PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A multidisciplinary approach to mental illness: do inflammation, telomere length and microbiota form a loop? A protocol for a cross-sectional study on the complex relationship between inflammation, telomere length, gut microbiota and psychiatric disorders
AUTHORS	Manchia, Mirko; Paribello, Pasquale; Arzedi, Carlo; Bocchetta, Alberto; Caria, Paola; Cocco, Cristina; Congiu, Donatella; Cossu, Eleonora; Dettori, Tinuccia; Frau, Daniela Virginia; Garzilli, Mario; Manca, Elias; Meloni, Anna; Montis, Maria Antonietta; Mura, Andrea; Nieddu, Mariella; Noli, Barbara; Pinna, Federica; Pisanu, Claudia; Robledo, Renato; Severino, Giovanni; Sogos, Valeria; Chillotti, Caterina; Carpiniello, Bernardo; Del Zompo, Maria; Ferri, Gian Luca; Vanni, Roberta; Squassina, Alessio

VERSION 1 - REVIEW

REVIEWER	Peter M Nilsson Lund University, Sweden
REVIEW RETURNED	03-Aug-2019

GENERAL COMMENTS	This is an observational study on the role of telomere lenght. inflammation and microbiota patterns in patients with psychiatric disorders as compared to healthy controls, in Sardinia, Italy. The recruitment is ongoing. The research question is adequate and the methods mostly valid. However, I have the following questions and comments to the authors: 1. There is a need af appropriate references for the methods used to determine telomere length (qPCR and qFISH) as well as the methods for assessment of microbiota patterns. This is standard and also customary. 2. The power calculation for estimation of sample size was based on a previous study measuring microbiota patterns before and after treatment with an anti-psychotic drug. This may be less relevant for comparing different psychiatric disorders. I fear that the numbers needed to screen will prove to be higher than the authors have expected. 3. Obesity is a major confounder and often differ between patients with mental disorders and healthy controls, both due to the disease itself and to its treatment as for example antipsychotic drugs may increase body weight. This may also influence telomere length, chronic inflammation and microbiota composition.

	Therefore different measures of obesity (BMI, WHR) are needed to calculate and to adjust for when groups are compared. 4. The role of TMAO is not so straight forward as previously thought. Will it be measured in the study or not? Please explain and also provide a more balanced view on this biomarker. 5. Participants will be excluded if they used antiobiotics during the previous month before screening. However, modern studies tell that this effect may reside up to 3 (even 6) months. Please modify and provide more exact intstructions. 6. Data on diet is very important when analysing microbiota patterns, but not much is revealed about this. Please specify more. 7. So far only 19 subjects with major depressive disorder has been recruited, and this is far from the goal of 40. How will this goal be achieved? Will recruitment also take place at other hospitals, if needed? 8. Please refer to the CONSORT, STROBE or PRISMA checklists in epidemiological studies. Minor: 1. The dates of the study must be mentioned (starting date, screening period) 2. In key words, "sybr green" is not possible to understand - please explain or delete

REVIEWER	Feng Zhang Xi'an Jiaotong University,China
REVIEW RETURNED	27-Sep-2019

GENERAL COMMENTS	In this current study, the authors investigate the complex interaction/s between inflammation, age-related co-morbidities, telomere shortening and the gut microbiota in psychiatric disorders. Some comments are as follows. 1. Page 6 line 18. It is recommended to add more descriptions about MD and SCZ in the introduction section. 2. Page 6, line39-40. Please add the detailed description of " involvement of altered telomere and inflammatory dynamics in the neurobiological underpinnings of severe psychiatric disorders and suggest that signatures of these processes might be detectable in peripheral tissues" 3. Page 11, line 3. The recruitment criteria for healthy controls are only described by the mouth of the surrounding people, and lack of convincing power. A series of medical health checks should be performed on the health controls so that they can be determined. 4. In cross-sectional studies, the determination of the size of the sample is very important, which is directly related to the authenticity of the results. In this study, the lack of sample size leads to insufficient credibility of the results, and it is recommended to increase the sample size in the subsequent research

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

Reviewer Name: Peter M Nilsson

Institution and Country: Lund University, Sweden

Please state any competing interests or state ‘None declared’: No competing interest.

Please leave your comments for the authors below

This is an observational study on the role of telomere length, inflammation and microbiota patterns in patients with psychiatric disorders as compared to healthy controls, in Sardinia, Italy. The recruitment is ongoing.

The research question is adequate and the methods mostly valid. However, I have the following questions and comments to the authors:

1. There is a need of appropriate references for the methods used to determine telomere length (qPCR and qFISH) as well as the methods for assessment of microbiota patterns. This is standard and also customary.

R. We thank the reviewers for the precious comments provided and issues raised, which we tried to address at our best. More details about the laboratory procedures and appropriate references were added.

2. The power calculation for estimation of sample size was based on a previous study measuring microbiota patterns before and after treatment with an anti-psychotic drug. This may be less relevant for comparing different psychiatric disorders. I fear that the numbers needed to screen will prove to be higher than the authors have expected.

R. As correctly pointed out by the reviewer, the estimates of the sample size needed to achieve an adequate statistical power is based on a prospective longitudinal study of 41 patients affected by schizophrenia (Yuan et al. 2018). Although we partly agree with the point raised, we would like to highlight that previous research of microbiota variation in SCZ (particularly in case-controls studies) has been performed in samples of lower or comparable size: Schwarz et al. (2018), N= 28, Castro-Nallar et al. (2015), N=16, Yolken et al. (2015), N=41. Similarly, in MDD a recent systematic review (Cheung et al. 2019) shows that sample sizes of published studies ranged from 10 to 63 individuals. Further, our study remains proof of principle (as stated in the Discussion) and does not necessarily need a power and sample size calculation. We highlight the exploratory spin of this part of our study in our revision of the sample size estimation section: “Even if the microbiota analysis remains mainly exploratory it is important to note that previous research was performed in samples of comparable size both for SCZ 28 and for MDD29.”

References

Castro-Nallar E, Bendall ML, Pérez-Losada M, Sabuncyan S, Severance EG, Dickerson FB, Schroeder JR, Yolken RH, Crandall KA. Composition, taxonomy and functional diversity of the oropharynx microbiome in individuals with schizophrenia and controls. PeerJ. 2015;3:e1140.

Cheung SG, Goldenthal AR, Uhlemann AC, Mann JJ, Miller JM, Sublette ME. Systematic Review of Gut Microbiota and Major Depression. Front Psychiatry. 2019;10:34.

Schwarz E, Maukonen J, Hyytiäinen T, Kieseppä T, Orešič M, Sabunciyan S, Mantere O, Saarela M,

Yolken RH, Severance EG, Sabunciyan S, Gressitt KL, Chen O, Stallings C, Origoni A, Katsafanas E, Schweinfurth LA, Savage CL, Banis M, Khushalani S, Dickerson FB. Metagenomic Sequencing Indicates That the Oropharyngeal Phageome of Individuals With Schizophrenia Differs From That of Controls. Schizophr Bull. 2015;41(5):1153-61

Yuan X, Zhang P, Wang Y, Liu Y, Li X, Kumar BU, Hei G, Lv L, Huang XF, Fan X, Song X. Changes in metabolism and microbiota after 24-week risperidone treatment in drug naïve, normal weight patients with first episode schizophrenia. Schizophr Res. 2018;201:299-306.

3. Obesity is a major confounder and often differ between patients with mental disorders and healthy controls, both due to the disease itself and to its treatment as for example antipsychotic drugs may increase body weight. This may also influence telomere length, chronic inflammation and microbiota composition. Therefore different measures of obesity (BMI, WHR) are needed to calculate and to adjust for when groups are compared.

R. We agree with the reviewer that obesity, as well as any other comorbidity associated with increased inflammation, chronic stress and modifications on the gut microbiota, might have an impact on the findings and represent a confounding factor. For these reasons, we will implement statistical models accounting for the effect of clinical variables related to the comorbid conditions. As far as obesity is concerned, BMI is being collected for each patient and control and is used as the sole measure to define obesity in the context of this study.

4. The role of TMAO is not so straight forward as previously thought. Will it be measured in the study or not? Please explain and also provide a more balanced view on this biomarker.

R. We thank the reviewer for this input. As TMAO will not be analyzed in our study and is actually less relevant in modulating microbiota, we have removed this section from the Introduction.

5. Participants will be excluded if they used antibiotics during the previous month before screening. However, modern studies tell that this effect may reside up to 3 (even 6) months. Please modify and provide more exact instructions.

R. We completely agree with the reviewer, and updated our exclusion criteria by increasing the period with no antibiotics from 1 to 3 months. All patients already recruited fulfilled this criterium.

6. Data on diet is very important when analysing microbiota patterns, but not much is revealed about this. Please specify more.

R. We added more details in the characterization of diet as follows: “, weight, body mass index, cardiovascular and metabolic co-morbidity, presence of physical activity. We are also gathering data on diet, as this is relevant to the analysis of microbiota, by asking participants to complete the food frequently questionnaires (FFQ) and 72 hours narrative summary of dietary intake prior to sampling, based on which all subjects will be characterized as belonging to one or more of the following categories: Mediterranean, junk food, high meat consumption, high consumption of baked products, high carbohydrates diet, vegetarian, and vegan”.

7. So far only 19 subjects with major depressive disorder has been recruited, and this is far from the goal of 40. How will this goal be achieved? Will recruitment also take place at other hospitals, if needed?

R. We thank the reviewer for this observation. At the moment of the submission of the revised version of the paper, the recruitment has reached 32 patients and we do not expect difficulties in reaching the target sample size.

8. Please refer to the CONSORT, STROBE or PRISMA checklists in epidemiological studies.

R. The STROBE checklist for observational case/control studies was checked and the paper was found to be consistent with it.

Minor:

1. The dates of the study must be mentioned (starting date, screening period)

R. The starting and end date were added in the main text, in the section “status of recruitment”

2. In key words, "sybr green" is not possible to understand - please explain or delete

R. We removed the keyword “sybr green”

Reviewer: 2

Reviewer Name: Feng Zhang

Institution and Country: Xi'an Jiaotong University,China

Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below

In this current study, the authors investigate the complex interaction/s between inflammation, age-related co-morbidities, telomere shortening and the gut microbiota in psychiatric disorders.

Some comments are as follows.

1. Page 6 line 18. It is recommended to add more descriptions about MD and SCZ in the introduction section.

R. We thank the reviewers for the comments provided, and issues raised, which we have tried to address at our best. We have added the following paragraph: “Both disorders are clinically severe, with SCZ characterized by positive symptoms such as delusions and hallucinations, negative symptoms (with avolition and social withdrawal) and significant permanent cognitive impairment4, and MDD by single or recurrent episodes of low mood and decreased energy often associated with high levels of anxiety and cognitive impairment5.

2. Page 6, line39-40. Please add the detailed description of “involvement of altered telomere and inflammatory dynamics in the neurobiological underpinnings of severe psychiatric disorders and suggest that signatures of these processes might be detectable in peripheral tissues”

R. We agree with the reviewer that this sentence was unclear. We changed it and removed some details, which we thought were already provided in the previous paragraph. The new sentence is as follows: “…, suggesting that the interaction and crosstalk between these two biological pathways might play a central role in the neurobiology of psychiatric disorders”.

3. Page 11, line 3. The recruitment criteria for healthy controls are only described by the mouth of the surrounding people, and lack of convincing power. A series of medical health checks should be performed on the health controls so that they can be determined.

R. We thank the reviewer for this observation. We added the following sentence: “Further, they will undergo a standard medical and laboratory test (including CBC, liver and kidney function) to verify their health status.”

4. In cross-sectional studies, the determination of the size of the sample is very important, which is directly related to the authenticity of the results. In this study, the lack of sample size leads to insufficient credibility of the results, and it is recommended to increase the sample size in the subsequent research.

R. We have updated the sample size estimation section which shows that most of the published research exploring the role of microbiota in SCZ and in MDD has been done in samples of lower or comparable size. Further, this study, at least for the investigation of the microbiota, remains largely exploratory.

VERSION 2 – REVIEW

REVIEWER	Peter M Nilsson Lund University, Sweden
REVIEW RETURNED	24-Dec-2019

GENERAL COMMENTS	The manuscript has now improved following revision. If statistical analyses will not be powered enough to reveal group differences, in spite of power calculations as described, the authors are recommended to plan for further enlargement of the study groups by recruiting more participants. The study period so far is estimated to take one year of inclusion, and this can be prolonged if needed.