

# BMJ Open 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

Mirko Manchia <sup>1,2,3</sup> Pasquale Paribello,<sup>1,2</sup> Carlo Arzedi,<sup>1,2</sup> Alberto Bocchetta,<sup>4,5</sup> Paola Caria,<sup>6</sup> Cristina Cocco,<sup>7</sup> Donatella Congiu,<sup>5</sup> Eleonora Cossu,<sup>1,2</sup> Tinuccia Dettori,<sup>6</sup> Daniela V Frau,<sup>6</sup> Mario Garzilli,<sup>1,2</sup> Elias Manca,<sup>7</sup> Anna Meloni,<sup>5</sup> Maria A Montis,<sup>1,2</sup> Andrea Mura,<sup>1,2</sup> Mariella Nieddu,<sup>6</sup> Barbara Noli,<sup>7</sup> Federica Pinna,<sup>1,2</sup> Claudia Pisanu,<sup>5</sup> Renato Robledo,<sup>6</sup> Giovanni Severino,<sup>5</sup> Valeria Sogos,<sup>8</sup> Caterina Chillotti,<sup>4</sup> Bernardo Carpiniello,<sup>1,2</sup> Maria Del Zompo,<sup>4,5</sup> Gian Luca Ferri,<sup>7</sup> Roberta Vanni,<sup>6</sup> Alessio Squassina <sup>5</sup>

**To cite:** Manchia M, Paribello P, Arzedi C, *et al.* 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. *BMJ Open* 2020;**10**:e032513. doi:10.1136/bmjopen-2019-032513

► Prepublication history and additional material for this paper are available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2019-032513>).

Received 21 June 2019  
Revised 13 December 2019  
Accepted 07 January 2020



© Author(s) (or their employer(s)) 2020. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

**Correspondence to**  
Dr Alessio Squassina;  
squassina@unica.it

## ABSTRACT

**Introduction** Severe psychiatric disorders are typically associated with a significant reduction in life expectancy compared with the general population. Among the different hypotheses formulated to explain this observation, accelerated ageing has been increasingly recognised as the main culprit. At the same time, telomere shortening is becoming widely accepted as a proxy molecular marker of ageing. The present study aims to fill a gap in the literature by better defining the complex interaction/s between inflammation, age-related comorbidities, telomere shortening and gut microbiota in psychiatric disorders.

**Methods and analysis** A cross-sectional study is proposed, recruiting 40 patients for each of three different diagnostic categories (bipolar disorder, schizophrenia and major depressive disorder) treated at the Section of Psychiatry and at the Unit of Clinical Pharmacology of the University Hospital Agency of Cagliari (Italy), compared with 40 age-matched and sex-matched non-psychiatric controls. Each group includes individuals suffering, or not, from age-related comorbidities, to account for the impact of these medical conditions on the biological make-up of recruited patients. The inflammatory state, microbiota composition and telomere length (TL) are assessed.

**Ethics and dissemination** The study protocol was approved by the Ethics Committee of the University Hospital Agency of Cagliari (PG/2018/11693, 5 September 2018). The study is conducted in accordance with the principles of good clinical practice and the Declaration of Helsinki, and in compliance with the relevant Italian national legislation. Written, informed consent is obtained from all participants. Participation in the study is on a voluntary basis only. Patients will be part of the

## Strengths and limitations of this study

- This is a novel approach proposing a systematic investigation of the complex interplay between inflammation, microbiota and telomere shortening in modulating the liability for major psychiatric disorders.
- The balanced recruitment of individuals affected and unaffected by age-related comorbidities among the four study groups, allows for a clearer definition of the possible confounding effects of these chronic medical conditions on telomere shortening, inflammatory status and microbiota composition.
- The cross-sectional design does not allow for causal inference of any possible finding on the explored psychiatric conditions, as these may simply represent the epiphenomena of these disorders.
- The small sample size and the relatively restricted catchment area may limit the generalisability of the results.

dissemination phase of the study results, during which a local conference will be organised and families of patients will also be involved. Moreover, findings will be published in one or more research papers and presented at national and international conferences, in posters or oral communications.

## INTRODUCTION

Bipolar disorder (BD) is a severe and chronic psychiatric disorder characterised by



alternating manic and depressive episodes interspersed with periods of well-being. Its prevalence in the general population ranges from 0.8% to 1.2%<sup>1</sup> and is associated with high levels of disability and premature mortality.<sup>2</sup> Indeed, patients with BD have a 10–20 years reduction in their life expectancy compared with the general population, an epidemiological mark shared with other severe mental disorders, including schizophrenia (SCZ) and major depressive disorder (MDD).<sup>3</sup> Both disorders are clinically severe, with SCZ characterised by positive symptoms such as delusions and hallucinations, negative symptoms (with avolition and social withdrawal) and significant permanent cognitive impairment,<sup>4</sup> and MDD by single or recurrent episodes of low mood and decreased energy often associated with high levels of anxiety and cognitive impairment.<sup>5</sup> A vast body of data suggests that the excess mortality of these severe psychiatric disorders is accounted for by a higher prevalence of comorbid chronic somatic disorders compared with individuals without mental illnesses.<sup>6</sup> In fact, age-related illnesses, such as cardiovascular and metabolic disorders, whose pathophysiology carries an inflammatory component, present a significantly higher incidence in psychiatric patients than in the general population.<sup>7,8</sup> This evidence has led to the hypothesis that accelerated ageing and inflammation may play a central role in the aetiopathogenesis of mental disorders. Biological signatures of these events are represented by telomere shortening (TS) and increased levels of proinflammatory markers. Several studies have reported shorter leucocyte telomere length (LTL) as well as increased levels of circulating proinflammatory cytokines in patients with BD, SCZ and MDD compared with controls,<sup>9–11</sup> suggesting that the interaction and cross-talk between these two biological pathways might play a central role in the neurobiology of psychiatric disorders. TS is a hallmark of cellular ageing, and although telomere length reduces with each cell division, the shortening rate is increased by allostatic load and inflammation,<sup>12</sup> thus affecting the ageing process. Consistently, a recent study evaluating mortality in >64 000 subjects from the general population showed that short telomeres in peripheral blood leucocytes were associated with high mortality.<sup>13</sup> So far, findings of shorter telomeres in psychiatric patients have been contradictory,<sup>14</sup> likely due to underpowered study samples and limitations in methodologies, whereas the interaction between inflammatory processes, telomeres shortening and psychiatric disorders has been scarcely investigated. There is evidence that proinflammatory cytokines are important mediators of the association between depressive and anxiety disorders and LTL.<sup>15</sup> Moreover, the impact of comorbid age-related disorders has been often overlooked in previous telomere studies in psychiatry. Although current data suggest that TS, inflammation and accelerated ageing may play a central role in modulating the liability for severe psychiatric disorders, differences and similarities in their involvement in mental illnesses have yet to be elucidated and therefore need comprehensive investigations.

Alterations of these biological pathways in severe psychiatric disorders might also influence other relevant physiological components. It is known that an estimate of 40 000 bacterial species and 1800 phyla inhabits our body, paralleling the number of human cells and approaching a ratio of nearly 1:1 according to recent estimates.<sup>16</sup> As a whole, the microorganisms living in our body are collectively referred to as the microbiota and include bacteria, fungi and protozoa. The microbiota serves multiple physiological functions, although its overall importance has not been fully grasped yet. A state of balance between ‘good’ bacteria and ‘harmful’ bacteria (called ‘eubiosis’) is therefore paramount to maintain the homeostasis of its hosting organism. In contrast, the perturbation of this balance (called ‘dysbiosis’) is related to the development of several pathological conditions. For example, the microbiota works synergistically with the immune system to avoid colonisation from pathogenic bacteria and viruses. The microbiota also plays a role in metabolism, participating in the digestion by aiding micronutrients absorption (in particular amino acids and short-chain fatty acids) and part of their further degradation as well.

Although dysbiosis has been clearly associated with numerous ailments, it is not clear whether a causal relationship exists between these two phenomena or, instead, if anomalies in the microbiota composition represent an epiphenomenon of the underlying pathological processes. Similarly, the eventual pre-emptive or even therapeutic effects deriving from the microbiota manipulation remain unclear. However, there is now consisting evidence that the microbiota, particularly gut microbiota, exerts a modulatory function on the brain, possibly even modulating behaviour.<sup>17</sup> Indeed, experimental data in animal models show that central nervous system neurotransmission can be profoundly disturbed by the absence of a normal gut microbiota<sup>18</sup> and that alterations of eubiosis with induced acute bacterial infection might induce cognitive alterations.<sup>19</sup> In humans, data have correlated alterations of microbiota to the manifestation of autism spectrum disorder (ASD).<sup>20</sup> Interestingly, a recent translational study operated transplants of gut microbiota from human donors with ASD controls into germ-free mice revealing that colonisation with ASD microbiota was sufficient to induce hallmark autistic behaviours.<sup>21</sup> Other severe psychiatric disorders, such as SCZ<sup>22</sup> and MDD,<sup>23</sup> show substantial microbiota imbalances. Relevant to this study protocol is the evidence that a proportion of this altered gut–brain communication in severe mental disorders, determined by a pathological microbiota, is determined through an immunomodulatory action, which includes a raise in the activity of inflammatory pathways.<sup>24</sup>

### Study objectives

The main objective of the present protocol is to clarify the relationship between telomere length (TL) and inflammation in the aetiopathogenesis of BD, SCZ and MDD. Furthermore, we evaluate the impact of the age-related medical comorbidities on these biological markers.

Contextually, we aim to determine the presence of alterations in the gut microbiota composition in individuals affected by SCZ, MDD and BD, as compared with healthy controls (HC). As reported previously, several lines of evidence suggest that the microbiota might play a significant role in the brain–gut axis, and microbial derangement may result in the development of several psychiatric conditions.<sup>25</sup>

Specifically, our study tests the following primary and secondary hypotheses:

Primary:

- ▶ To determine the differences in TL among individuals affected by SCZ, MDD, BD and HC.
- ▶ To test for the presence of differences in inflammatory markers among patients affected by SCZ, MDD, BD and HC.
- ▶ To assess whether microbiota composition differs among individuals affected by SCZ, MDD, BD and HC.

Secondary:

- ▶ To clarify the impact of medical comorbidities related to ageing on the differences in TL and in the number of short telomeres, as well as on inflammatory markers levels and on microbiota.

## METHODS

### Study design

This is a cross-sectional study in the context of which we are performing the recruitment of three cohorts, each comprised of 40 patients affected by SCZ, MDD and BD diagnosed with the Italian version of the Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID).<sup>26</sup> In addition, we are recruiting a sample of 40 HC.

### Patient and public involvement

There was no involvement of patients in the development of this study protocol. The patients were asked to report their impression on the time required to participate in the study and the burden of the sampling procedure as well. An effort is constantly made to facilitate the participation of the eligible individuals, taking into account their indications and minimising the potential disruption during sampling procedures. Patients were not involved in the development of the recruitment process, but they will be part of the dissemination phase of the study results.

### Recruitment process

The sample is recruited from patients followed-up and treated at the community mental health centre of the Section of Psychiatry of the Department of Medical Science and Public Health, University of Cagliari and University Hospital Agency of Cagliari and the Unit of Clinical Pharmacology, University Hospital Agency, Cagliari, Italy. The recruitment process is performed on the basis of the presence or absence of medical comorbidities related to ageing (eg, cardiovascular diseases, diabetes mellitus type 2 and so on). Approximately half of the subjects for each group are recruited on the basis

of the presence of such comorbidities. HC are recruited by word of mouth among hospital staff, their families and university students. Further, they will undergo a standard medical and laboratory test (including complete blood count, liver and kidney function) to verify their health status.

### Inclusion and exclusion criteria

The recruitment process is based on the following inclusion criteria: (1) patients affected by SCZ, BD, MDD according to DSM-IV-TR<sup>27</sup> criteria; (2) able to express a consent to participate formulated by signing the consent form; (3) age between 18 and 70 years; (4) in euthymic phases for BD and MDD and with at least the 6 months of stability before recruitment for SCZ. We also apply the following exclusion criteria: (1) presence of acute infections; (2) presence of chronic autoimmune inflammatory conditions (eg, rheumatoid arthritis and thyroiditis); (3) presence of eating disorders; (4) presence of post-traumatic stress disorder; (5) presence of current substance use disorders; (6) presence of neurological disorders; (7) past traumatic brain injury; (8) presence of severe comorbidities that may influence molecular testing (such as cancer and HIV infection). The inclusion criteria for HC comprise: (1) the absence of a personal history of mental disorders, (2) the willingness to participate in the study, (3) absence of acute infections; (4) absence of chronic autoimmune inflammatory conditions (eg, rheumatoid arthritis and thyroiditis); (5) absence of past traumatic brain injury; (6) absence of severe comorbidities that may influence molecular testing (such as cancer and HIV infection). In addition to the above-mentioned exclusions criteria for HC and patients, the following are considered in relation to the microbiota study: use of antibiotics in the 3 months preceding the sampling procedure and chronic use of probiotics.

### Sample size estimation

Considering the magnitude of the effect size reported in the literature regarding the correlation between LTL and mood disorders (Cohen  $d=0.67$ ,<sup>28</sup>) our sample has an 85% statistical power to identify the existing differences between the diagnostic groups (SCZ, BD and MDD) and HC at an  $\alpha=0.05$ . 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<sup>29</sup> and for MDD.<sup>30</sup>

### Clinical assessment

Recruited subjects are assessed by trained mental-health professionals (psychiatry residents or senior clinical staff). Clinical information is collected through direct interview of the patient as well as through a systematic assessment of existing medical records. Whenever possible, we are collecting collateral information from at least one first degree relative or significant other, after obtaining the consent from the participant. Specific to patients' groups are the following clinical data: age of onset, history of

suicide attempt, history of violent attempt, type of onset, number of episodes, including depressive, hypomanic/manic, mixed recurrences (for BD and MDD), family history of psychiatric disorders, presence of past and current substance and/or alcohol use disorder, past and current pharmacological treatments, duration of pharmacological treatment and type of drugs. Treatment resistance for patients with MDD and SCZ is defined according to the criteria of Souery *et al*<sup>31</sup> and Kane *et al*<sup>32</sup> and is based on the clinical course and evaluation of treatment response patterns. In addition, we are collecting, for patients and HC, detailed data on the presence of past and current cigarette smoking status, number of cigarettes per day (categorised according to Fagerström criteria<sup>33</sup>), height, weight, body mass index, cardiovascular and metabolic comorbidity, and 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-hour narrative summary of dietary intake prior to sampling, based on which all subjects will be characterised 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. Finally, for HC, the screening study visit includes a clinical interview (1.5 hours long). Each subject will be interviewed using the Italian version of the SCID-I/NP<sup>26</sup> to rule out the presence of axis I psychiatric disorders.

### Psychopathological measures

Some psychopathological measures are used to establish the stability of the illness at the moment of recruitment: the Positive and Negative Scale for Schizophrenia (PANSS)<sup>34</sup> and the Clinical Global Impression Scale for Schizophrenia (CGI-SCH),<sup>35</sup> which have both shown to be valid assessment tools for the identification of patients in remission, particularly in routine clinical practice.<sup>36 37</sup> In patients with BD, we assess the response to mood stabilisers using the 'Retrospective Criteria of Long-Term Treatment Response in Research Subjects with Bipolar Disorder' scale.<sup>38</sup>

### Blood sampling and assay procedures

Fasting peripheral venous blood samples are taken from all patients within a similar time window (8:00 to 10:00 AM). Blood samples will be collected in a euthymic phase (for patients with MDD and BD), or at least 6 months after their latest exacerbation of psychopathological symptoms (for patients with SCZ). Specimens will be divided into three aliquots: (1) for measurement of plasma C reactive protein (high-sensitivity C reactive protein assay) and the proinflammatory cytokines TNF- $\alpha$  and IL-6, using enzyme-linked immunosorbent assays (ELISA); (2) for DNA extraction and the subsequent measurement of LTL with qPCR, performed according to the method described by Cawthon<sup>39</sup>; (3) for measurement of TL using quantitative fluorescent in situ hybridisation on the

metaphase chromosomes in lymphocytes, carried out as described in Cantara *et al*.<sup>40</sup>

### Fecal microbiota analysis methods

The 16S RNA Microbial Profiling is a test capable of exploiting the recent technological advances of metagenomic in microbiota ecology: it is based on the sequencing of the regions V3 and V4 of the 16S rRNA (the gene responsible for the production of the prokaryote small ribosomal subunit), a gene that represents an important marker widely used in bacterial taxonomy. Starting from biological material (a stool sample), we are isolating bacterial DNA using the QIAamp DNA Stool Mini Kit (Qiagen, Milan, Italy). DNA is then used for amplification of the V3–V4 hypervariable regions of the bacterial 16S ribosomal RNA (rRNA) with next generation sequencing using a MiSeq Instrument (Illumina Inc, San Diego, California, USA), as previously described.<sup>41</sup> The analysis is completed thereafter by sequencing the 16S rRNA gene pool corresponding to the different microorganisms present in the microbiota, subsequently identified through the use of bioinformatic tools (Metagenomics Illumina Inc. and Kraken APP).

### Data analysis plan

The association between TL or cytokine levels and demographic/clinical variables is tested using parametric or non-parametric tests according to the observed distribution. The presence of statistically significant differences in the TL and in the cytokine plasma levels among the different study groups (SCZ, BD, MDD and HC) is tested through linear regression. Furthermore, the presence of medical comorbidities related to ageing, and the other demographic and clinical variables significantly associated with the outcome are inserted in the regression model. The analyses will be conducted using R<sup>42</sup> and IBM Statistical Package for Social Science (SPSS). As for the microbiota analysis, the position, dispersion and shape indexes will be calculated for the quantitative variables, while the relative frequency for each class will be calculated for categorical variables. In addition, the correlations between the different analysed variables will be evaluated by using the aforementioned univariate and multivariate analyses methods.

### Ethics and dissemination

#### Ethics, consent and permissions

This study protocol was approved by the Ethics Committee of the University Hospital Agency of Cagliari (PG/2018/11693) on 5 September 2018. The study is conducted in accordance with the principles of good clinical practice, with the Declaration of Helsinki and in compliance with the national legislation. Written, informed consent is being obtained from all participants. Participation to the study is voluntary and patients may be able to withdraw consent at any point with no disadvantage to their treatments. A psychiatric assessment

establishes that patients' ability to consent is not compromised by their psychopathological status.

### Dissemination

We plan to describe the main findings of the study to the participants and their families organising a local conference using lay language. Moreover, we will also organise a public event open to the community where we will explain and discuss the potential impact of the findings of our project on the management of the BP. Findings will also be published in one or more research papers, and presented at national and international conferences, in posters or oral communications.

### Status of recruitment

The enrolment of patients and controls started in January 2019. Collection of clinical data and biological samples is currently undergoing and at the moment of the preparation of the protocol 87% of the sample has been recruited (104/120). Clinical data have been already cleaned and inputted into a database in preparation for data analysis. The main sociodemographic and clinical characteristics for the BD, MDD, SCZ and HC samples are summarised in the online supplementary table 1. The planned end date for the study is 29 February 2020.

## DISCUSSION

In this study, we sought to explore the relationship between TL, inflammation, the microbiota and the impact of their interaction on the risk of developing a severe mental disorder such as SCZ, BD or MDD. In addition, a corollary objective is to establish similarities and differences in how abnormal molecular dynamics among telomeres, inflammation and microbiota manifest in SCZ, BD and MDD. A novel approach in our study is to ascertain the impact of the age-related medical comorbidities on such molecular dynamics. Previous research has shown that accelerated biological ageing is a hallmark of severe psychiatric disorders.<sup>43–45</sup> Molecular signatures of this accelerated decay are currently being identified and point to TS as well as to a series of inflammatory markers. In the case of SCZ, a recent meta-analysis has found that patients with SCZ have a highly statistically significant shortening of LTL compared with HC.<sup>46</sup> Further, a recent qualitative synthesis of the literature has found that consisting evidence points to elevated levels of proinflammatory cytokines and chemokines in SCZ, including interleukin (IL)-1 $\beta$ , tumour necrosis factor alpha (TNF- $\alpha$ ), eotaxin-1, eotaxin-2, monocyte chemoattractant protein-1, macrophage inflammatory protein-1 $\beta$ , thymus-regulated and activation-regulated chemokine, macrophage-derived chemokine, as well as a decline in the levels of the anti-inflammatory cytokine IL-2.<sup>43</sup> In patients with BD, there is evidence of a significant LTL shortening compared with HC irrespective of mood state.<sup>47</sup> Similarly to SCZ, the inflammatory patterns in BD are characterised by increased levels of TNF- $\alpha$ , the soluble TNF receptor

type 1 (sTNF-R1) and the soluble IL-2 receptor (sIL-2R) compared with HC.<sup>48</sup> Of note, there are intriguing findings, although still at the level of experimental preclinical data, suggesting that perturbations of the gut microbiota composition and the functional metagenome may be associated with accelerated ageing in animal models.<sup>49</sup> In summary, the literature shows that: (1) there is a plausible interaction between inflammation and TS in modulating the risk of severe psychiatric disorders, and (2) this association might reflect (or be modulated) by specific detrimental alterations of the microbiota. However, available data also tell that: (1) no previous studies have so far examined the role of confounders (mainly the presence of age-related medical comorbidities) in influencing the dynamics between accelerated biological ageing and inflammation in psychiatric disorders, and (2) there is a gap in the knowledge on how microbiota might influence these dynamics in humans.

Our project has been set in this context. We expect to demonstrate that a shortening of TL and/or a higher number of short telomeres will be present in the diagnostic groups (SCZ, MDD and BD) as compared with HC. We also expect that the proinflammatory cytokines levels will be higher among patients and will be negatively correlated to the TL. Further, we anticipate that patients with medical comorbidities related to ageing will present shorter telomeres and higher proinflammatory cytokines levels as compared with patients bereft of such comorbidities. Finally, we expect to identify specific microbiota clusters associated with the diagnostic groups (SCZ, BD and MDD) and related to specific biological markers of inflammation and accelerated ageing.

Clearly, our study findings will need to be interpreted in the context of several limitations. First, due to the cross-sectional design, we will not be able to establish causality (ie, whether biological markers of ageing resulted from an increased inflammatory load or vice versa). Due to the relatively small sample size, our study should be considered as a hypothesis generator, capable of clarifying the association strength between the different variables. The resulting data will be of great importance for planning future studies in this area of research.

Notwithstanding the existing limitation deriving from the study design and the small sample size, this project appears unique and original. The role exerted by the interaction between inflammation and telomeres, and the contribution of medical comorbidities related to ageing in influencing the effect of this interaction, have been hypothesised but never tested in a systematic manner. Moreover, there are no available data linking these biological markers to the microbiota derangements. By using an accurate methodology to better characterise the recruited patients, the synergy between different research groups with renowned and complementary skills allows for a reliable study concerning the three-sided interplay between telomeres, inflammation and microbiota. Obtaining a deeper understanding of the influence exerted on these systems could help us

in developing novel ways of managing these severely disabling conditions.

#### Author affiliations

- <sup>1</sup>Unit of Psychiatry, Department of Public Health, Clinical and Molecular Medicine, University of Cagliari, Cagliari, Italy  
<sup>2</sup>Unit of Clinical Psychiatry, University Hospital Agency of Cagliari, Cagliari, Italy  
<sup>3</sup>Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada  
<sup>4</sup>Unit of Clinical Pharmacology, University Hospital Agency of Cagliari, Cagliari, Italy  
<sup>5</sup>Department of Biomedical Sciences, Section of Neuroscience and Clinical Pharmacology, University of Cagliari, Cagliari, Italy  
<sup>6</sup>Unit of Biology and Genetics, Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy  
<sup>7</sup>Department of Biomedical Sciences, NEF Laboratory, University of Cagliari, Cagliari, Italy  
<sup>8</sup>Department of Biomedical Sciences, Section of Cytomorphology, University of Cagliari, Cagliari, Italy

**Acknowledgements** The authors wish to thank all the patients who are participating to this study. They also thank the nurses Ms Flavia Orrù (Unit of Clinical Pharmacology, University Hospital Agency of Cagliari), and Ms Anna Greca Floris, Mr Gianluca Carta, Mr Sergio Deiana, Ms Gioia Gabriela Delogu, Mr Giovanni Dessanai, Ms Maria Elisabetta Loche, Mr Giampiero Neri, Ms Maria Cristina Olmi, Ms Enrica Pintus, Ms Loredana Satta (Unit of Clinical Psychiatry of the University Hospital Agency of Cagliari) for helping with the recruitment procedures.

**Contributors** MM: contributed to the design of the study protocol and wrote the first draft of the manuscript. PP: co-wrote the first draft of the manuscript. AS designed the study protocol and co-wrote the first draft of the manuscript. CA, AB, PC, CC, DC, EC, TD, DVF, MG, EM, AMel, MAM, AMur, MN, BN, FP, CP, RR, GS, VS: critically revised the manuscript and contributed to the discussion. CChill, BC, MDZ, GLF, RV: contributed to the design of the study protocol and critically revised the manuscript.

**Funding** This work was supported by Fondazione di Sardegna (“Convenzione triennale 2015–2017”) and by the Autonomous Region of Sardinia (“Legge Regionale 7 agosto 2007, n. 7 – call 2016”), grant number F72F16003090002. This work was supported by the Open Access Publishing Fund of the University of Cagliari, with the funding of the Regione Autonoma della Sardegna - L.R. n. 7/2007

**Competing interests** None declared.

**Patient consent for publication** Not required.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

#### ORCID iDs

Mirko Manchia <http://orcid.org/0000-0003-4175-6413>  
 Alessio Squassina <http://orcid.org/0000-0001-7415-7607>

#### REFERENCES

- Merikangas KR, Jin R, He J-P, *et al.* Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative. *Arch Gen Psychiatry* 2011;68:241–51.
- Roshanaei-Moghaddam B, Katon W. Premature mortality from general medical illnesses among persons with bipolar disorder: a review. *PS* 2009;60:147–56.
- Chesney E, Goodwin GM, Fazel S. Risks of all-cause and suicide mortality in mental disorders: a meta-review. *World Psychiatry* 2014;13:153–60.
- Owen MJ, Sawa A, Mortensen PB. Schizophrenia. *Lancet* 2016;388:86–97.
- Malhi GS, Mann JJ. Depression. *Lancet* 2018;392:2299–312.
- Walker ER, McGee RE, Druss BG. Mortality in mental disorders and global disease burden implications: a systematic review and meta-analysis. *JAMA Psychiatry* 2015;72:334–41.
- Newcomer JW, Hennekens CH. Severe mental illness and risk of cardiovascular disease. *JAMA* 2007;298:1794–6.
- De Hert M, Correll CU, Bobes J, *et al.* Physical illness in patients with severe mental disorders. I. Prevalence, impact of medications and disparities in health care. *World Psychiatry* 2011;10:52–77.
- Lindqvist D, Epel ES, Mellon SH, *et al.* Psychiatric disorders and leukocyte telomere length: underlying mechanisms linking mental illness with cellular aging. *Neurosci Biobehav Rev* 2015;55:333–64.
- Young JJ, Bruno D, Pomara N. A review of the relationship between proinflammatory cytokines and major depressive disorder. *J Affect Disord* 2014;169:15–20.
- Leboyer M, Oliveira J, Tamouza R, *et al.* Is it time for immunopsychiatry in psychotic disorders? *Psychopharmacology* 2016;233:1651–60.
- Zhang J, Rane G, Dai X, *et al.* Ageing and the telomere connection: an intimate relationship with inflammation. *Ageing Res Rev* 2016;25:55–69.
- Rode L, Nordestgaard BG, Bojesen SE. Peripheral blood leukocyte telomere length and mortality among 64 637 individuals from the general population. *JNCI J Natl Cancer Inst* 2015;107:djv074.
- Darrow SM, Verhoeven JE, Révész D, *et al.* The association between psychiatric disorders and telomere length: a meta-analysis involving 14,827 persons. *Psychosom Med* 2016;78:776–87.
- Révész D, Verhoeven JE, Milaneschi Y, *et al.* Depressive and anxiety disorders and short leukocyte telomere length: mediating effects of metabolic stress and lifestyle factors. *Psychol Med* 2016;46:2337–49.
- Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol* 2016;14:e1002533.
- Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci* 2012;13:701–12.
- Clarke G, Grenham S, Scully P, *et al.* The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol Psychiatry* 2013;18:666–73.
- Gareau MG, Wine E, Rodrigues DM, *et al.* Bacterial infection causes stress-induced memory dysfunction in mice. *Gut* 2011;60:307–17.
- Coretti L, Paparo L, Riccio MP, *et al.* Gut microbiota features in young children with autism spectrum disorders. *Front Microbiol* 2018;9.
- Sharon G, Cruz NJ, Kang D-W, *et al.* Human gut microbiota from autism spectrum disorder promote behavioral symptoms in mice. *Cell* 2019;177:1600–18.
- Shen Y, Xu J, Li Z, *et al.* Analysis of gut microbiota diversity and auxiliary diagnosis as a biomarker in patients with schizophrenia: a cross-sectional study. *Schizophr Res* 2018;197:470–7.
- Dash S, Clarke G, Berk M, *et al.* The gut microbiome and diet in psychiatry: focus on depression. *Curr Opin Psychiatry* 2015;28:1–6.
- Forsythe P, Bienenstock J. Immunomodulation by commensal and probiotic bacteria. *Immunol Invest* 2010;39:429–48.
- Alam R, Abdolmaleky HM, Zhou J. Microbiome, inflammation, epigenetic alterations, and mental diseases. *Am J Med Genet* 2017;174:651–60.
- First MB, Spitzer RL, Gibbon M, *et al.* *Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition. (SCID-I/P)*. New York: Biometrics Research, New York State Psychiatric Institute, 2002.
- American Psychiatric Association. Diagnostic and statistical manual of mental disorders DSM-IV-TR fourth edition (text revision) 2000.
- Simon NM, Smoller JW, McNamara KL, *et al.* Telomere shortening and mood disorders: preliminary support for a chronic stress model of accelerated aging. *Biol Psychiatry* 2006;60:432–5.
- Yuan X, Zhang P, Wang Y, *et al.* 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.
- Cheung SG, Goldenthal AR, Uhlemann A-C, *et al.* Systematic review of gut microbiota and major depression. *Front Psychiatry* 2019;10:34.
- Souery D, Papakostas GI, Trivedi MH. Treatment-resistant depression. *J Clin Psychiatry* 2006;67:16–22.
- Kane J, Honigfeld G, Singer J, *et al.* Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. *Arch Gen Psychiatry* 1988;45:789–96.
- Fagerstrom K-O, Schneider NG. Measuring nicotine dependence: a review of the Fagerstrom tolerance questionnaire. *J Behav Med* 1989;12:159–82.
- Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull* 1987;13:261–76.
- Haro JM, Kamath SA, Ochoa S, *et al.* The clinical global Impression-Schizophrenia scale: a simple instrument to measure the diversity of symptoms present in schizophrenia. *Acta Psychiatr Scand* 2003;107:16–23.

- 36 Pinna F, Bosia M, Cavallaro R, *et al.* Consensus five factor PANSS for evaluation of clinical remission: effects on functioning and cognitive performances. *Schizophr Res* 2014;1:187–92.
- 37 Pinna F, Deriu L, Diana E, *et al.* Clinical global Impression-severity score as a reliable measure for routine evaluation of remission in schizophrenia and schizoaffective disorders. *Ann Gen Psychiatry* 2015;14:6.
- 38 Grof P, Duffy A, Cavazzoni P, *et al.* Is response to prophylactic lithium a familial trait? *J Clin Psychiatry* 2002;63:942–7.
- 39 Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res* 2002;30:47e–47.
- 40 Cantara S, Pisu M, Frau DV, *et al.* Telomere abnormalities and chromosome fragility in patients affected by familial papillary thyroid cancer. *J Clin Endocrinol Metab* 2012;97:E1327–31.
- 41 Klindworth A, Pruesse E, Schweer T, *et al.* Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 2013;41:e1.
- 42 R Core Team. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing, 2019.
- 43 Nguyen TT, Eyler LT, Jeste DV. Systemic biomarkers of accelerated aging in schizophrenia: a critical review and future directions. *Schizophr Bull* 2018;44:398–408.
- 44 Squassina A, Pisanu C, Corbett N, *et al.* Telomere length in bipolar disorder and lithium response. *Eur Neuropsychopharmacol* 2017;27:560–7.
- 45 Squassina A, Pisanu C, Vanni R. Mood disorders, accelerated aging, and inflammation: is the link hidden in telomeres? *Cells* 2019;8:52.
- 46 Russo P, Prinzi G, Proietti S, *et al.* Shorter telomere length in schizophrenia: evidence from a real-world population and meta-analysis of most recent literature. *Schizophr Res* 2018;202:37–45.
- 47 Huang Y-C, Wang L-J, Tseng P-T, *et al.* Leukocyte telomere length in patients with bipolar disorder: an updated meta-analysis and subgroup analysis by mood status. *Psychiatry Res* 2018;270:41–9.
- 48 Munkholm K, Vinberg M, Vedel Kessing L. Cytokines in bipolar disorder: a systematic review and meta-analysis. *J Affect Disord* 2013;144:16–27.
- 49 Peng W, Yi P, Yang J, *et al.* Association of gut microbiota composition and function with a senescence-accelerated mouse model of Alzheimer's disease using 16S rRNA gene and metagenomic sequencing analysis. *Aging* 2018;10:4054–65.