31
32 expression (36) nor has subsequent individual studies on cytokines (44;102), BDNF (39;102), gene
33
34 expression (46) or DNA and RNA damage (40;41). Effects of medication on electronic smartphone
35
36 generated data as well as on neuropsychological and brain imaging data are poorly investigated and
37
38 warrants further studies (103).
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46 Fourth, due to the large number of biological and statistical tests included in the BIO study, chance
47
48 findings may occur in relation to the individual biological test. However, the aim of the BIO study
49
50 is to identify a composite biomarker measure related to bipolar illness, depression and mania using
51
52 cross validation or alternatively split sample designs in the development and validation of the
53
54 composite biomarker.
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9 Finally, the BIO study does not include (randomised) interventions limiting causal interpretations of
10 the results. Nevertheless, with the BIO prospective, repeated measures design it is possible to
11 identify valid associations between the composite measures (of biological, electrical,
12 neuropsychological and brain imaging data) and depressive and manic symptoms and states.
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19 Strengths

20
21 The BIO study is the first study aiming to identify 1) a composite blood-based biomarker, 2) a
22 composite electronic smartphone-based biomarker and 3) a neurocognitive signature for bipolar
23 disorder as well as to measure the same biomarkers in newly diagnosed/first episode bipolar
24 patients and their healthy first-generation relatives. It is possible to recruit newly diagnosed/first
25 episode patients with mania/bipolar as all such patients from the entire Capital Region of Denmark
26 are referred to the Copenhagen Affective Disorder Clinic and routinely asked for inclusion in the
27 BIO study. Long-term attrition is supposed to be low as all patients will be followed by the
28 Copenhagen Affective Disorder Clinic for the first two years and subsequently in other treatment
29 settings in the Capital Region of Denmark. Including longitudinal assessment of healthy individuals
30 is of paramount importance to control for the effect of timing and ageing (104), but among all
31 studies on biomarkers in bipolar disorder, this has been done only in two studies from our group
32 (40;44). The study will be performed by an experienced international research group, combining
33 expertise from all areas of the study.
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53 Conclusion

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4 The BIO study is a large long-term cohort study on biomarkers in bipolar disorder and we expect
5
6 that the findings for the first time will be representative of biomarkers in bipolar disorder in general
7
8 as no prior study on newly diagnosed/first episode bipolar disorder has been conducted. It is
9
10 expected that the BIO cohort will provide valid biological, electronically, neuropsychological and
11
12 brain imaging longitudinal data.
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21 Authors' contribution

22 LVK designed the study together with MV, KM, KWM and MFJ. LVK drafted the study protocol
23 and the manuscript. All authors contributed to development of the study protocol and to editing the
24 manuscript and read and approved the final version.

25 KM, LBN, RFS, CE, BKP, HEP, RSM, FK, WFG, and MV contributed specifically to BIO-1 on
26 peripheral blood-based biomarkers. MFJ, OW, JB, MF and OM contributed specifically to BIO-2
27 on smartphone-based electronic biomarkers. KWM, GMK and MP contributed specifically to BIO-
28 3 on neurocognitive and brain imaging signatures. MV contributed specifically to BIO-4 on at risk
29 or prodromal phase of bipolar disorder (in addition to BIO-1).
30
31
32

33 Funding

34 The study is funded by grants from the Mental Health Services, Capital Region of Denmark, The
35 Danish Council for Independent Research, Medical Sciences (DFF – 4183-00570), Weimans Fund,
36 Markedmodningsfonden (the Market Development Fund, (2015-310), Gangstedfonden (A29594),
37 Helsefonden (16-B-0063), Innovation Fund Denmark (the Innovation Fund, Denmark, 5164-
38 00001B), Copenhagen Center for Health Technology (CACHET), EU H2020 ITN (EU project
39 722561), Augustinusfonden (16-0083), Lundbeck foundation (R215-2015-4121).
40
41

42 Competing interests

43 LVK has within the preceding three years been a consultant for Lundbeck, AstraZeneca and
44 Sunovion. KWM has received consultancy fees in the past three years from Lundbeck and Allergan.
45 MFJ has been a consultant for Eli Lilly and Lundbeck. MV has within the preceding three years
46 been a consultant for Astra Zeneca and Servier. FK has been a speaker for Ache, Daiichi Sankyo
47 and Janssen. MP is a consultant for Roche Pharmaceuticals.

48 RSM: Advisory Boards: Lundbeck, Pfizer, AstraZeneca, Elli-Lilly, Janssen, Ortho Purdue, Johnson
49 & Johnson, Moksha8, Sunovion, Mitsubishi, Takeda, Forest, Otsuka, Bristol-Myers Squibb, Shire,
50 Speakers Fees: Lundbeck, Pfizer, AstraZeneca, Elli-Lilly, JanssenOrtho, Purdue, Johnson &
51 Johnson, Moksha8, Sunovion, Mitsubishi, Takeda, Forest, Otsuka, Bristol-Myers Squibb, Shire,
52 Research Grants: Lundbeck, JanssenOrtho, Shire, Purdue, AstraZeneca, Pfizer, Otsuka, Allergan.
53 HEP has received a research Grant from Boeringer Ingelheim. GMK has received honoraria as
54 Field Editor of the International Journal of Neuropsychopharmacology and as scientific advisor for
55 H Lundbeck A/S. JEB and MF are co-founders and shareholders in Monsenso.
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3
4 LBN, RFS and WFG declare no competing interests.
5

6 The validity of the research cannot be influenced by any of these potential secondary interest (such
7 as financial gain or personal relationship).
8

9
10 Patient consent
11 Obtained

12
13 Ethical approval

14 The study has been approved by the Local Ethical Committee (H-7-2014-007) and the data agency,
15 Capital Region of Copenhagen (RHP-2015-023).
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18 Study registration

19 The BIO study has been registered at clinicaltrials.gov with Trial Registration Number:
20 NCT02888262.
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Table 1. Overview of the longitudinal assessments during risk periods and following onset of bipolar disorder in the four sub-studies of the BIO study.

Course	Healthy first-gen. relatives	First episode	Remission	First relapse	Remission	Second relapse	Etc.
BIO-1	x	x	x	x	x	x	x
BIO-2	x	x	x	x	x	x	x
BIO-3	x		x		x		x
BIO-4	x	x	x	x	x	x	x

BIO-1: Peripheral blood-based biomarker in bipolar disorder

BIO-2: Smartphone-based electronic biomarker in bipolar disorder

BIO-3: Neurocognitive and brain imaging signatures in bipolar disorder

BIO-4: At risk or prodromal phase for bipolar disorder

Reference List

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