STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official Title: A Probiotic Intervention to Prevent Relapse Following Hospitalization for Mania

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The University of Texas Southwestern Medical Center at Dallas
Institutional Review Board

Protocol

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1. Introduction and Purpose:

A probiotic containing the microorganisms Lactobacillus GG and Bifidobacteria lactis strain Bb12 has recently been evaluated in a population hospitalized for acute mania. Findings from this trial indicated that those taking the adjunctive probiotic had a reduced rate of relapse, as defined by hospital readmission, compared to those taking adjunctive placebo. We will conduct a replication trial to confirm the clinical findings and extend understanding of the mechanism by which the probiotic affects these clinical outcomes.

Primary Aim 1. To determine if adjunctive probiotic administration can reduce relapse for participants first hospitalized for mania. *Hypothesis*: Participants receiving adjunctive probiotic microorganisms vs. adjunctive placebo will have a lower rate of relapse as defined by a re-hospitalization (e.g., admission to an inpatient unit) during the 24 week study period.
Secondary Outcomes. The number of new mood episodes, the severity of psychiatric symptoms, and changes in cognitive scores over the 24 week study period will be evaluated.

Exploratory Aim 1. To study the effect of probiotic therapy in lowering the levels of inflammatory markers following an acute episode of mania. *Hypothesis:* Participants receiving adjunctive probiotic microorganisms vs. adjunctive placebo will have reduced levels of antibodies to casein, gliadin, and the NMDA receptor, and reduced levels of C-Reactive protein and the cytokine TNF alpha following 24 weeks of probiotic therapy.
Exploratory Aim 2. To evaluate changes in the gut microbiota following probiotic administration. *Hypothesis*: Probiotic administration will enrich the gut microbiota of participants with the given microorganisms and these changes may correlate to changes in the peripheral inflammatory markers being measured.

2. Background:

Mania is an abnormal mood state and the defining characteristic of bipolar disorder. Individuals with mania are at high risk for ongoing mood symptoms and the recurrence of new mood episodes, which contribute to social disability and reduced quality of life (1).The etiology of mania is largely unknown. Although genetic factors play a major role in the etiology of mania and bipolar disorder, most have relatively low odds ratios (2). The role of gut bacteria in the expression of mania and the use of probiotics to modulate existing gut bacteria have been explored for psychiatric illness, including mania (3-5). Most probiotic products contain bacteria from the genera Lactobacillus or Bifidobacteria, specifically Lactobacillus strain GG and Bifidobacteria lactis strain Bb12. These microorganisms are found in breast milk and may contribute to the healthier status of infants who were breast-fed compared to infants who were not breast-fed (6). Probiotic microorganisms are also found in fermented dairy products such as yogurt as well as in several fermented food products such as sausages. However, the concentration of probiotic organisms in these foods is not consistent and the organisms are subject to degradation during food processing and storage. Therefore, in this trial, probiotic compounds will be prepared by a specialty company (Chr. Hansen) that can guarantee the high concentration and quality of the probiotic organisms. Probiotic microorganisms are considered highly safe due to the non-pathogenic nature of the bacteria. The preparations are generally well-tolerated and serious complications even in immune incompetent individuals are extremely rare (7).

Immunological abnormalities have been identified in individuals with mania and may contribute to the pathophysiology of mania (3, 4, 8-10) as well as to bipolar disorder more broadly (11). With further understanding of their role in mania and bipolar disorder, measures of immune activation may

help to define disease states and predict clinical course. The identification of inflammatory markers that contribute to mania also opens new possibilities for treatment. Increased immune reactivity to food antigens is one type of immunologic abnormality which has been previously described in psychiatric populations including bipolar disorder (12) and mania (4). Of particular interest in terms of food antigens are bovine casein, the main component of cow's milk, and gliadin, a glycoprotein which is the main antigen derived from gluten, the principal glycoprotein in wheat. In a recent study (4), 60 individuals were assessed during a hospital stay for acute mania, 39 of whom were assessed at a 6 month follow-up. At baseline, individuals with mania had increased levels of IgG antibodies to gliadin but not other markers of gluten sensitivity compared with 143 controls ($F=4.99$, $p<.027$). However, these levels were not significantly different from those of the controls at the 6 month follow-up. Individuals who were re-hospitalized during the follow-up period (28% of the sample) were more likely to have a higher level of IgG antibodies to gliadin at the follow-up (hazard ratio 1.04; 95% CI 1.02, 1.07; $p=.001$).

Cytokines are another type of inflammatory marker and there is also evidence that cytokines are elevated in individuals with mania (6, 11). There is also an association between inflammatory markers and cognitive functioning. In a recent study of 1112 individuals with bipolar disorder, significantly increased odds of low cognitive scores, as measured by the total RBANS score, for individuals who had a CRP level higher than the 90th percentile level (OR= 8.1; 95 % CI 1.8, 35.2; $p=.006$) and the 75th percentile level (OR=4.8, 95 % CI 1.62, 14.1; $p=.005$) of the control group. Probiotics provide a safe and well-tolerated means for the modulation of the immune response to harmful antigens such as food-derived proteins. Probiotic microorganisms have been shown to reduce levels of autoantibodies (13, 14) and cytokines (15, 16).

3. Concise Summary of Project:

We will conduct a 24-week, randomized, double-blind, placebo-controlled trial of adjunctive probiotic therapy in 66 persons hospitalized with a manic or mixed episode. The study will take place at UT Southwestern Medical Center, Dallas, TX and John Peter Smith (JPS) Health Network, Fort Worth, TX. The active study compound will consist of capsules containing approximately 10^9 colony forming units of the probiotic organisms, Lactobacillus GG and Bifidobacteria lactis strain Bb12. The dose has been selected because it has been used safely in other probiotic trials, was well-tolerated by the participants in two previous trials of individuals with schizophrenia or mania, and was utilized in the original trial on which this replication is based. This dose is higher than that available in most commercially-sold health food supplements. Following hospital discharge, participants will be randomized to receive adjunctive probiotic or placebo for a 24 week period. It is anticipated that of the 66 participants randomized, ~50 (75%) will complete the full 24 weeks of the study. The primary outcome is relapse, defined as re-hospitalization (e.g., admission to an inpatient unit) for psychiatric symptoms following a previous hospital discharge by at least 2 weeks. The occurrence of new mood episodes, the severity of psychiatric symptoms, and any changes in cognitive test scores over the course of the study will also be evaluated. Changes in the levels of inflammatory markers as well as changes in gut microbiota will be evaluated at three time intervals over the course of the study.

4. Study Procedures:

Potential participants will be approached during the inpatient hospital stay, if possible, and, if s/he is eligible, interested and willing, sign consent at that time. It is expected that many participants will elect to sign consent at that time and schedule the baseline visit. The baseline visit will be completed within 4 weeks of discharge after the hospital stay.

Baseline: For this Baseline visit (~3-3.5 hours), the psychiatric diagnosis will be confirmed by the structured clinical interview for DSM-*5* (SCID), and background information including age, race, gender, educational attainment, psychiatric and medical history, and current medications will be collected. Mood and suicidality will be assessed via the Montgomery-Asberg Depression Rating Scale (MADRS), the Young Mania Rating Scale (YMRS), Brief Psychiatric Rating Scale (BPRS), the Columbia-Suicide Severity Rating Scale (C-SSRS), the Internal State Scale (ISS) and quality of life via the Quality of Life Enjoyment and Satisfaction Questionnaire (QLESQ) and the Sheehan Disability

Scale. Cognitive function will be assessed by the Trail Making Test (TMT), the Controlled Oral Word Association Test (COWAT), Stroop task, and the Ray Auditory Verbal Learning Test (RAVLT). The Treatment Impression Inventory (TII) will evaluate the participant's views and feelings about medical treatment. Dietary intake for the past 24 hours will be assessed.

Blood will be drawn for complete blood count (CBC), Comprehensive Metabolic Panel (CMP, includes a liver panel with AST, ALT). A urine sample for drug screen, pregnancy test (if applicable), urinalysis and later analysis of immune markers, throat swabs to examine the oral microbiota, stool specimens to examine the gut microbiota (these can be collected by the participant at home and returned to the clinic within 1 week of the in-person visit), psychiatrist assessment, physical exam, collection of height, weight and vitals will be completed. Drug screening may be done at any time during the study at discretion of research staff. If available, data from the hospital admission work-up, performed within the past 30 days, may be used to establish medical eligibility. We will also ask to obtain the hospital admission record to determine reason for admission. We will obtain consent from the participant to contact the participant's outpatient treatment providers to inform them of the study and establish communication with the providers. In the case of participants who do not yet have outpatient treatment providers at the time of the baseline visit, consent to contact the participant's outpatient treatment providers will be obtained once the providers have been identified.

Abnormal lab values will be likely be detected in some participants. In the event that the study physician determines that a participant's lab value is clinically significant, the participant will be made aware of any abnormal laboratory findings and will be referred for follow-up with his/her primary care physician or other appropriate medical care. Clinically significant abnormal laboratory findings may be repeated. Lab results may be sent to participant's primary care physician or other treatment providers if requested and copies of lab-work will be made available to participants upon their request. In the case of a serious medical illness detected by lab values or other clinical information, the study physician may determine that a participant should not be enrolled in the study or be terminated from the study if participation has already begun.

Randomization

Double-blind randomization will occur after eligibility has been established at the baseline visit. Each participant will be randomly assigned to receive either the probiotics compound or placebo, which they will begin to receive at the week 0 in-person visit. Block randomization based on number of previous hospitalizations in the last year will be used to assign participants to the two treatment groups. Only the statistician and the study staff which prepare the medications will be aware of participant assignment; the principal investigators and other research staff will remain blinded until all participants have completed the study.

The double-blind treatment phase will last from randomization at Week 0 until the end of 24 weeks. After randomization, the participant will be seen in-person again at weeks 4, 8, 12, 16, 20, and 24 when symptom evaluations and assessments of dietary intake will be performed. Phone contact will be made with the participant every week between in-person study visits to promote adherence to the study and to identify any study-related problems.

Week 0: Once the participant is deemed eligible to participate in the study, they will be randomized and receive study compound. The coordinator will ask about any changes to medications, current treatment or any re-hospitalizations. They will also complete the ISS to evaluate mood.

Phone contacts: The weekly phone contacts (~20mins, Weeks 1-3, 5-7, 9-11, 13-15, 17-19, 21-23) will collect reports of any adverse events, including current pregnancy, medication compliance by participant self-report, report on current treatment and any re-hospitalizations, updates to the medication list, and assessment to determine the current diagnosis and add any new mood episodes. Participants will be asked to complete the ISS via a link sent to their email via REDCap.

Virtual visits, Weeks 4, 8, 16, and 20 (~1hr): These in-person visits will collect reports of any adverse events, including pregnancy, updates on current treatment and medications and notation of any re-hospitalizations, pill counts and/or participant report of compliance, assessment to ascertain the

current diagnosis and any new mood episode, the YMRS, MADRS, BPRS, QLESQ, CSSRS, ISS. Weight and vitals will also be collected, and a brief evaluation with a psychiatrist will be conducted.

In-person visits, Weeks 12 and 24 (~2hrs): These in-person visits will collect reports of any adverse events, including pregnancy, updates on current treatment and medications and notation of any re-hospitalizations, pill counts and/or participant report of compliance, assessment to ascertain the current diagnosis and any new mood episode, YMRS, MADRS, BPRS, QLESQ, the Sheehan Disability Scale, CSSRS, ISS, TMT, COWAT, Stroop task, and RAVLT, and assessment of dietary intake of participant based on a 24 hour recall of food intake. During the recall, each participant will be shown a selection of portion sizes to obtain the most accurate information. The participant will also be questioned about his/her intake of any foods or supplements containing probiotics. Blood, throat swabs, stool and urine samples, as well as weight and vitals will also be collected. A brief evaluation with a psychiatrist will be conducted. At week 24 (or, in case of early discontinuation, the last study visit done by participant), an exit survey will be administered to evaluate participant's perception of in-person vs. virtual study visits.

Follow-up Phone Call, 6 months following completion of study compound (~15 min): This call will collect information about any (re)hospitalization for either psychiatric or medical reasons, any current treatment, and complete the ISS and the short-form CSSRS.

	Baseline	Week 0	Weeks 1 -3	Week 4	Weeks 5-7	Week 8	Weeks 9-11	Week 12	Weeks 13-15	Week 16	Weeks 17-19	Week 20	Weeks 21-23	Week 24
SCID	X													
Physical Exam & Psych Eval	X													
Blood draw, stool collection, urine collection	X							X						X
Height (BL only), Weight, and Vitals, Clinician Follow-up	X			X		X		X		X		X		X
Tx &/or Hospitalization, ISS	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Dispense Study Compound		X		X		X		X		X		X		
New Mood Episode		X	X	X	X	X	X	X	X	X	X	X	X	X
BPRS, YMRS, MADRS, QLESQ, CSSRS	X			X		X		X		X		X		X
TMT, COWAT, RAVLT, Stroop	X							X						X
TII	X													
Sheehan Disability Scale	X							X						X
Dietary Questionnaire	X							X						X
Exit survey														X

The participants will receive:

- $40 for completion of the Baseline Visit.

- $40 for Week 0.
- $180 for phone visits (18 visits, $10 each).
- $180 for in-person visits (6 visits, $30 each).
- $100 for additional lab work and assessments at Weeks 12 and 24 (2 visits, $50 each):
- $50 for a completion bonus provided at the completion of the Week 24 visit.
- The total compensation received by participants who complete all study visits will be $590.

5. Criteria for Inclusion of Subjects:

- Age 18-65.
- Capacity for written informed consent
- Currently (or within the last 4 weeks) admitted to inpatient hospital for symptoms of mania.
- Primary Axis I diagnosis (DSM-5) of bipolar I (single manic episode, most recent episode manic, or most recent episode mixed) OR schizoaffective disorder, bipolar type (manic or mixed state).
- Proficient in the English language.
- Available to come to the Research Clinic for follow-up visits.

6. Criteria for Exclusion of Subjects:

- Substance- or medically-induced symptoms of mania at time of assessment.
- HIV infection or other immunodeficiency condition (such as receiving cancer chemotherapy).
- A serious medical condition that affects brain or cognitive functioning (e.g., epilepsy, serious head injury, concussion involving loss of consciousness, brain tumor, or other neurological disorder). Note that Hepatitis-C is not an exclusion criterion unless the participant has an acute infection.
- Poorly controlled comorbid medical condition
- Major surgery in the last year
- History of weight loss surgery
- Diagnosis of Intellectual Disability or history of severe learning disorder.
- Diagnosis of alcohol or substance use disorder (moderate/severe) according to DSM-5 criteria within the last 3 months, or has a positive drug toxicity screen (except for THC) proximate to the time of recruitment.
- History of IV drug use in the last 6 months.
- Participated in any investigational drug trial in the past 30 days.
- AST or ALT > 3 times upper limit of normal
- Abnormal electrolyte levels
- Pregnant, breastfeeding, or planning to become pregnant during the study period.
- Documented celiac disease (as such persons should be on a gluten-free diet as this is the standard care). Of note, we are not limiting the study to individuals with elevated levels of gliadin or casein antibodies as we intend to look at these levels as a predictor of response.

<u>Discontinuation from the Study</u>

A participant may be discontinued from the study, at any time, at the discretion of the study principal investigator. The participant will be asked to complete the final study visit if discontinued.

A participant may be discontinued for the following reasons:

- If the participant does not follow the study directions (for example, not taking the minimum number of study supplement)
- If it is the judgment of the study physician, in an effort to improve the participant's medical care or if the participant develops a serious medical illness
- If the participant has a positive drug screen for drugs other than THC, staff will inform the participant that a repeat test will be done at the next in-person visit; if this is positive then he/she will be removed from the study.
- If the participant has unexpected or serious side effects.

- If the participant reports she is pregnant.

7. Sources of Research Material:

For our clinical assessments, we will use the SCID, MADRS, YMRS, BPRS, CSSRS, QLESQ, the Sheehan Disability Scale, TMT, COWAT, Stroop task, RAVLT, and a dietary recall over the last 24hrs.

For our biological measures, we will obtain stool samples at three different time points for analysis of the gut microbiota, multiple urine samples of approximately 5 mLs each time for urinalysis, urinary drug and pregnancy screening (if necessary), and to analyze the levels of neuroactive peptides derived from casein or gliadin. For our blood markers, we will collect samples approximately 31.5mLs blood at baseline for analysis of peripheral immune markers, metabolic factors, and blood cell count analysis. Additional blood draws at Weeks 12 and 24 will be 2 mLs for analysis of peripheral immune markers. A redraw may be requested if the first sample cannot be processed. Any abnormal ranges will be reviewed by a clinician and must be deemed not clinically significant in order to proceed with enrollment in the study. Those blood samples not sent to Quest Diagnostics, urine samples, throat swabs, and stool specimens will be analyzed in the Johns Hopkins laboratory of Dr. Robert Yolken, one of the study collaborators. Samples will be identified by participant number only when they are sent to laboratories for analysis. The clinical investigators will be blind to these results of the participant's research laboratory tests during the course of the trial.

8. Recruitment Methods and Consenting Process:

UT Southwestern and John Peter Smith (JPS)

The UT Southwestern study site will conduct their recruitment primarily at the inpatient psychiatric hospital programs at Zale Lipshy, Parkland Memorial Hospital, and Texas Health Resources Presbyterian Dallas. The JPS study site will recruit through the JPS Behavioral Service, adult inpatient unit at Trinity Springs Pavilion and Long Term Care Alternative, as well as the JPS Psychiatric Emergency Center, and JPS outpatient Behavioral Health clinics. Research coordinators will contact admitting staff at the inpatient facilities to determine if any potential participants are present, it is currently expected that researchers will contact the facility at least once weekly. If the admitted staff confirm that a potential participant is present, the research coordinator will then travel to the appropriate inpatient facility and speak with the patient. Only patients who are fully able to understand the consent form, have a conversation with the research coordinator, and be able to give consent will be contacted. The patient will have all aspects of the study explained to them and, if the patient determines they would like to participate, they can sign consent at that time. If possible, the baseline visit will be scheduled for a date after the participant is discharged from the hospital.

Texas Health Resources

Recruitment flyers will be placed into the general discharge paperwork for all patients being discharged from the adult psychiatric inpatient unit at Texas Health Resources facilities (Texas Health Recovery and Wellness Center, Texas Health Arlington Memorial Behavioral Health, Texas Health Seay Behavioral Health Plano, Texas Health Springwood Behavioral Health Hospital HEB). If interested in study participation, patients discharged from the listed THR facilies will contact the listed UT Southwestern study staff on the study recruitment flyer for study eligibility screening.

9. Potential Risks:

Adverse Effects and Drug Interactions

Probiotic compounds have been widely used in clinical trials with an excellent safety record. Over-the-counter probiotic preparations have received GRAS (Generally Recognized As Safe) status from the United States Food and Drug Administration. An IND has been obtained for this study. While the administration of live organisms poses some theoretical risks, serious complications of probiotic administration are extremely rare (17) and no major side effects are expected in this study. Safety information provided by the manufacturer confirms the favorable safety profile of the probiotic product. Side effects, if they occur, tend to be mild digestive symptoms such as gas or bloating (18), any mild

gastrointestinal disruption can generally be minimized by administering the organisms with food which will be the advice given to participants this study. Generally, anaerobic organisms residing in the gastrointestinal tract are only minimally affected by most antibiotics in common usage. Once enrolled in the study, any participant who is subsequently prescribed antibiotics will not be removed from the study.

The probiotic organisms may have difficulty establishing colonization in the presence of antibiotics. It is thus preferable that the probiotic compound be administered to an individual who has not received systemic or broad acting antibiotics over the previous 24 hours. On the other hand, the administration of antibiotics is unlikely to affect organisms which have already colonized the intestinal tract. It is thus not necessary to withhold antibiotics to an individual who is already in the study or to remove individuals from the study who are receiving antibiotics. Any administration of antibiotics will be noted and reported descriptively as part of the analyses.

Psychological Stress

Some of the questions asked as part of this study may make the participant feel uncomfortable. They may refuse to answer any of the questions, take a break or stop participation in the study at any time.

Risks of Blood Drawing

Risks associated with drawing blood from the arm include minimal discomfort and/or bruising. Infection, excess bleeding, clotting, and/or fainting also are possible, although unlikely.

Placebo

Receiving a placebo means that no active medication will be administered for a health problem; however, in this study, the placebo is administered as adjunctive treatment to the participants normally prescribed medications. Nevertheless, if the problem becomes worse, participation in the research could stop. If this happens, the study doctor can discuss alternative care with the participant.

Loss of Confidentiality

Any time information is collected; there is a potential risk for loss of confidentiality. Every effort will be made to keep the participant's information confidential; however, this cannot be guaranteed.

10. Subject Safety and Data Monitoring:

Adverse Events and Reporting

For the purposes of collecting and evaluating all information found during this clinical study, an adverse event (AE) is any undesirable or unexpected experience that occurs after informed consent has been obtained without regard to the possibility of a causal relationship and without regard to treatment group assignment. A serious adverse event (SAE) is any adverse event occurring that: results in death, is life threatening, results in inpatient hospitalization or prolongation of existing hospitalization, results in a persistent or significant disability/incapacity, or results in congenital anomaly/birth defect. UT Southwestern requires AEs/SAEs to be reported if they are unexpected, definitely study-related, and put the participant and the participant pool at an increased risk of harm. The non-reportable AEs/SAEs will be tracked and summarized on the continuing review.

Each participant will be seen or spoken to on a weekly basis and assessed for the presence of any adverse reactions. AEs and SAEs will be monitored using standard definitions. A participant with AEs or SAEs will be followed until they return to baseline or stabilize regardless of whether the participant has discontinued or completed the study. AEs and SAEs will be reported to the Data Safety Monitoring Board, comprised of clinicians with a keen awareness of the risks for acutely ill psychiatric patients and the general disease course for those hospitalized with mania or bipolar disorder. Details can be found in the separate DSMB Charter.

Worsening of Illness

Given that re-hospitalization is the primary outcome of this study, it is expected that some proportion of participants will again require admission for stabilization of their psychiatric symptoms. Research study staff will continuously monitor the status of psychiatric symptoms and ensure that participant safety is of upmost concern. Each participant will be instructed and expected to continue

treatment with his/her mental health providers and to remain on his/her prescribed psychotropic medications throughout the study. Psychiatric treatments that the participant receives will be noted and included in the analyses. We note that the participant's usual psychiatric treatment will be uncontrolled in this study; to standardize such treatment would not be practical or ethically feasible given the individualized nature of treatment regimens and responses. If a participant reports psychiatric symptoms that raise concerns about safety such as imminent, potentially dangerous behavior, research staff will consult with the study PI and/or study physician and may contact the participant's current treatment providers, as permission to contact these providers will be obtained as early in the study as is available. Research staff will encourage participants to seek immediate help in these cases. Additionally, research staff may arrange for emergency assessment if a participant presents as a danger to self or others, in consultation with the study PI and/or the study physician. If the participant is deemed an acute risk for serious consequences, including suicide, the physician will direct the measures necessary to get the participant to safety, using clinic specific crisis management procedures which may include getting the participant to the Psychiatric Emergency Room. The staff at the Psychiatric ER would then determine the necessary steps and would decide if the participant's symptoms warrant admission to the hospital.

11. Procedures to Maintain Confidentiality:
Blood specimens for UT Southwestern patients will be provided to Quest Diagnostics for analysis, and identifying information (e.g., name) that could be linked to the patient's identity will be removed prior to sending for analysis. The blood provided to Quest will have the participant's year of birth to allow for accurate analysis and correctly link the results to the participant. The results of this test will be de-identified and added to the rest of the de-identified data. Once de-identified, only the identified investigators can learn/discover the names of participant. All samples which are sent to Dr. Yolken's lab at Johns Hopkins for analysis of the biological factors will also be de-identified. Similar to the blood specimen data, the results will be combined with the wider data set in which the participants identifying information is not present.

Blood specimens for JPS patients will be assessed within JPS's central laboratory and reported per usual clinical procedure in the JPS electronic medical record. No identifying information for patients will travel outside the JPS system for these laboratory analyses.

Data Management Plan
All data related to the participant's participation in this study will be given a study ID which cannot be traced back to the patient except by study staff. All personal information which can identify the patient's identity will be kept separately from study documents and assessments. All clinical assessments will be collected in paper form and scores transferred to a computer database maintained on a password protected computer, on a secure server behind a firewall. Remote data entry at JPS will be done via encrypted, direct data entry from paper form data to the RedCap only study program. The paper forms will be kept in binders marked only with the patient's study ID and will be kept in a locked room for the required time period following the completion of this study.

12. Potential Benefits: The original study on which this replication study is based indicated that adjunctive probiotic therapy was able to reduce the rate of relapse in the patient population. If the results of this current study can confirm and extend these original findings, this would provide important evidence supporting the use of probiotics in treatment interventions to improve clinical outcomes.

13. Biostatistics: The primary outcome of the study will be re-hospitalization for mania or for other psychiatric symptoms. The placebo- and probiotic-supplemented groups will be compared by means of Kaplan-Meyer analysis using time to re-hospitalization as the primary variable. Censoring will be performed to account for individuals who do not complete the study so all who have at least one post-randomization visit will be included in the analyses. Additional variables such as gender, age, and diagnosis will be included in the analysis by the use of Cox Proportional Hazards Estimates. The

previous study on which this study is based was able to find variables which correlated significantly with risk of re-hospitalization with a sample size of n=66, indicating that this sample size is adequate for the detection of an effect of the probiotic therapy. Secondary and exploratory outcomes, such as the number of new illness episodes, symptom severity, changes in cognitive test scores, or the effect of treatment on the levels of inflammatory markers over the course of the study will be performed by means of chi-square, analysis of variance, and other analyses comparing the placebo- and probiotic-supplemented groups.

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