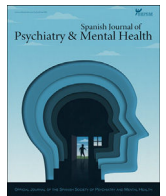




Available online at www.sciencedirect.com

Spanish Journal of Psychiatry and Mental Health

journal homepage: <http://www.elsevier.es/saludmental>



Clinical trials and protocols

Intestinal permeability and low-grade chronic inflammation in schizophrenia: A multicentre study on biomarkers. Rationale, objectives, protocol and preliminary results

Gerard Anmella^{a,b,c,1}, Silvia Amoretti^{a,b,c,d,1}, Gemma Safont^{b,e}, Ana Meseguer^{a,b,c}, Eduard Vieta^{a,b}, Maria Teresa Pons-Cabrera^{a,b,c}, Miquel Alfonso^f, Carla Hernández^f, Monica Sanchez-Autet^f, Ferran Pérez-Baldellou^e, Leticia González-Blanco^{g,h}, Maria Paz García-Portilla^{g,h}, Miquel Bernardo^{b,c,*}, Belén Arranz^{b,f}

^a Bipolar and Depressive Disorders Unit, Hospital Clínic de Barcelona, Institute of Neuroscience, August Pi i Sunyer Biomedical Research Institute (IDIBAPS), University of Barcelona, Barcelona, Catalonia, Spain

^b Biomedical Research Networking Center for Mental Health Network (CIBERSAM), Barcelona, Spain

^c Barcelona Clinic Schizophrenia Unit, Hospital Clínic de Barcelona, Institute of Neuroscience, University of Barcelona, IDIBAPS, Spain

^d Group of Psychiatry, Mental Health and Addictions, Vall d'Hebron Research Institute (VHIR), Psychiatric Genetics Unit, Vall d'Hebron Research Institute (VHIR), Barcelona, Catalonia, Spain

^e Department of Psychiatry, Hospital Universitari Mutua de Terrassa, Universitat de Barcelona, Barcelona, Spain

^f Department of Psychiatry, Hospital Parc Sanitari Sant Joan de Déu, Barcelona, Spain

^g Department of Psychiatry, University of Oviedo, Servicio de Salud Mental del Principado de Asturias, Oviedo, Spain

^h Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), INEUROPA, Biomedical Research Networking Center for Mental Health Network (CIBERSAM), Oviedo, Spain

ARTICLE INFO

Article history:

Received 21 December 2022

Accepted 18 September 2023

Available online xxx

Keywords:

Intestinal permeability

Microbiota–gut–brain axis

Inflammation

Schizophrenia

Biomarkers, Protocol

ABSTRACT

Background: Altered intestinal permeability and low-grade chronic inflammation disrupt the integrity of the blood–brain barrier (microbiota–gut–brain axis), probably playing a role in the pathophysiology of schizophrenia-spectrum disorders. However, studies assessing the microbiota–gut–brain axis are inconsistent. This article describes the rationale, objectives, protocol, and presents descriptive results for a new project.

Methods: The sample of this study came from an observational, cross-sectional and multisite study including four centers in Spain (PI17/00246) recruiting adult patients with DSM-5 schizophrenia-spectrum disorders at any stage of the disease. The aims of the project are to assess the interrelation between intestinal permeability and low-grade chronic inflammation in schizophrenia-spectrum disorders and the role of peripheral biomarkers, diet, exercise, metabolic syndrome, disease severity and functioning as well as cognition. Assessments included the following variables: (1) anthropometric, (2) intestinal permeability, diet, and physical exercise, (3) clinical and functional, (4) neuropsychological and cognitive reserve, and (5) peripheral biomarkers from blood.

Results: A total of 646 patients were enrolled (257, 39.7% female). Mean age was 43.2 ± 13.6 years, illness duration 15.1 ± 11.5 years. 55.8% consumed tobacco. Positive PANSS score was 13.68 ± 6.55 , and 20.38 ± 8.69 in the negative symptoms. CGI was 4.16 ± 2.22 and GAF was 60.00 ± 14.84 .

Conclusion: The results obtained by this project are expected to contribute toward the understanding of the physiopathology of schizophrenia-spectrum disorders. This will likely aid to personalize treatments in real-world clinical practice, potentially including variables related to intestinal permeability and inflammation.

© 2023 Sociedad Española de Psiquiatría y Salud Mental (SEPSM). Published by Elsevier España, S.L.U. All rights reserved.

* Corresponding author.

E-mail address: bernardo@clinic.cat (M. Bernardo).

¹ The first two authors contributed equally to this work.

<https://doi.org/10.1016/j.sjpmh.2023.09.005>

2950-2853/© 2023 Sociedad Española de Psiquiatría y Salud Mental (SEPSM). Published by Elsevier España, S.L.U. All rights reserved.

Please cite this article as: G. Anmella, S. Amoretti, G. Safont et al., Intestinal permeability and low-grade chronic inflammation in schizophrenia: A multicentre study on biomarkers. Rationale, objectives, protocol and preliminary results, *Spanish Journal of Psychiatry and Mental Health*, <https://doi.org/10.1016/j.sjpmh.2023.09.005>

Background

Low-grade chronic inflammation is present in the pathophysiology of numerous chronic conditions, such as obesity, cancer, diabetes, cardiovascular diseases and neurodegenerative pathologies (Castro, Macedo-de la Concha and Pantoja-Meléndez, 2017). In the latest years, the role of inflammation in mental disorders has become more and more clear,¹ especially in schizophrenia-spectrum disorders.^{2–6}

Common key elements of this low-grade systemic inflammation among different chronic pathologies include elevation levels of proinflammatory cytokines^{5,7} and macrophage infiltration in peripheral tissue.⁸ The production of these molecules by adipocytes, coupled with the destruction of these cells, induces the inflammation to become chronic, and influences other systems by altering their functions, which leads to different diseases, such as insulin resistance, metabolic syndrome,⁹ diabetes, and cardiovascular atherosclerosis.¹⁰ These diseases, especially metabolic syndrome and type-2 diabetes, are highly comorbid with schizophrenia-spectrum disorders,^{11,12} with cardiovascular disease being the most important cause of premature mortality among these patients.¹³

Apart from the role of low-grade chronic inflammation in the pathophysiology of chronic conditions, its main causes are related to a lifestyle discordant with our physiological needs, such as physical inactivity, obesity (visceral adiposity), chronic stress,¹⁴ vitamin D deficiency, pollution, tobacco use, unhealthy/hypercaloric diet,¹⁵ oxidative stress, alterations in the intestinal microbiota, and altered intestinal permeability “leaky-gut”.^{16–19}

There is increasing evidence of the importance of the intestinal barrier and its permeability in relation with (mental) health conditions.^{20,21} The destruction of epithelial tight junctions between mucosal cells in the intestinal lumen leads to disruption of the intestinal barrier (permeable intestine or leaky-gut), which in turn leads to penetration of antigens and other inflammatory substances into the circulatory system, and thus to activation of the immune system and an immune response.²² Cytokines such as TNF and IL-6 increase the permeability of brain microvascular endothelial cells. This low-grade inflammatory state may, in turn, alter the blood–brain barrier’s integrity,²³ leading to decreased central nervous system protection and increased permeability of proinflammatory (e.g., cytokines, reactive oxygen species) substances from the peripheral blood into the brain.¹⁸ There is accumulating evidence on the close bidirectional relationship between the gut microbiota and the brain, the microbiota–gut–brain (MBG) axis, involving the nervous, hormonal, and immune systems,^{24,25} with the gut bacteria strongly regulating this axis.^{26,27} Several studies have examined the relationship between the gut environment and various neuropsychiatric diseases, especially schizophrenia-spectrum disorders.^{28–30} The most involved factors of the MBG axis in the development of schizophrenia-spectrum disorders include alterations in the intestinal microbiota and leaky-gut syndrome.²⁸

There is evidence of an increase in inflammation markers in the intestinal mucosa in schizophrenia-spectrum disorders.^{21,31–33,34} However, the results assessing gut permeability in schizophrenia-spectrum disorders are inconsistent,^{31,32,35} which may be due to the difference in the methods used.³⁶ Recent studies showed an abnormally increased gut permeability in patients with schizophrenia-spectrum disorders compared to healthy controls, and also that gut permeability may be related to the cognitive and cellular immunity function of patients with schizophrenia.³⁵ Another recent study adds evidence to these findings suggesting a direct mechanistic link (and potential therapeutic target) between abnormal microbiota and cognitive alterations in schizophrenia-spectrum disorders (Thirion et al., 2023).³⁷

Moreover, sex-related differences in schizophrenia-spectrum disorders have been found in several domains, including clinical and functional status,³⁸ but also inflammatory^{39–41} and metabolic-related parameters⁴² in association with psychopathological symptoms.⁴³ To our knowledge, sex related-differences in intestinal permeability and their interplay with inflammation in schizophrenia-spectrum disorders have not yet been studied.

Low-grade chronic inflammation in patients with schizophrenia-spectrum disorders has also been associated with comorbid cardio-metabolic medical conditions,^{12,44} which result from an interaction of heritable factors and environmental influences, including drug treatment.⁴⁵ Growing evidence suggests that a low-grade inflammatory state is present before the first psychotic symptoms, which may ease the first psychotic event in individuals with genetic vulnerability.⁴⁶ During the course of the psychotic illness, this low-grade chronic inflammation may worsen the illness course through facilitating the development of comorbidities,⁴⁷ which in turn may increase the inflammatory state. Also, unhealthy behaviors derived and associated with schizophrenia, such as smoking, poor nutrition, and physical inactivity, aggravate this vicious circle. Furthermore, antipsychotics have an impact on inflammation, particularly cytokine levels.⁴⁸ Antipsychotic agents might exert different effects on the immune system, exhibiting both direct anti-inflammatory activity and indirect pro-inflammatory activity, mediated by their effect on weight-gain and increased adiposity.⁴⁹ Alteration of these immune markers has been implicated in the pathophysiology of schizophrenia-spectrum disorders.^{50,51} Cause or consequence, all the prior influence and are influenced by intestinal microbial unbalance and altered intestinal permeability.²⁸

In fact, many of the risk factors for the development of schizophrenia-spectrum disorders (including early-life stress,^{52,53} inflammation, exposure to toxoplasma gondii, gluten sensitivity, etc.) can be linked through a common origin in the gastrointestinal tract, thus affecting the gut microbiome and leading to leaky gut.^{20,28,54} So far, there are few non-invasive markers of intestinal permeability, which have not been studied in schizophrenia-spectrum disorders (i.e. lipopolysaccharides (LPS) and LPS-binding protein (LBS),⁵⁵ zonulin,⁵⁶ citrulline,⁵⁷ and intestinal fatty-acid binding protein (I-FABP)⁵⁸).

To our knowledge, no study has been conducted to examine the relationship between low-grade chronic inflammation and gut permeability in schizophrenia using the aforementioned biomarkers. Studying the impact and interrelation of low-grade chronic inflammation and intestinal permeability is necessary to understand the physiopathology of schizophrenia-spectrum disorders. This article describes the rationale, objectives, protocol, and presents descriptive results of a new project in this line.

Material and methods

Study design and objectives

The sample of this study (N° PI17/00246, PI Belen Arranz) came from an observational, cross-sectional and multisite study including four centers in Spain: Hospital Clínic de Barcelona, University Hospital Mutua Terrassa, Hospital Parc Sanitari Sant Joan de Déu, and Mental Health Centers La Eria and La Corredoria, Oviedo. The aims of the project are:

- 1) To assess the state of intestinal permeability in patients with schizophrenia-spectrum disorders throughout the combination of 5 factors: tobacco or alcohol use, pharmacological drugs, diet, altered vitamin A and D, and evaluation of the intestinal permeability (state of the tight-junctions).⁵⁹

- 2) To identify the consequences of a high intestinal permeability with the determination of serum inflammatory biomarkers, glucose metabolism, one-carbon metabolism, metabolic syndrome, vitamin D and A concentrations, lead metabolism, oxidative stress biomarkers and presence of systemic inflammatory diseases.
- 3) To assess the interrelation between intestinal permeability, chronic inflammation, metabolic syndrome, and lifestyle habits including diet factors.
- 4) To assess the interrelation between intestinal permeability and chronic inflammation with disease severity, including psychopathological status, and patient's functionality.
- 5) To assess the interrelation between lifestyle habits, including diet factors, with the severity of the disease.
- 6) To assess the role of cognition and cognitive reserve in the interplay between intestinal permeability, chronic inflammation, diet, lifestyle habits, drug use, disease severity, functioning, pharmacological treatments, and peripheral biomarkers.

Sub-analyses will be performed in order to assess potential sex-related differences for the former aims, and also potential differences between diagnostic groups (i.e. first-episode psychosis and schizoaffective disorder).

Sample

A total of 646 adult patients with DSM-5 (Diagnostic and Statistical Manual of Mental Disorders)⁶⁰ schizophrenia-spectrum disorder at any stage of the disease have been included.

The inclusion criteria for patients were: (1) adults over 18 years of age; (2) ability to speak Spanish correctly; and (3) signed informed consent. Exclusion criteria were: (1) history of head trauma with loss of consciousness and (2) organic disease with mental repercussions; (3) presence of an acute inflammatory process: (3.1) fever ($>38^{\circ}\text{C}$) or infection in the two weeks prior to the baseline interview, or (3.2) have received vaccinations in the past 4 weeks.

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and Good Clinical Practice and the Ethics and Research Boards from all recruiting centers. All participants provided written informed consent prior to their inclusion in the study.

Assessment

Sociodemographic assessment

The following sociodemographic variables were collected: age, gender and drug misuse habits, years of illness duration, first-degree relatives with schizophrenia-spectrum disorder and diagnoses. Diagnostic was determined according to the diagnostic criteria of DSM-5.

Anthropometric assessment

Anthropometric measurements included weight, height, body mass index (BMI), body circumference, blood pressure and percentage of fat mass and lean body mass measured by bioelectrical impedance scale.

Intestinal permeability, diet, and physical exercise assessment

Lifestyle habits were assessed with the Mediterranean Diet Adherence Screener (MEDAS),^{61,62} a validated questionnaire of Mediterranean diet adherence consisting of 14-items (intake and amount of extra-virgin olive oil, frequency of fruit, vegetables, nuts, legumes, red meat, poultry, fish, animal fat, sweetened beverages, sweets and fried food), used in the Prevención con Dieta Mediterránea (PREDIMED) study.⁶³ MEDAS score was calculated

by assigning a score of 1 and 0 for each item. The Short Scale of Physical Activity (IPAQ)⁶⁴ assesses specific types of activity such as walking, moderate-intensity activities and vigorous intensity activities. Frequency (measured in days per week) and duration (time per day) are collected separately for each specific type of activity. Continuous Score IPAQ results are expressed as MET-min per week and calculated by multiplying the MET assigned to it (vigorous—8 MET, moderate—4 MET and walking—3.3 MET) by the number of days it was performed during a week, where MET corresponds to O_2 consumption during the rest and equals $3.5 \text{ mL O}_2/\text{kg}$ of the body mass per minute. Finally, a questionnaire from the symptoms of permeable-intestine-syndrome was created and administered to each patient. The questionnaire consisting of 19 items, using 4-point Likert scale ranging from 0 “never” to 3 “everyday”, included questions about the presence or absence of chronic diarrhea, constipation, or bloating, fatigue, headaches, difficulty concentrating, skin problems, such as acne, rashes, or eczema, asthma, food allergies, use of non-steroid anti-inflammatory drugs or antibiotics, gluten or lactose intolerance, probiotic use, joint pain or widespread inflammation. Higher scores indicate higher severity of permeable intestine syndrome.

Clinical and functional assessment

A psychopathological assessment was carried out with the Spanish version of the Positive and Negative Syndrome Scale (PANSS)⁶⁵ and the Clinical Global Impression-Severity (CGI-S).⁶⁶ Higher scores indicate greater severity.

The functioning level was assessed by Global Assessment of Functioning (GAF).⁶⁷ It is a scale used to assess the severity of symptoms and the level of functioning, on a numeric scale from 1 to 100. Higher scores indicate better functioning.

Neuropsychological and cognitive reserve assessment

To assess cognitive impairment the Screen for Cognitive Impairment in Psychiatry (SCIP)⁶⁸ has been administered. It has five subtests for evaluating immediate (VLT-I) and delayed verbal learning (VLT-D), working memory (WMT), verbal fluency (VFT), and processing speed (PST). The range score goes from 0 to 30 in VLT-I, 0–24 in WMT, ≥ 0 in VFT, 0–10 in VLT-D and 0–30 in PST. In all neurocognitive domains higher scores correspond to better performance.

To assess cognitive reserve (CR), the Cognitive Reserve Assessment Scale in Health (CRASH) was used.⁶⁹ It has showed optimal psychometric properties and it has been demonstrated to be a valid tool to assess CR. The scale's maximum total score is 90. Higher score in this scale indicate greater CR.

Blood collection

Two tubes of 10 mL of peripheral blood from all participants were collected. Either plasma or serum was separated from the blood cells by centrifugation. An aliquot of the collected samples was frozen at -80°C until further specific analyses.

Inflammatory markers

A complete blood analysis was performed, including the analytical parameters reported in Table S1. Inflammatory parameters included C-reactive protein, white cells and platelets, erythrocyte sedimentation rate (ESR), vitamin A, ferritin, transferrin, lipoprotein A, magnesium, fibrinogen, cortisol, prolactin, folate, cyanocobalamin, homocysteine, immunoglobulin A, anti-transglutaminase antibodies (ATA), toxoplasma immunoglobulins, and apolipoproteins A and B. Insulin resistance and metabolic syndrome were assessed using glucose, fasting insulin, Hemoglobin A1c, triglycerides, LDL and HDL cholesterol, along with anthropometric parameters.

Intestinal permeability markers

Lipopolysaccharides (LPS), LPS-binding protein (LBS), citrulline, zonuline, and intestinal fatty-acid binding protein (I-FABP) will be measured as peripheral blood markers of bacterial translocation and gut barrier dysfunction.

Statistical analyses

Descriptive analyses of the main variables were conducted to characterize the study population, as shown in the Results section. Categorical variables were expressed as absolute and relative frequencies and quantitative variables were expressed using means and standard deviations. Statistical Package for the Social Sciences (SPSS v26) was used to analyze data.

To reach the objectives of the project, Pearson Chi-square, and Fisher's exact test in contingency tables (χ^2), will be used to analyze categorical data. The level of significance will be set at $p \leq 0.05$ (two-tailed). The General Linear Model (univariate) procedure will be used to perform analysis of covariance (ANCOVA) applying a logarithmic conversion of variables that do not follow a normal distribution. A factorial analysis will be carried out to determine the combination of factors (including inflammatory markers) related to the degrees (low, medium, high) of intestinal permeability. The more significant variables will be extracted through dimension-reduction techniques and multivariate analyses (such as principal component analysis) will be carried out to determine how the intestinal permeability predicts metabolic syndrome and the severity of disease. Multivariate linear regression models and logistic regression models will be performed to assess the interrelation between the aforementioned variables, intestinal permeability, chronic inflammation, metabolic syndrome, diet factors, disease severity, patient's functionality, the severity of the diseases, cognition, and cognitive reserve. Sub-analyses excluding the population taking anti-inflammatory drugs, probiotics or other drugs potentially affecting the systemic inflammation status or the MBG axis will be performed.

Results

A total of 646 patients with schizophrenia-spectrum disorder were enrolled in the study. 451 (69.7%) had schizophrenia, 112 (17.2%) schizoaffective disorder, and 83 (12.8%) first-episode psychosis. A total of 257 (39.7%) were female with a mean age of 43.19 ± 13.58 years (illness duration of 15.1 ± 11.5 years). A percentage as high as 55.8% consumed tobacco. Regarding clinical status, patients obtained a mean score of 13.68 ± 6.55 in the positive PANSS and 20.38 ± 8.69 in the negative PANSS subscale. The mean CGI was 4.16 ± 2.22 (moderately ill) and GAF was 60.00 ± 14.84 (moderate difficulty in functioning). Table 1 provides a summary of the characteristics of the sample.

Discussion

In recent years, there is growing interest in the study of the MBG axis as well as low-grade chronic inflammation in mental illness.^{23,70} As mentioned in the introduction, the interaction between leaky gut, systemic inflammation, their influence in the blood-brain barrier and the central nervous system, and their role in the aetiopathogenesis of mental illness is still not clear.^{16,28} Furthermore, the interplay between leaky gut-inflammation with diet and lifestyle habits, drug use, disease severity and symptoms, cognition and cognitive reserve⁷¹ has not been elucidated.^{26,29} In addition, the ideal biomarkers to assess intestinal permeability in a clinical setting are still under discussion,³⁶ and the most representative peripheral biomarkers to assess low-grade

Table 1

Characteristics of the sample (N = 646).

Sociodemographic variables	
Age (M $\pm$ SD)	43.19 $\pm$ 13.58
Sex: Females N (%)	257 (39.7)
Illness duration (years)	15.1 $\pm$ 11.5
First episode psychosis N (%)	83 (12.8%)
Schizophrenia N (%)	451 (69.7%)
Schizoaffective disorder N (%)	112 (17.2)
First-degree relative with schizophrenia: Yes N (%)	98 (22.0)
Tobacco use: Yes N (%)	330 (55.8)
Cannabis use: Yes N (%)	58 (12.0)
Alcohol use: Yes N (%)	97 (20.3)
Obstetric complications: Yes N (%)	66 (15.9)
Anthropometric assessment (M $\pm$ SD)	
Weight (kg)	81.0 $\pm$ 17.8
Height (m)	1.69 $\pm$ 0.1
Body mass index (kg/m ²)	28.4 $\pm$ 5.8
Body circumference (cm)	100.4 $\pm$ 16.1
Intestinal permeability (M $\pm$ SD)	
MEDAS	7.70 $\pm$ 2.18
Intestinal permeability scale	3.47 $\pm$ 2.48
IPAQ	1953.24 $\pm$ 2461.23
Clinical and functional variables (M $\pm$ SD)	
PANSS positive	13.68 $\pm$ 6.55
PANSS negative	20.38 $\pm$ 8.69
PANSS general	32.10 $\pm$ 12.15
PANSS total	66.16 $\pm$ 24.19
CGI	4.16 $\pm$ 2.22
GAF	60.00 $\pm$ 14.84
Antipsychotic treatment	
No	5 (0.8)
One	377 (59.0)
Two	257 (40.2)
Neurocognitive variables and cognitive reserve (M $\pm$ SD)	
Immediate verbal learning	17.28 $\pm$ 5.27
Delayed verbal learning	15.00 $\pm$ 5.88
Working memory	13.49 $\pm$ 5.34
Verbal fluency	4.27 $\pm$ 2.67
Processing speed	7.78 $\pm$ 4.02
CRASH	30.53 $\pm$ 14.11

M: mean; SD: standard deviation; N: number; kg: kilograms; m: meters; cm: centimeters; MEDAS: Mediterranean Diet Adherence Screener: Questionnaire of Adherence to Mediterranean Diet; IPAQ: Short Scale of Physical Activity; PANSS: Positive and Negative Syndrome Scale; CGI: Clinical Global Impression Scale; GAF: Global Assessment of Functioning; SCIP: Screen for Cognitive Impairment in Psychiatry; CRASH: Cognitive Reserve Assessment Scale in Health.

chronic inflammation in schizophrenia are common to other medical comorbidities,^{10,16} and are also influenced by psychotropic medications,¹² and intestinal permeability.²⁷

The current project aims to generate knowledge to shed light on the aforementioned unsolved research questions. To our knowledge, this will be the first study assessing the relation among intestinal permeability, low-grade chronic inflammation, peripheral biomarkers, clinical, functional and sociodemographic variables, as well as diet and lifestyle in a large sample of patients with a schizophrenia-spectrum disorder.

In the coming articles, we expect to show that (1) patients with schizophrenia present altered intestinal permeability and secondary low-grade chronic inflammation, which mediates metabolic syndrome and other systemic inflammatory diseases. (2) Inflammatory biomarkers are related to intestinal permeability, Mediterranean-diet adherence, exercise and metabolic syndrome. (3) Altered intestinal permeability is related to a worse disease severity and functionality and to more impaired cognition and lower cognitive reserve. (4) High adherence with Mediterranean-diet will be associated with less altered intestinal permeability and secondarily to less inflammation markers and better functionality in schizophrenia.

This project has many limitations. To start with, there is no control group. This is due to the fact that peripheral analytical parameters have reference (normal) values for the healthy population. Also, the external validity of the results is high due to unrestrictive inclusion criteria and the main goal of the project is not to compare a population with healthy controls, but to establish the status of intestinal permeability in a specific population and to find the most predictive factors for this quantification. Moreover, an inherent limitation in clinical and neuropsychological assessments lies in the differences between observers. Therefore, semi-structured clinical interviews were included and periodical meetings were held to reduce the differences between neuropsychological assessors. Even the observational design brings certain strengths to the project, there is a wide variability in variables, such as psychotropic medications, disease severity, or diet. Some variables, such as cognition and intestinal permeability, can be influenced by symptoms/drug treatment and diet respectively. Notwithstanding, all the variables that were deemed to influence the main outcomes have been registered and their effect will be taken into account in the analyses, and the sample is large enough as to perform sub-analyses to assess sex-related differences, differences in specific subpopulations (such as first episode psychoses, schizoaffective disorder) or to specific variables (such as specific pharmacological treatments). The fact that the sample is heterogeneous with broad inclusion criteria ensures that this project includes people with schizophrenia representative from our everyday clinical practice and that results derived may be generalizable to a real-world setting.⁷² Despite the aforementioned limitations, this is an innovative project analyzing a large quantity of clinical, functional,⁷³ cognitive, lifestyle, and biomarker variables, which can allow for a deeper understanding of the concept of intestinal permeability and low-grade chronic inflammation and their interplay in people with schizophrenia-spectrum disorders.

In conclusion, the results obtained by this project are hoped to contribute toward the understanding of the physiopathology of intestinal permeability and inflammation in schizophrenia-spectrum disorders. This will likely aid to personalize treatments in real-world clinical practice, potentially including variables related to intestinal permeability and inflammation.

Authors' contributions

BA and GS designed the project. BA, GS, MB, and MGP coordinated the project development. GA and SA drafted the manuscript. GA, SA, GS, AM, MTPC, MA, CH, MSA, FPB, LGB, MGP, and BA participated in the recruitment. SA and GA performed the statistical analyses. All authors reviewed and approved the final version of the manuscript.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

Consent for publication

All participants were asked to provide written informed consent prior to their inclusion in the study.

Funding

This project is funded by the *Instituto de Salud Carlos III (ISCIII)*, in the grant call for the year 2017 as part of the Fondo de Investigación

Sanitaria (FIS) (N° PI17/00246), modality “Proyectos de Investigación en Salud de la Acción Estratégica en Salud 2013–2016”.

Conflicts of interest

Gerard Anmella has received CME-related honoraria, or consulting fees from Angelini, Casen Recordati, Janssen-Cilag, Lundbeck, Lundbeck/Otsuka, and Rovi, with no financial or other relationship relevant to the subject of this article.

Eduard Vieta has received research support from or served as consultant, adviser or speaker for AB-Biotics, Abbott, Abbvie, Adamed, Angelini, Biogen, Celon, Dainippon Sumitomo Pharma, Ferrer, Gedeon Richter, GH Research, Glaxo SimthKline, Janssen, Lundbeck, Organon, Otsuka, Rovi, Sage pharmaceuticals, Sanofi-Aventis, Shire, Sunovion, Takeda, and Viartis, out of the submitted work.

Leticia González-Blanco has received honoraria for lecturing, research and/or travel grants for attending conferences from the Spanish Foundation of Psychiatry and Mental Health, Instituto de Salud Carlos III, Otsuka-Lundbeck, Janssen-Cilag, Angelini, Casen Recordati and Pfizer.

Maria Paz Garcia-Portilla has been a consultant to and/or has received honoraria/grants from Adamed, Angelini, Casen Recordati, Alianza Otsuka-Lundbeck, Janssen-Cilag, Lundbeck, Otsuka, and SAGE Therapeutics.

Miquel Bernardo has been a consultant for, received grant/research support and honoraria from, and been on the speakers/advisory board (unrelated to the present work) of AB-Biotics, Adamed, Angelini, Casen Recordati, Janssen-Cilag, Lundbeck, Otsuka, Menarini and Takeda.

All other authors report no financial or other relationship relevant to the subject of this article.

Acknowledgements

We are grateful to all participants.

We would also like to thank the Carlos III Healthcare Institute, the Spanish Ministry of Science, Innovation and Universities, the European Regional Development Fund (ERDF/FEDER) (PI080208, PI11/00325 and PI14/00612); CIBERSAM; CERCA Program; Catalan Government, the Secretariat of Universities and Research of the Department of Enterprise and Knowledge (2017SGR1355); PERIS (SLT006/17/00345), Institut de Neurociències, Universitat de Barcelona, and the Government of the Principality of Asturias PCTI-2021–2023 IDI/2021/111.

Gerard Anmella is supported by a Rio Hortega 2021 grant (CM21/00017) and M-AES mobility fellowship (MV22/00058), from the Spanish Ministry of Health financed by the Instituto de Salud Carlos III (ISCIII) and co-financed by the Fondo Social Europeo Plus (FSE+).

SA has been supported by Sara Borrell doctoral programme (CD20/00177) and M-AES mobility fellowship (MV22/00002), from the Instituto de Salud Carlos III (ISCIII), and co-funded by European Social Fund “Investing in your future”.

Eduard Vieta thanks the support of the Spanish Ministry of Science, Innovation and Universities (PI15/00283, PI18/00805, PI19/00394, CPII19/00009) integrated into the Plan Nacional de I+D+I and co-financed by the Instituto de Salud Carlos III (ISCIII)-Subdirección General de Evaluación and the Fondo Europeo de Desarrollo Regional (FEDER); the ISCIII; the CIBER of Mental Health (CIBERSAM); the Secretaria d'Universitats i Recerca del Departament d'Economia i Coneixement (2017 SGR 1365), and the CERCA Programme/Generalitat de Catalunya. We would like to thank the Departament de Salut de la Generalitat de Catalunya for the PERIS grant SLT006/17/00357.

Appendix A. Supplementary material

Supplementary material associated with this article can be found in the online version available at <https://doi.org/10.1016/j.sjpmh.2023.09.005>.

References

1. Lasić D, Bevanda M, Bošnjak N, Uglešić B, Glavina T, Frani T. Metabolic syndrome and inflammation markers in patients with schizophrenia and recurrent depressive disorder. *Psychiatr Danub*. 2014;26:214–219.
2. Müller N, Weidinger E, Leitner B, Schwarz MJ. The role of inflammation in schizophrenia. *Front Neurosci*. 2015;9:372.
3. Halstead S, Siskind D, Amft M, et al. Alteration patterns of peripheral concentrations of cytokines and associated inflammatory proteins in acute and chronic stages of schizophrenia: a systematic review and network meta-analysis. *Lancet Psychiatry*. 2023;10:260–271.
4. Parksepp M, Haring L, Kilk K, et al. A marked low-grade inflammation and a significant deterioration in metabolic status in first-episode schizophrenia: a five-year follow-up study. *Metabolites*. 2022;12:983.
5. Roomruangwong C, Noto C, Kanchanatawan B, et al. The role of aberrations in the Immune-Inflammatory Response System (IRS) and the Compensatory Immune-Regulatory Reflex System (CIRS) in different phenotypes of schizophrenia: the IRS-CIRS theory of schizophrenia. *Mol Neurobiol*. 2020;57:778–797.
6. Leza JC, Bueno B, Bioque M, et al. Inflammation in schizophrenia: a question of balance. *Neurosci Biobehav Rev*. 2015;55:612–626.
7. Upthegrove R, Khandaker GM. Cytokines, oxidative stress and cellular markers of inflammation in schizophrenia. *Curr Top Behav Neurosci*. 2019:44.
8. Hope S, Ueland T, Steen NE, et al. Interleukin 1 receptor antagonist and soluble tumor necrosis factor receptor 1 are associated with general severity and psychotic symptoms in schizophrenia and bipolar disorder. *Schizophr Res*. 2013;145:36–42.
9. Foiselle M, Barbosa S, Godin O, et al. Immuno-metabolic profile of patients with psychotic disorders and metabolic syndrome. Results from the FACE-SZ cohort. *Brain Behav Immun Health*. 2022:22.
10. Castro AM, Macedo-de la Concha LE, Pantoja-Meléndez CA. Low-grade inflammation and its relation to obesity and chronic degenerative diseases. *Revista Médica del Hospital General de México*. 2017;80:101–105.
11. Holt RIG, Bushe C, Citrome L. Diabetes and schizophrenia 2005: are we any closer to understanding the link? *J Psychopharmacol*. 2005;19:56–65.
12. Greenhalgh AM, Gonzalez-Blanco L, Garcia-Rizo C, et al. Meta-analysis of glucose tolerance, insulin, and insulin resistance in antipsychotic-naïve patients with nonaffective psychosis. *Schizophr Res*. 2017;179:57–63.
13. Tiihonen J, Lönnqvist J, Wahlbeck K, et al. 11-Year follow-up of mortality in patients with schizophrenia: a population-based cohort study (FIN11 study). *Lancet*. 2009;374:620–627.
14. Romero-Pardo V, Mascayano F, Susser ES, Martínez-Alés G. Schizophrenia incidence in Spain: more questions than facts. *Rev Psiquiatr Salud Ment*. 2022;15:61–62.
15. Zurrón Madera P, Casaprima Suárez S, García Álvarez L, García-Portilla González MP, Junquera Fernández R, Canut MTL. Eating and nutritional habits in patients with schizophrenia. *Rev Psiquiatr Salud Ment*. 2022;15:54–60.
16. Fasano A. Gut permeability, obesity, and metabolic disorders: who is the chicken and who is the egg? *Am J Clin Nutr*. 2017;105:3–4.
17. Kelly JR, Kennedy PJ, Cryan JF, Dinan TG, Clarke G, Hyland NP. Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Front Cell Neurosci*. 2015:9.
18. Patel JP, Frey BN. Disruption in the blood–brain barrier: the missing link between brain and body inflammation in bipolar disorder? *Neural Plast*. 2015:2015.
19. Bischoff SC, Barbara G, Buurman W, et al. Intestinal permeability – a new target for disease prevention and therapy. *BMC Gastroenterol*. 2014;14:189.
20. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol*. 2020;19:55–71.
21. Naveed M, Zhou QG, Xu C, et al. Gut–brain axis: a matter of concern in neuropsychiatric disorders. *Prog Neuropsychopharmacol Biol Psychiatry*. 2021;104:110051.
22. García Bueno B, Caso JR, Madrigal JLM, Leza JC. Innate immune receptor Toll-like receptor 4 signalling in neuropsychiatric diseases. *Neurosci Biobehav Rev*. 2016;64:134–147.
23. Powell N, Walker MM, Talley NJ. The mucosal immune system: master regulator of bidirectional gut–brain communications. *Nat Rev Gastroenterol Hepatol*. 2017;14:143–159.
24. Grenham S, Clarke G, Cryan JF, Dinan TG. Brain–gut–microbe communication in health and disease. *Front Physiol*. 2011;2:94.
25. Obermeier B, Daneman R, Ransohoff RM. Development, maintenance and disruption of the blood–brain barrier. *Nat Med*. 2013;19:1584–1596.
26. Callaghan BL, Fields A, Gee DG, et al. Mind and gut: associations between mood and gastrointestinal distress in children exposed to adversity. *Dev Psychopathol*. 2020;32:309–328.
27. Cryan JF, O’riordan KJ, Cowan CSM, et al. The microbiota–gut–brain axis. *Physiol Rev*. 2019;99:1877–2013.
28. Karakula-Juchnowicz H, Dzikowski M, Pelczarska A, Dzikowska I, Juchnowicz D. The brain–gut axis dysfunctions and hypersensitivity to food antigens in the etiopathogenesis of schizophrenia. *Psychiatr Pol*. 2016;50:747–760.
29. Rodrigues-Amorim D, Rivera-Baltanás T, Regueiro B, et al. The role of the gut microbiota in schizophrenia: current and future perspectives. *World J Biol Psychiatry*. 2018;19:571–585.
30. Zhuo C, Yao Y, Xu Y, et al. Schizophrenia and gut–flora related epigenetic factors. *Prog Neuropsychopharmacol Biol Psychiatry*. 2019;90:49–54.
31. Wood NC, Hamilton I, Axon ATR, et al. Abnormal intestinal permeability. An aetiological factor in chronic psychiatric disorders? *Br J Psychiatry*. 1987;150:853–856.
32. Lambert MT, Bjarnason I, Connelly JB, et al. Small intestine permeability in schizophrenia. *Br J Psychiatry*. 1989;155:619–622.
33. Severance EG, Alaedini A, Yang S, et al. Gastrointestinal inflammation and associated immune activation in schizophrenia. *Schizophr Res*. 2012;138:48–53.
34. Pu Z, Sun Y, Jiang H, et al. Effects of berberine on gut microbiota in patients with mild metabolic disorders induced by olanzapine. *Am J Chin Med (Gard City NY)*. 2021;49:1949–1963.
35. Ishida I, Ogura J, Aizawa E, et al. Gut permeability and its clinical relevance in schizophrenia. *Neuropsychopharmacol Rep*. 2022;42:70.
36. Vojdani A. For the assessment of intestinal permeability, size matters. *Altern Ther Health Med*. 2013;19:12–24.
37. Thirion F, Speyer H, Hansen TH, et al. Alteration of gut microbiome in patients with schizophrenia indicates links between bacterial tyrosine biosynthesis and cognitive dysfunction. *Biol Psychiatry Global Open Sci*. 2023;3:283.
38. Bucci P, Giordano GM, Mucci A, et al. Sex and gender differences in clinical and functional indices in subjects with schizophrenia and healthy controls: data from the baseline and 4-year follow-up studies of the Italian Network for Research on Psychoses. *Schizophr Res*. 2023;251:94–107.
39. Zhu M, Liu Z, Guo Y, et al. Sex difference in the interrelationship between TNF- α and oxidative stress status in first-episode drug-naïve schizophrenia. *J Neuroinflamm*. 2021:18.
40. He J, Wei Y, Li J, et al. Sex differences in the association of treatment-resistant schizophrenia and serum interleukin-6 levels. *BMC Psychiatry*. 2023:23.
41. Yang X, Yang H, Li N, Li C, Liang W, Zhang X. Increased serum homocysteine in first episode and drug-naïve individuals with schizophrenia: sex differences and correlations with clinical symptoms. *BMC Psychiatry*. 2022:22.
42. Zhou Y, Song X, Guo Y, Lang X, Li Z, Zhang XY. Sex differences in metabolic disorder patterns of first-episode drug-naïve patients with schizophrenia. *Psychoneuroendocrinology*. 2021:124.
43. Cyran A, Piotrowski P, Samochowiec J, Grądzewski T, Misiak B. Risk factors of deficit and non-deficit schizophrenia: results from a cross-sectional study. *Rev Psiquiatr Salud Ment*. 2022;15:223–229.
44. Arranz B, Rosel P, Ramírez N, et al. Insulin resistance and increased leptin concentrations in noncompliant schizophrenia patients but not in antipsychotic-naïve first-episode schizophrenia patients. *J Clin Psychiatry*. 2004;65:1335–1342.
45. Orhilly S. Human genetics illuminates the paths to metabolic disease. *Nature*. 2009;462:307–314.
46. Trépanier MO, Hopperton KE, Mizrahi R, Mechawar N, Bazinet RP. Postmortem evidence of cerebral inflammation in schizophrenia: a systematic review. *Mol Psychiatry*. 2016;21:1009–1026.
47. Balótšev R, Koido K, Vasar V, et al. Inflammatory, cardio-metabolic and diabetic profiling of chronic schizophrenia. *Eur Psychiatry*. 2017;39:1–10.
48. Zajkowska Z, Mondelli V. First-episode psychosis: an inflammatory state? *Neuroimmunomodulation*. 2014;21:102–108.
49. Mondelli V, Howes O. Inflammation: its role in schizophrenia and the potential anti-inflammatory effects of antipsychotics. *Psychopharmacology (Berl)*. 2013;231:317–318.
50. Poggi A, Benelli R, Venè R, et al. Human gut-associated natural killer cells in health and disease. *Front Immunol*. 2019;10:961.
51. Miller BJ, Goldsmith DR. Towards an immunophenotype of schizophrenia: progress, potential mechanisms, and future directions. *Neuropsychopharmacology*. 2017;42:299–317.
52. Zhu F, Guo R, Wang W, et al. Transplantation of microbiota from drug-free patients with schizophrenia causes schizophrenia-like abnormal behaviors and dysregulated kynurenine metabolism in mice. *Mol Psychiatry*. 2019;25:2905–2918.
53. El Aidy S, Ramsteijn AS, Dini-Andreote F, et al. Serotonin transporter genotype modulates the gut microbiota composition in young rats, an effect augmented by early life stress. *Front Cell Neurosci*. 2017;11:222.
54. Lionetti E, Leonardi S, Franzonello C, Mancardi M, Ruggieri M, Catassi C. Gluten psychosis: confirmation of a new clinical entity. *Nutrients*. 2015;7:5532–5539.
55. Jayashree B, Bibin YS, Prabhu D, et al. Increased circulatory levels of lipopolysaccharide (LPS) and zonulin signify novel biomarkers of proinflammation in patients with type 2 diabetes. *Mol Cell Biochem*. 2014;388:203–210.
56. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. *Physiol Rev*. 2011;91:151–175.
57. Kong W, Wang J, Ping X, et al. Biomarkers for assessing mucosal barrier dysfunction induced by chemotherapy: identifying a rapid and simple biomarker. *Clin Lab*. 2015;61:371–378.
58. Jensen SB, Sheikh MA, Akkouch IA, et al. Elevated systemic levels of markers reflecting intestinal barrier dysfunction and inflammasome activation are correlated in severe mental illness. *Schizophr Bull*. 2023;49:635–645.

59. Arranz B, Sanchez-Autet M, San L, et al. Are plasma 25-hydroxyvitamin D and retinol levels and one-carbon metabolism related to metabolic syndrome in patients with a severe mental disorder? *Psychiatry Res.* 2019;273:22–29.
60. American Psychiatric Association. *DSM-5: Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Washington, DC: APA; 2013.
61. García-Conesa MT, Philippou E, Pafilas C, et al. Exploring the validity of the 14-item Mediterranean diet adherence screener (MEDAS): a cross-national study in seven European countries around the Mediterranean Region. *Nutrients.* 2020;12:1–18.
62. Schröder H, Fitó M, Estruch R, et al. A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women. *J Nutr.* 2011;141:1140–1145.
63. Martínez-González MA, García-Arellano A, Toledo E, et al. A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. *PLoS ONE.* 2012;7:e43134.
64. Booth M. Assessment of physical activity: an international perspective. *Res Q Exerc Sport.* 2000;71(suppl 2):114–120.
65. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull.* 1987;13:261–276.
66. Guy W. *ECDEU Assessment Manual for Psychopharmacology, Revised. US Department of Health, Education, and Welfare Publication (ADM)*. Rockville, MD: National Institute of Mental Health. Scientific Research Publishing; 1976.
67. Jones SH, Thornicroft G, Coffey M, Dunn G. A brief mental health outcome scale-reliability and validity of the Global Assessment of Functioning (GAF). *Br J Psychiatry.* 1995;166:654–659.
68. Pino O, Guilera G, Rojo JE, et al. Spanish version of the Screen for Cognitive Impairment in Psychiatry (SCIP-S): psychometric properties of a brief scale for cognitive evaluation in schizophrenia. *Schizophr Res.* 2008;99:139–148.
69. Amoretti S, Cabrera B, Torrent C, et al. Cognitive Reserve Assessment Scale in Health (CRASH): its validity and reliability. *J Clin Med.* 2019;8:586.
70. Khalfallah O, Barbosa S, Martinuzzi E, Davidovic L, Yolken R, Glaichenhaus N. Monitoring inflammation in psychiatry: caveats and advice. *Eur Neuropsychopharmacol.* 2022;54:126–135.
71. Amoretti S, Cabrera B, Torrent C, et al. Cognitive reserve as an outcome predictor: first-episode affective versus non-affective psychosis. *Acta Psychiatr Scand.* 2018;138:441–455.
72. Taipale H, Schneider-Thoma J, Pinzón-Espinosa J, et al. Representation and outcomes of individuals with schizophrenia seen in everyday practice who are ineligible for randomized clinical trials. *JAMA Psychiatry.* 2022;79:210–218.
73. Escandell MJ, Prat G, Garcia-Franco M, et al. Clinical symptoms and social functioning in schizophrenia. *Rev Psiquiatr Salud Ment.* 2022;15:251–258.