Acta Neuropsychiatrica

cambridge.org/neu

Gut microbiota alterations in stable outpatients with schizophrenia: findings from a case–control study

Błażej Misiak[1], Patryk Piotrowski[1], Agnieszka Cyran[1], Krzysztof Kowalski[1], Jerzy Samochowiec[2], Marcin Jabłoński[2], Piotr Plichta[2], Łukasz Łaczmański[3], Paulina Żebrowska[3], Dorota Kujawa[3], Igor Łoniewski[4,5] and Mariusz Kaczmarczyk[6]

[1]Department of Psychiatry, Division of Consultation Psychiatry and Neuroscience, Wroclaw Medical University, Wroclaw, Poland; [2]Department of Psychiatry, Pomeranian Medical University, Szczecin, Poland; [3]Laboratory of Genomics & Bioinformatics, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland; [4]Department of Biochemical Sciences, Pomeranian Medical University, Szczecin, Poland; [5]Departmenrt of Human Nutrition and Metabolomics, Pomeranian Medical University, Szczecin, Poland and [6]Department of Clinical and Molecular Biochemistry, Pomeranian Medical University, Szczecin, Poland

Original Article

Cite this article: Misiak B, Piotrowski P, Cyran A, Kowalski K, Samochowiec J, Jabłoński M, Plichta P, Łaczmański Ł, Żebrowska P, Kujawa D, Łoniewski I, and Kaczmarczyk M. (2023) Gut microbiota alterations in stable outpatients with schizophrenia: findings from a case–control study. *Acta Neuropsychiatrica* **35**:147–155. doi: 10.1017/neu.2022.38

Received: 14 July 2022
Revised: 30 November 2022
Accepted: 1 December 2022
First published online: 12 December 2022

Key words:
gastrointestinal microbiome; psychotic disorders; cognition

Author for correspondence:
Błażej Misiak,
Email: blazej.misiak@umw.edu.pl

Abstract

Objective: The pathogenesis of schizophrenia is multidimensional and intensively studied. The gut–brain axis disturbances might play a significant role in the development of schizophrenia. *Methods:* We compared the gut microbiota of 53 individuals with schizophrenia and 58 healthy controls, using the 16S rRNA sequencing method. Individuals with schizophrenia were assessed using the following scales: the Positive and Negative Syndrome Scale, the Calgary Depression Scale for Schizophrenia, the Social and Occupational Functioning Assessment Scale and the Repeatable Battery for the Assessment of Neuropsychological Status. *Results:* No significant between-group differences in α-diversity measures were observed. Increased abundance of Lactobacillales (order level), Bacilli (class level) and Actinobacteriota (phylum level) were found in individuals with schizophrenia regardless of potential confounding factors, and using two independent analytical approaches (the distance-based redundancy analysis and the generalised linear model analysis). Additionally, significant correlations between various bacterial taxa (the Bacteroidia class, the Actinobacteriota phylum, the Bacteroidota phylum, the Coriobacteriales order and the Coriobacteria class) and clinical manifestation (the severity of negative symptoms, performance of language abilities, social and occupational functioning) were observed. *Conclusions:* The present study indicates that gut microbiota alterations are present in European patients with schizophrenia. The abundance of certain bacterial taxa might be associated with the severity of negative symptoms, cognitive performance and general functioning. Nonetheless, additional studies are needed before the translation of our results into clinical practice.

Significant outcomes

- The present study found an increased abundance of Lactobacillales (order level), Bacilli (class level) and Actinobacteriota (phylum level) in patients with schizophrenia.
- The abundance of Bacteroidia (class level), Actinobacteriota (phylum level), Bacteroidota (phylum level), Coriobacteriales (order level) and Coriobacteria (class level) was related to the severity of negative symptoms, general functioning and performance of language abilities.

Limitations

- All patients in our sample were medicated.
- Lifestyle characteristics, other than dietary habits, were not controlled in this study.

Introduction

Schizophrenia is a complex neurodevelopmental disorder manifesting in multidimensional psychopathology that includes positive symptoms (delusions and hallucinations), negative symptoms (social withdrawal, diminished emotional reactivity, anhedonia, alogia and avolition) and mood symptoms (Owen *et al.*, 2016). Moreover, individuals with schizophrenia show

impairments across several cognitive domains that are also present in subjects at clinically high risk of psychosis and unmedicated individuals with first-episode psychosis (Moustafa *et al.*, 2016). Although current strategies of pharmacological treatment offer acceptable efficacy with respect to positive symptoms, cognitive deficits and negative symptoms are often resistant to available treatments and largely contribute to impairments of social functioning.

There is a general consensus that schizophrenia is a multi-systemic disorder with a number of biological alterations detectable outside the brain (Pillinger *et al.*, 2019). Among them, subclinical inflammation represents one of the most widely replicated phenomena in subjects with schizophrenia. In this population, subclinical inflammation manifests in altered levels of various leukocyte populations (Karpiński *et al.*, 2018), together with elevated blood and cerebrospinal fluid levels of C-reactive protein (CRP) (Fernandes *et al.*, 2016), pro-inflammatory cytokines (Miller *et al.*, 2011), complement cascade components (Laskaris *et al.*, 2019) as well as specific and non-specific antibodies (Ezeoke *et al.*, 2013). Moreover, there is evidence that subclinical inflammation is associated with neurostructural alterations (Pasternak *et al.*, 2016), cognitive impairments (Bora, 2019; Misiak *et al.*, 2018) and poor response to antipsychotic treatment (Mondelli *et al.*, 2015). Also, a recent meta-analysis by Bora (2019) showed that elevated blood levels of CRP are modestly but significantly associated with impairments of global cognition, verbal and working memory, processing speed as well as planning.

Although a great progress in understanding subclinical inflammation in schizophrenia can be observed in recent years, the exact mechanisms underlying this phenomenon remain unclear. It has been proposed that aberrant immune-inflammatory responses in schizophrenia might be attributed to gut microbiota alterations (Samochowiec & Misiak, 2021). Moreover, gut microbiota might communicate with the central nervous system through interactions with the autonomic nervous system, and the bloodstream mediators related to gut hormones, the hypothalamic-pituitary-adrenal axis and metabolites released by microbial species (e.g., short-chain fatty acids) (Misiak *et al.*, 2020). Experimental studies show that the faecal microbiota transplantation from unmedicated patients with schizophrenia induces hyperkinetic behaviours, impairs social interactions and leads to various biological alterations (e.g., up-regulation of the kynurenine-kynurenic acid pathway in the brain and bloodstream as well as altered neurotransmission) in the recipient germ-free mice (Zheng *et al.*, 2019; Zhu *et al.*, 2020a, b). A recent systematic review revealed that individuals with schizophrenia show lower levels of bacterial genera that produce short-chain fatty acids as well as higher levels of the bacteria that produce lactic acid and the bacteria associated with glutamate and GABA metabolism (McGuinness *et al.*, 2022). Although these alterations overlap with those observed in patients with bipolar disorder or major depression, some observations might be specific to individuals with schizophrenia. These include higher abundance of *Prevotell*a as well as lower abundance of *Bacteroides*, *Haemophilus* and *Streptococcus* (findings consistent across at least 20% of studies included in systematic review).

It should be noted that findings form studies in this field are characterised by relatively low replicability. This observation might originate from the effects of potential confounding factors that are rarely controlled. These include, i.e., recruitment of patients at various stages of illness, medication effects, comorbidities and lifestyle characteristics (e.g., dietary habits and substance use). To the best of our knowledge, recent studies on gut microbiota in schizophrenia were mainly based on the U.S. or Chinese populations, with only few studies representing the European population (Schwarz *et al.*, 2018; Manchia *et al.*, 2021). Importantly, there is evidence that geography and ethnicity serve as important determinants of gut microbiota composition (Gupta *et al.*, 2017). A lack of methodological homogeneity is also an important reason underlying low replicability of findings (Nguyen *et al.*, 2021). Moreover, it has been shown that gut microbiota alterations might be associated with psychopathological symptoms of schizophrenia, including positive, negative and depressive symptoms (Li *et al.*, 2020; Nguyen *et al.*, 2019; Zhu *et al.*, 2021). Also, only one study investigated as to whether oropharyngeal microbiota alterations are associated with cognitive impairment observed in schizophrenia (Yolken *et al.*, 2021). In light of these shortcomings and research gaps, we aimed to compare gut microbiota composition between stable outpatients with schizophrenia and healthy controls (HCs), controlling for the effects of various confounding factors. Moreover, we aimed to investigate as to whether gut microbiota composition is associated with clinical manifestation and cognitive impairment.

Material and methods

Participants

A total of 53 individuals with schizophrenia and 58 HCs were enrolled at two university hospitals in Wroclaw and Szczecin (Poland) as the convenience sample. All participants were non-consanguineous and represented Caucasian ethnicity. The inclusion criteria were (1) age between 18 and 65 years; (2) a diagnosis of schizophrenia according to the DSM-IV criteria, validated using the Operational Criteria for Psychotic Illness (OPCRIT) checklist (McGuffin *et al.*, 1991); (3) maintenance of a stable antipsychotic regimen over the period of at least 6 preceding months and (4) symptomatic remission of positive and disorganisation symptoms based on the Positive and Negative Syndrome Scale (PANSS) items (P1 – delusions, P2 – conceptual disorganisation, P3 – hallucinatory behaviour, G5 – mannerisms/posturing and G9 – unusual thought content rated ≤3) (Andreasen *et al.*, 2005). The daily dosage of antipsychotics was expressed as chlorpromazine equivalents (CPZeq). In addition, the current use of antidepressants and mood stabilisers was recorded for all patients.

In turn, HCs had never received psychiatric diagnosis or treatment. They reported no family members affected by psychotic and mood disorders in first- and second-degree relatives. All of them were screened for psychiatric disorders using the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Sheehan, 1998). Recruitment of HCs was performed through advertisements.

Both groups of participants were matched for age, sex and the level of parental education. The latter one was applied as the proxy measure of socio-economic status.

Clinical assessment

The clinical manifestation was recorded using the following scales: (1) the Positive and Negative Syndrome Scale (PANSS) (Kay *et al.*, 1987); (2) the Social and Occupational Functioning Assessment Scale (Smith *et al.*, 2011) and (3) the Calgary Depression Scale for Schizophrenia (Addington *et al.*, 1994). Two items (N5 – difficulty in abstract thinking and N7 – stereotyped thinking) were excluded from the PANSS score of negative symptoms as there

https://doi.org/10.1017/neu.2022.38 Published online by Cambridge University Press

is evidence that they measure other psychopathological constructs (N5 – cognitive symptoms and N7 – thought disorganisation) (Galderisi *et al.*, 2021). Due to clinical stability, the associations with positive symptoms were not analysed.

The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) was used to assess cognitive performance (Randolph *et al.*, 1998). The RBANS is based on 12 tasks that allow to examine the following domains of cognition: (1) immediate memory (list learning and story memory); (2) visuospatial/constructional abilities (figure copy and line orientation); (3) language (picture naming and semantic fluency); (4) attention (digit span and coding) and (5) delayed memory (list recall, list recognition, story memory and figure recall). Higher RBSANS scores indicate better cognitive performance.

Dietary habits

The Food Frequency Questionnaire 6 (FFQ-6) was administered to record dietary habits (Niedzwiedzka *et al.*, 2019). It is composed of 62 items that record self-reported frequency for the consumption of various food products in the preceding 12 months. Each item measures the frequency of consuming specific food products according to a 6-point scale: 1 – 'never or almost never'; 2 – 'once a month or less'; 3 – 'several times a month'; 4 – 'several times a week'; 5 – 'daily' and 6 – 'several times a day'. In the present study, we conceptualised dietary habits as the adherence to the Mediterranean diet using the aMED score (Hawrysz *et al.*, 2020; Krusinska *et al.*, 2018). The aMED score can be calculated based on the frequency of consuming the following food products: (1) vegetables; (2) fruits; (3) whole grains; (4) fish; (5) legumes; (6) nuts and seeds; (7) the ratio of vegetable oils to animal fat and (8) red and processed meat. Participants with the intake above (or below, in case of red and processed meat) the median intake among HCs receive 1 point; otherwise, they receive 0 points. The aMED score ranges between 0 and 8, with higher scores corresponding to greater adherence to the Mediterranean diet.

Stool collection and sequencing

Participants received home stool collection kits and detailed instructions on how to collect stool samples. Returned samples were immediately frozen at –80°C before DNA extraction.

Subsequently, DNA was extracted from frozen faecal samples using the QIAamp PowerFecal Pro DNA Kit (Qiagen) and concentration was measured with the fluorometric method (Quantus, Promega). The libraries were prepared using the QIAseq 16S/ITS region panels according to the standard protocol (Qiagen) for the V3V4 region. The QC libraries were created using the High Sensitivity D1000 ScreenTape System on the TapeStation (Agilent Technologies). The fluorometric technique was applied to measure the library's concentration (Quantus, Promega). Libraries were diluted according to the QIAseq® 16S/ITS Panel Handbook (Qiagen), pooled and sequenced on the MiSeq (Illumina). Paired-end sequencing was performed using the MiSeq Reagent Kits v3 (600 cycles).

Data processing

Raw sequence data quality was checked using the FastQC application (ver. 0.11.8) and the multiQC application (ver. 1.10.1). The 16S rRNA V3 and V4 primers (341F CCTACGGGNGG CWGCAG and 785R GACTACHVGGGTATCTAATCC) were trimmed off all reads using the *cutadapt* (version 3.5). The 16S rRNA gene sequencing data were processed using the QIIME2 (Bolyen *et al.*, 2019). Sequence denoising and error correction were carried out with the QIIME2 DADA2 plugin (ver. 1.18.0) (Callahan *et al.*, 2016). The forward and reverse sequences were truncated at positions 235 and 220, respectively. The 5' ends of the forward and reverse sequences were trimmed off at the first six bases. Otherwise, the default DADA2 parameter settings were used which resulted in the reconstruction of 5036 features and associated amplicon sequence variants (ASVs). Low-frequency features (<10) were removed. The ASVs were then classified using the naïve Bayesian classifier trained on the target (V3–V4) SILVA v138 99% reference sequence database. Sequences classified as chloroplasts, mitochondria, Archaea or Eukarya, and those that could not be classified at the phylum level were removed. Resulting taxa were collapsed by their taxonomic assignment into six levels (i.e., species, genus, family, order, class and phylum). The final table of ASVs, after taxonomy-based filtering and including samples with available metadata ($n = 111$), contained 3770 features with the median frequency (a total feature count per sample) of 40 921 (range: 7367–157 488). The α-alpha diversity measures were calculated at the level of ASVs and the sampling depth of 7367. The β-diversity (the Bray-Curtis distance) was calculated at each taxonomic level using rarefied tables at depths expressed by the minimum frequency per sample. The feature count tables were rarefied using the *rtk* package (Saary *et al.*, 2017).

Statistical analysis and bioinformatics

Between-group differences in general characteristics were assessed using the χ^2 test (categorical variables) and the Mann–Whitney U test (continuous variables). The level of significance in bivariate tests of general characteristics was set at $p < 0.05$.

Multivariate analysis included the distance-based redundancy analysis (dbRDA) based on the Bray–Curtis dissimilarities using the *capscale* function, while the selection of significant variables was carried out with the *ordistep* function from the *vegan* package (Oksanen *et al.*, 2020) using the combined (forward and backward) stepwise selection algorithm, only for models that were significant in the global test (when all variables were included). The *ordistep* function enables automatic model building for constrained ordination methods (such as the dbRDA). The scores from the simplified dbRDA models (with significant explanatory variables) were used to create ordination diagrams using the scaling with the main interest focused on the relationship between bacterial features and explanatory variables. In this scaling, relative direction of arrows approximates the linear correlation between the bacterial features, between the bacterial features and characteristics of participants (explanatory variables) as well as between explanatory variables.

To examine the gut microbiome compositional differences between patients with schizophrenia and HCs, we performed the differential abundance analysis using both univariate and multivariate analyses based on the generalised linear model with a negative binomial distribution and the log-link function. The generalised linear models for multivariate abundance data were fitted using the *mvabund* package (Wang *et al.*, 2012). The default family, when fitting multivariate model, was negative binomial, assuming a quadratic mean-variance relationship and a log-linear relationship between the dependent and independent variables. A multivariate hypothesis of the difference in the community composition between groups was tested using the analysis of deviance and the log-likelihood ratio test statistic. The family-wise error

https://doi.org/10.1017/neu.2022.38 Published online by Cambridge University Press

rates were analysed with the Westfall and Young's stepdown resampling algorithm (SRA) in the generalised linear model univariate analysis. The relationships between bacterial features and clinical characteristics were assessed using the Spearman correlation coefficients followed by the false discovery rate (FDR) procedure.

Results

The general characteristics of the sample are reported in Table 1. Both groups did not differ significantly in terms of age, sex and the level of parental education. However, significant between-group differences with respect to the level of education were found. Individuals with schizophrenia also presented significantly higher BMI, higher level of nicotine dependence and worse cognitive performance across all domains, except for the RBANS language domain. The frequency of using of concomitant, non-psychiatric medications was similar in both groups.

There were no significant between-group differences with respect to α-diversity measures (Fig. 1). The dbRDA was used to assess the effect of patients' characteristics on the gut microbial community structure (at various taxonomic levels). The first dbRDA analysis was carried out in both subgroups of participants and included the group status (individuals with schizophrenia vs. HCs), age, sex, BMI and the level of education. Explained variance (expressed by adjusted R^2) for simplified models with significant explanatory variables only (selected by the *vegan ordistep* function) varied from 2.3% for the species to 11.3% for the class. Thus, explanatory power of these models was limited. The summary of the full and simplified models (with significant explanatory variables only), including the adjusted R^2, is presented in Supplementary Table 1. Group status (individuals with schizophrenia vs. HCs) was found to be significantly associated with the gut community composition at the higher taxonomic ranks, i.e., the order, class and phylum (Fig. 2). Triplots of the dbRDA output showed that the taxa that were enriched in subjects with schizophrenia represented three orders (Erysipelotrichales, Bifidobacteriales and Lactobacillales), two classes (Bacilli and Actinobacteria) and one phylum (Actinobacteriota). In turn, the taxa that were enriched in HCs represented the order Bacteroidales and the class Bacteroidia.

To identify the bacterial features that were differentially abundant in subjects with schizophrenia and HCs, a set of univariate tests were further performed. These tests revealed that the order Lactobacillales, classes Bacilli and Actinobacteria as well as the phylum Actinobacteriota were enriched in subjects with schizophrenia (Fig. 3). Between-group differences in the abundance of Lactobacillales, Bacilli and Actinobacteriota remained significant after including age, sex, BMI and education as covariates in the generalised linear model analysis (Supplementary Tables 2 and 3).

Finally, a series of bivariate correlations between clinical characteristics and bacterial features were tested using the Spearman coefficients and the FDR procedure (Fig. 4). At the order level, a significant negative correlation between the abundance of Coriobacteriales and the RBANS language score was observed. At the class level, significant correlations of the abundance of Bacteroidia with the SOFAS score, the score of PANSS negative subscale and the RBANS language score were found. Moreover, there was a significant negative correlation of the abundance of

Table 1. General characteristics of the sample

	Schizophrenia, $n = 53$	Healthy controls, $n = 58$	p
Age, years	43.0 ± 13.7	44.4 ± 14.2	0.622
Sex, males (%)	22 (41.5)	21 (36.2)	0.567
Education			
Primary	4 (7.5)	0 (0)	**0.011**
Vocational	8 (15.1)	3 (5.2)	
Secondary	29 (54.7)	47 (81.0)	
Higher	12 (22.7)	8 (13.8)	
Father's education			
Primary	10 (20.0)	9 (15.5)	0.119
Vocational	9 (18.0)	21 (36.2)	
Secondary	20 (40.0)	22 (38.0)	
Higher	11 (22.0)	6 (10.3)	
Mother's education			
Primary	14 (27.0)	7 (12.1)	0.063
Vocational	6 (11.5)	16 (27.6)	
Secondary	23 (44.2)	28 (48.3)	
Higher	9 (17.3)	7 (12.0)	
BMI, kg/m^2	29.9 ± 5.9	26.6 ± 4.2	**<0.001**
FTND	2.0 ± 2.8	1.0 ± 2.3	**0.027**
aMED	2.6 ± 1.1	3.0 ± 1.3	0.066
Antibiotics, *n* (%)	2 (3.8)	0 (0)	0.135
NSAIDs, *n* (%)	15 (28.3)	12 (20.7)	0.350
Probiotics or prebiotics, *n* (%)	3 (5.7)	0 (0)	0.066
Antacids, *n* (%)	5 (9.4)	5 (8.6)	0.881
Antidepressants, *n* (%)	10 (18.9)	–	–
Mood stabilisers, *n* (%)	7 (13.2)	–	–
CPZeq, mg/day	535.3 ± 309.3	–	–
PANSS – negative symptoms	13.2 ± 6.1	–	–
CDSS	2.1 ± 3.0	–	–
RBANS – immediate memory	36.6 ± 8.5	46.3 ± 6.1	**<0.001**
RBANS – visuospatial/ constructional abilities	32.4 ± 6.2	37.3 ± 2.8	**<0.001**
RBANS – language	31.6 ± 11.8	30.4 ± 5.5	0.487
RBANS – attention	44.4 ± 11.9	56.4 ± 12.0	**<0.001**
RBANS – delayed memory	43.2 ± 8.7	51.5 ± 8.3	**<0.001**
RBANS – global cognition	188.2 ± 29.9	221.9 ± 23.5	**<0.001**

aMED, the Adherence to Mediterranean Diet score; BMI, body mass index; CDSS, the Calgary Depression Scale for Schizophrenia; CPZeq, chlorpromazine equivalent dosage; FTND, the Fagerstrom Test for Nicotine Dependence (Pomerleau *et al.*, 1989), NSAIDs, non-steroid anti-inflammatory drugs; PANSS, the Positive and Negative Syndrome Scale; RBANS, the Repeatable Battery for the Assessment of Neuropsychological Status.
Significant differences ($p < 0.05$) were marked with bold characters.

https://doi.org/10.1017/neu.2022.38 Published online by Cambridge University Press

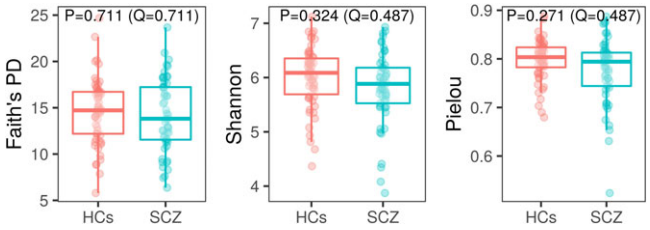

Fig. 1. The comparison of α-diversity measures in patients with schizophrenia (SCZ) and healthy controls (HCs).

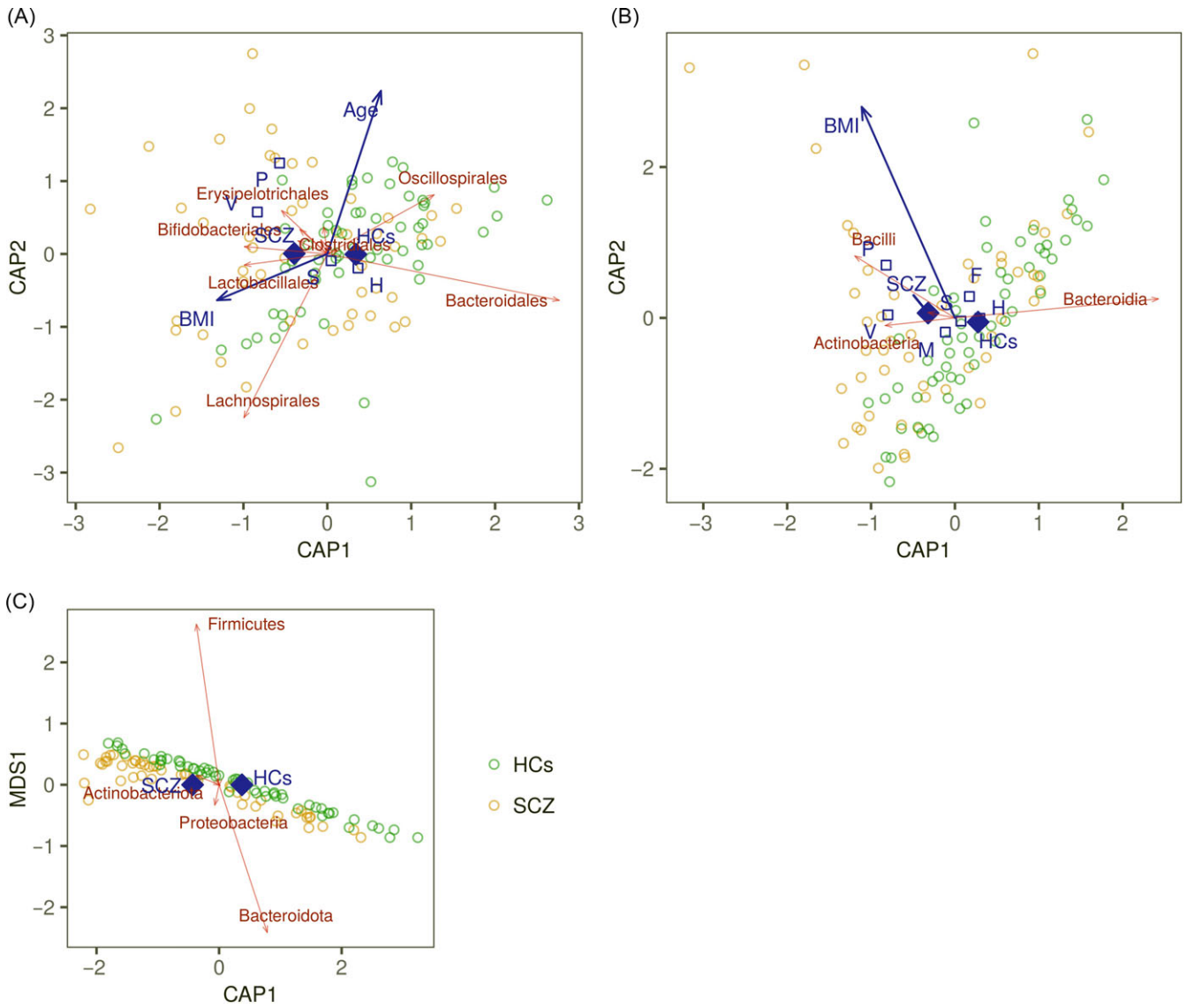

Fig. 2. The triplot ordination diagrams from the Bray–Curtis distance-based RDA analysis at the level of order (A), class (B) and phylum (C). The analysis includes the effects of age, body mass index (BMI), education and group status (schizophrenia vs. healthy controls). Diagrams show the simplified models limited to significant effects. The triplot shows correlations between gut microbes (red arrows) and explanatory variables (blue arrows). Projection of the group status centroids (blue diamonds) on gut microbe vectors approximates their abundance. Abbreviations: F – females; H – higher education; HCs – healthy controls; M – males; P – primary education; V – vocational education; S – secondary education; SCZ – patients with schizophrenia.

Coriobacteria with the RBANS language score. Finally, at the phylum level, significant correlations of the abundance of Bacteroidota with the SOFAS score, the PANSS negative symptoms score and the RBANS language score were found. The abundance of Actinobacteriota (phylum level) was significantly and negatively correlated with the RBANS language score. No significant associations with CPZeq as well as the use of antidepressants and mood stabilisers were found.

https://doi.org/10.1017/neu.2022.38 Published online by Cambridge University Press

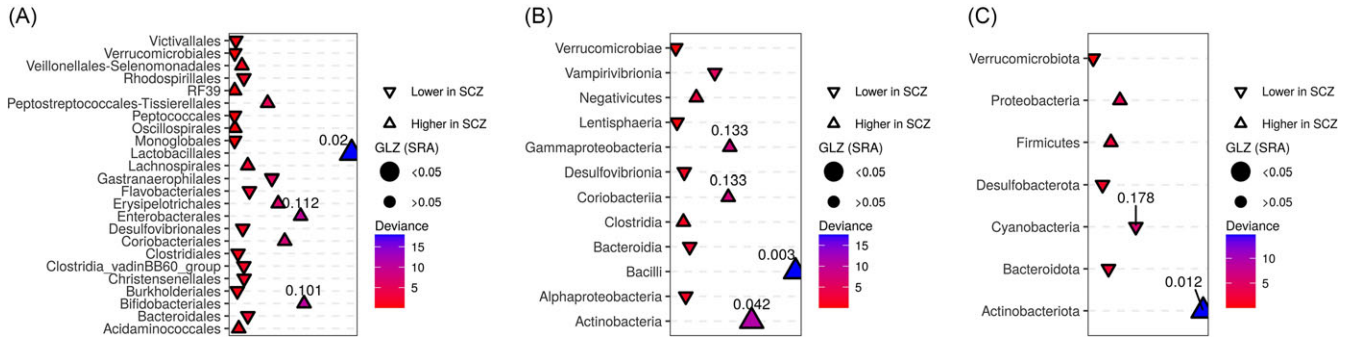

Fig. 3. Results of the differential abundance analysis at the level of order (A), class (B) and phylum (C). The significance of the individual taxa based on the generalised linear model analysis (assuming negative binomial distribution and log-link function) is indicated by the size of the point (triangle) – large triangle indicates the corrected *p*-value < 0.05. Abbreviations: GLZ – generalised linear model; SRA – Westfall and Young's stepdown resampling algorithm; SCZ – schizophrenia.

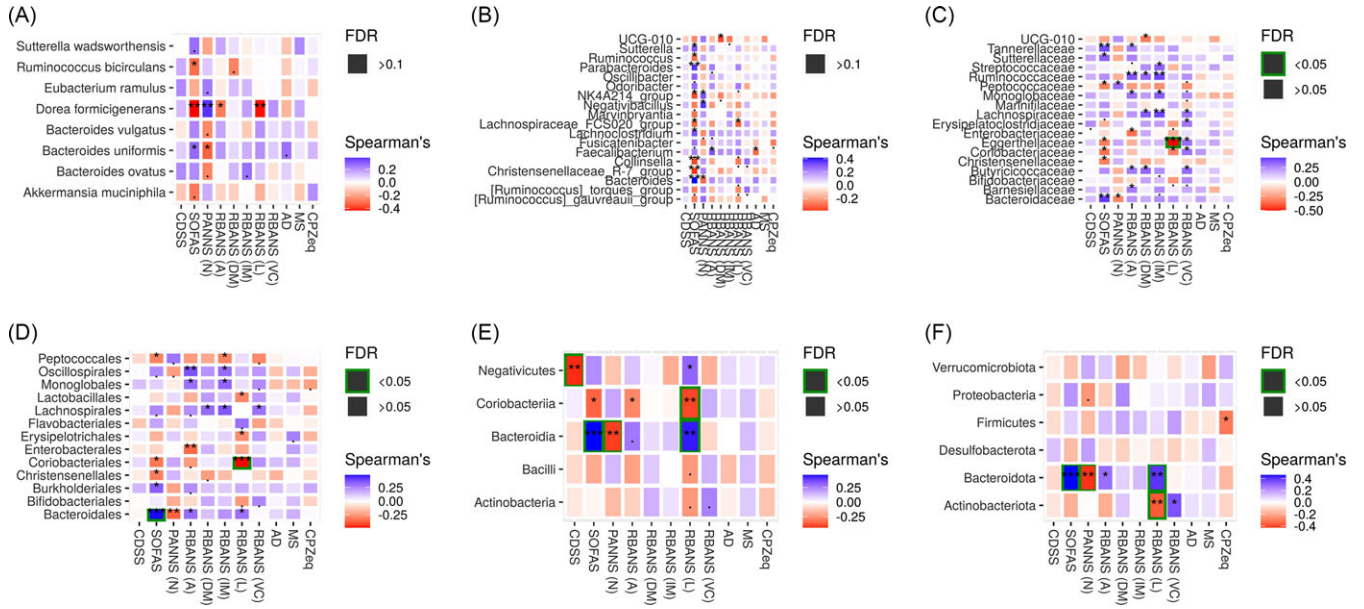

Fig. 4. Bivariate correlations of bacterial abundancies with clinical characteristics at the level of species (A), genus (B), family (C), order (D), class (E) and phylum (F). Abbreviations: AD – antidepressants; CDSS – the Calgary Depression Scale for Schizophrenia; CPZeq – chlorpromazine equivalent dosage; MS – mood stabilisers; PANSS (N) – the Positive and Negative Syndrome Scale – negative symptoms subscore; RBANS (A) – the Repeatable Battery for the Assessment of Neuropsychological Status – attention score; RBANS (DM) – the Repeatable Battery for the Assessment of Neuropsychological Status – delayed memory subscore; RBANS (IM) – the Repeatable Battery for the Assessment of Neuropsychological Status – immediate memory subscore; RBANS (VC) – the Repeatable Battery for the Assessment of Neuropsychological Status – visuospatial/constructional abilities subscore; SOFAS – the Social and Occupational Functioning Assessment Scale.

Discussion

Main findings of the present study indicate that a number of bacteria at various taxonomic levels might show altered abundance in patients with schizophrenia. However, only some of them might be present regardless of potential confounding factors and the method used to analyse the data. These include enrichment of Lactobacillales (the order level), Bacilli (the class level) and Actinobacteriota (the phylum level). Importantly, none of these observations appeared to be associated with the dosage of antipsychotics or the use of antidepressants and mood stabilisers. Also, these findings cannot be attributed to between-group differences in dietary habits operationalised as the aMED score. Our findings are partially in agreement with previous studies showing greater abundance of the phylum Actinobacteria (Li *et al.*, 2020), the family Lactobacillaceae (Ma *et al.*, 2020) and the genus Lactobacillus (Shen *et al.*, 2018) in patients with chronic schizophrenia. In the systematic review, McGuinness *et al.* (2022) found that increased abundance of the Lactobacillus genus is shared by schizophrenia, bipolar disorder and major depression. Therefore, this observation may not be associated with specific severe mental illness. However, it is important to note that Lactobacilli express glutamate decarboxylase that is responsible for the conversion of L-glutamate to GABA (Yogeswara *et al.*, 2020). In turn, Actinobacteriota play a number of physiological roles that might be relevant for the pathogenesis of schizophrenia. These include the regulation of glucose homeostasis, interactions with the immune system through the induction of regulatory T cells and the impact on neurotransmission through the involvement in the tryptophan metabolism pathway (Binda *et al.*, 2018; Kaur *et al.*, 2019). In parallel, the role of inflammation in the development of schizophrenia seems to be associated with microbiome alterations. Studies have shown that increased abundance of bacteria producing lactic acid, such as

https://doi.org/10.1017/neu.2022.38 Published online by Cambridge University Press

the Lactobacillus and Bifidobacterium genus may impact gut inflammation (Xu *et al*., 2020). Moreover, the Lactobacillus genus might stimulate the production of tumour necrosis factor in patients with schizophrenia (He *et al*., 2018). Finally, various bacteria from the class Bacilli can produce dopamine and noradrenaline, thereby influencing the gut–brain axis (Strandwitz, 2018).

The present study also demonstrated a number of correlations between various bacterial taxa (including the Bacteroidia class, the Actinobacteriota phylum, the Bacteroidota phylum, the Coriobacteriales order and the Coriobacteria class) and clinical characteristics (the severity of negative symptoms, performance of the language cognitive domain as well as the level of social and occupational functioning). However, it should be noted that most of them (except for the Actinobacteriota phylum) were not related to differentially abundant taxa. Moreover, although all of these bacterial taxa were related to performance of language abilities, this cognitive domain was not found to be impaired in patients with schizophrenia from our sample. To our knowledge, none of previous studies showed similar patterns of correlations between the abundance of specific bacterial taxa and symptomatic manifestation of schizophrenia. Interestingly, one study found enriched abundance of *Bifidobacterium longum* genus (Actinobacteriota phylum level) in schizophrenia and its correlation with impaired performance of several cognitive domains (Zhu *et al*., 2020b). Moreover, greater abundance of the Lactobacillus group (Lactobacillales order level) was associated with worse global functioning in patients with first-episode psychosis (Schwarz *et al*., 2018).

Importantly, this study is characterised by various limitations that need to be considered. Our sample was not large, and thus the likelihood of false positive and false negative findings cannot be excluded. Although both groups of participants were matched for age, sex and the level of parental education as the proxy measure of socio-economic position, the possibility of selection bias should be indicated. For instance, both groups did not differ significantly in terms of dietary habits. However, our previous study showed that poor adherence to Mediterranean diet appears only in patients with the deficit subtype of schizophrenia, characterised by primary and enduring negative symptoms (Kowalski *et al*., 2022). Another limitation is that all patients in our sample were medicated, although we controlled for a number of clinical characteristics related to medication effects. Moreover, certain lifestyle characteristics that might affect gut microbiota were not controlled in this study. These include, i.e., physical activity as well as the use of alcohol and illicit drugs. Deep whole-genome sequencing and metabolomic analysis could provide additional insights into the functional relationship between microbiota and symptoms of schizophrenia. Finally, a lack of longitudinal design does not allow to establish conclusions about causality.

In sum, our study shows altered gut microbiota composition in patients with schizophrenia from Europe. Specifically, our findings indicate increased abundance of Lactobacillales (order level), Bacilli (class level) and Actinobacteriota (phylum level) in this population after controlling for the effects of various confounding factors. Certain gut bacteria (the Bacteroidia class, the Actinobacteriota phylum, the Bacteroidota phylum, the Coriobacteriales order and the Coriobacteria class) might also be related to clinical manifestation of schizophrenia in terms of the severity of negative symptoms, general functioning and performance of language abilities. However, additional studies, adopting longitudinal designs, homogeneous methodology and controlling for latent confounding are needed before their translation into clinical practice.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/neu.2022.38

Acknowledgements. Authors are deeply grateful to all patients and HCs participating in this study.

Author contributions. BM – conceptualisation, study design, recruitment of patients and healthy controls, data analysis and manuscript writing; PŻ, DK and ŁŁ – analysis of gut microbiota and manuscript writing; KK and IŁ – manuscript writing; JS, MJ, AC and Patryk Piotrowski – recruitment and psychiatric assessment of patients, critical review of the manuscript; Piotr Plichta – assessment of neurocognition and manuscript editing; MK – bioinformatic analysis

Financial support. This study received funding from the OPUS grant awarded by National Science Centre, Poland (grant number: 2018/31/B/NZ5/00527).

Conflict of interest. I.Ł. is a shareholder of the company involved in probiotic selling and the multiomic analysis of microbiota. Other authors declare no conflict of interest.

Ethical statement. The study was approved by the Bioethics Committees at Wroclaw Medical University (Wroclaw, Poland; approval number: 512/2019) and Pomeranian Medical University (Szczecin, Poland; approval number: KB-0012/130/2019). It was performed in agreement with the Declaration of Helsinki and all participants gave written informed consent.

References

Addington D, Addington J and Matickatyndale E (1994) Specificity of the Calgary depression scale for schizophrenics. *Schizophrenia Research* **11**(3), 239–244.

Andreasen NC, Carpenter WT, Kane JM, Lasser RA, Marder SR and Weinberger DR (2005) Remission in schizophrenia: proposed criteria and rationale for consensus. *The American Journal of Psychiatry* **162**(3), 441–449.

Binda C, Lopetuso LR, Rizzatti G, Gibiino G, Cennamo V and Gasbarrini A (2018) Actinobacteria: a relevant minority for the maintenance of gut homeostasis. *Digestive and Liver Disease* **50**(5), 421–428.

Bora E (2019) Peripheral inflammatory and neurotrophic biomarkers of cognitive impairment in schizophrenia: a meta-analysis. *Psychological Medicine* **49**(12), 1971–1979.

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang K, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras, D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R and Caporaso JG (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* **37**(8), 852–857.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA and Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**(7), 581–583.

Ezeoke A, Mellor A, Buckley P and Miller B (2013) A systematic, quantitative review of blood autoantibodies in schizophrenia. *Schizophrenia Research* **150**(1), 245–251.

Fernandes BS, Steiner J, Bernstein HG, Dodd S, Pasco JA, Dean OM, Nardin P, Gonçalves CA and Berk M (2016) C-reactive protein is increased in schizophrenia but is not altered by antipsychotics: meta-analysis and implications. *Molecular Psychiatry* **21**(4), 554–564.

Galderisi S, Mucci A, Dollfus S, Nordentoft M, Falkai P, Kaiser S, Giordano GM, Vandevelde A, Nielsen MØ., Glenthøj LB, Sabé M, Pezzella P, Bitter I and Gaebel W (2021) EPA guidance on assessment of negative symptoms in schizophrenia. *European Psychiatry* **64**(1), 23.

Gupta VK, Paul S and Dutta C (2017) Geography, ethnicity or subsistence-specific variations in human microbiome composition and diversity. *Frontiers in Microbiology* **8**, 1162.

Hawrysz I, Wadolowska L, Slowinska MA, Czerwinska A and Golota JJ (2020) Adherence to prudent and Mediterranean dietary patterns is inversely associated with lung cancer in moderate but not heavy male Polish smokers: a case-control study. *Nutrients* **12**(12), 3788.

He Y, Kosciolek T, Tang J, Zhou Y, Li Z, Ma X, Zhu Q, Yuan N, Yuan L, Li C, Jin K, Knight R, Tsuang MT and Chen X (2018) Gut microbiome and magnetic resonance spectroscopy study of subjects at ultra-high risk for psychosis may support the membrane hypothesis. *European Psychiatry* **53**, 37–45.

Karpiński P, Samochowiec J, Frydecka D, Sąsiadek MM and Misiak B (2018) Further evidence for depletion of peripheral blood natural killer cells in patients with schizophrenia: a computational deconvolution study. *Schizophrenia Research* **201**, 243–248.

Kaur H, Bose C and Mande SS (2019) Tryptophan metabolism by gut microbiome and gut-brain-axis: an in silico analysis. *Frontiers in Neuroscience* **13**, 1365.

Kay SR, Fiszbein A and Opler LA (1987) The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophrenia Bulletin* **13**(2), 261–276.

Kowalski K, Bogudzińska B, Stańczykiewicz B, Piotrowski P, Bielawski T, Samochowiec J, Szczygieł K, Plichta P and Misiak B (2022) The deficit schizophrenia subtype is associated with low adherence to the Mediterranean diet: findings from a case-control study. *Journal of Clinical Medicine* **11**(3), 568.

Krusinska B, Hawrysz I, Wadolowska L, Slowinska MA, Biernacki M, Czerwinska A and Golota JJ (2018) Associations of Mediterranean diet and a posteriori derived dietary patterns with breast and lung cancer risk: a case-control study. *Nutrients* **10**(4), 470.

Laskaris L, Zalesky A, Weickert CS, Di Biase MA, Chana G, Baune BT, Bousman C, Nelson B, McGorry P, Everall I, Pantelis C and Cropley V (2019) Investigation of peripheral complement factors across stages of psychosis. *Schizophrenia Research* **204**, 30–37.

Li S, Zhuo M, Huang X, Huang Y, Zhou J, Xiong D, Li J, Liu Y, Pan Z, Li H, Chen J, Li X, Xiang Z, Wu F and Wu K (2020) Altered gut microbiota associated with symptom severity in schizophrenia. *PeerJ* **8**, e9574.

Ma X, Asif H, Dai L, He Y, Zheng W, Wang D, Ren H, Tang J, Li C, Jin K, Li Z and Chen X (2020) Alteration of the gut microbiome in first-episode drug-naïve and chronic medicated schizophrenia correlate with regional brain volumes. *Journal of Psychiatric Research* **123**, 136–144.

Manchia M, Fontana A, Panebianco C, Paribello P, Arzedi C, Cossu E, Garzilli M, Montis MA, Mura A, Pisanu C and Congiu D (2021) Involvement of gut microbiota in schizophrenia and treatment resistance to antipsychotics. *Biomedicines* **9**(8), 875.

McGuffin P, Farmer A and Harvey I (1991) A polydiagnostic application of operational criteria in studies of psychotic illness. Development and reliability of the OPCRIT system. *Archives of General Psychiatry* **48**(8), 764–770.

McGuinness AJ, Davis JA, Dawson SL, Loughman A, Collie F, O'Hely M, Simpson CA, Green J, Marx W, Hair C, Guest G, Mohebbi M, Berk M, Stupart D, Watters D and Jacka FN (2022) A systematic review of gut microbiota composition in observational studies of major depressive disorder, bipolar disorder and schizophrenia. *Molecular Psychiatry* **27**(4), 1920–1935.

Miller BJ, Buckley P, Seabolt W, Mellor A and Kirkpatrick B (2011) Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. *Biological Psychiatry* **70**(7), 663–671.

Misiak B, Łoniewski I, Marlicz W, Frydecka D, Szulc A, Rudzki L and Samochowiec J (2020) The HPA axis dysregulation in severe mental illness: can we shift the blame to gut microbiota? *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **102**, 109951.

Misiak B, Stańczykiewicz B, Kotowicz K, Rybakowski JK, Samochowiec J and Frydecka D (2018) Cytokines and C-reactive protein alterations with respect to cognitive impairment in schizophrenia and bipolar disorder: a systematic review. *Schizophrenia Research* **192**, 16–29.

Mondelli V, Ciufolini S, Belvederi Murri M, Bonaccorso S, Di Forti M, Giordano A, Marques TR, Zunszain PA, Morgan C, Murray RM, Pariante CM and Dazzan P (2015) Cortisol and inflammatory biomarkers predict poor treatment response in first episode psychosis. *Schizophrenia Bulletin* **41**(5), 1162–1170.

Moustafa AA, Garami JK, Mahlberg J, Golembieski J, Keri S, Misiak B and Frydecka D (2016) Cognitive function in schizophrenia: conflicting findings and future directions. *Reviews in the Neurosciences* **27**(4), 435–448.

Nguyen TT, Hathaway H, Kosciolek T, Knight R and Jeste DV (2021) Gut microbiome in serious mental illnesses: a systematic review and critical evaluation. *Schizophrenia Research* **234**, 24–40.

Nguyen TT, Kosciolek T, Maldonado Y, Daly RE, Martin AS, McDonald D, Knight R and Jeste DV (2019) Differences in gut microbiome composition between persons with chronic schizophrenia and healthy comparison subjects. *Schizophrenia Research* **204**, 23–29. doi: 10.1016/j.schres.2018.09.014.

Niedzwiedzka E, Wadolowska L and Kowalkowska J (2019) Reproducibility of a non-quantitative food frequency questionnaire (62-item FFQ-6) and PCA-driven dietary pattern identification in 13-21-year-old females. *Nutrients* **11**(9), 2183.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O.'Hara RB, Simpson GL, Solymos P, Stevens MH, Szoecs E and Wagner H (2020) vegan: Community Ecology Package. R package version 2.5-7. Available at https://CRAN.R-project.org/package=vegan 13 June 2022.

Owen MJ, Sawa A and Mortensen PB (2016) Schizophrenia. *Lancet* **388**, 86–97. doi: 10.1016/S0140-6736(15)01121-6.

Pasternak O, Kubicki M and Shenton ME (2016) In vivo imaging of neuroinflammation in schizophrenia. *Schizophrenia Research* **173**(3), 200–212.

Pillinger T, D'Ambrosio E, McCutcheon R and Howes OD (2019) Is psychosis a multisystem disorder? A meta-review of central nervous system, immune, cardiometabolic, and endocrine alterations in first-episode psychosis and perspective on potential models. *Molecular Psychiatry* **24**(6), 776–794.

Pomerleau CS, Majchrzak MJ and Pomerleau OF (1989) Nicotine dependence and the Fagerström tolerance questionnaire: a brief review. *Journal of Substance Abuse* **1**(4), 471–477.

Randolph C, Tierney MC, Mohr E and Chase TN (1998) The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS): preliminary clinical validity. *Journal of Clinical and Experimental Neuropsychology* **20**(3), 310–319.

Saary P, Forslund K, Bork P and Hildebrand F (2017) RTK: efficient rarefaction analysis of large datasets. *Bioinformatics* **33**(16), 2594–2595.

Samochowiec J and Misiak B (2021) Gut microbiota and microbiome in schizophrenia. *Current Opinion in Psychiatry* **34**(5), 503–507.

Schwarz E, Maukonen J, Hyytiäinen T, Kieseppä T, Orešič M, Sabunciyan S, Mantere O, Saarela M, Yolken R and Suvisaari J (2018) Analysis of microbiota in first episode psychosis identifies preliminary associations with symptom severity and treatment response. *Schizophrenia Research* **192**, 398–403.

Sheehan D (1998) The Mini International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview. *The Journal of Clinical Psychiatry* **59**, 22.

Shen Y, Xu J, Li Z, Huang Y, Yuan Y, Wang J, Zhang M, Hu S and Liang Y (2018) Analysis of gut microbiota diversity and auxiliary diagnosis as a biomarker in patients with schizophrenia: a cross-sectional study. *Schizophrenia Research* **197**, 470–477.

Smith GN, Ehmann TS, Flynn SW, MacEwan GW, Tee K, Kopala LC, Thornton AE, Schenk CH and Honer WG (2011) The assessment of symptom severity and functional impairment with DSM-IV axis V. *Psychiatric Services* **62**(4), 411–417.

https://doi.org/10.1017/neu.2022.38 Published online by Cambridge University Press

Strandwitz P (2018) Neurotransmitter modulation by the gut microbiota. *Brain Research* **1693**, 128–133.

Wang Y, Naumann U, Wright ST and Warton DI (2012) mvabund - an R package for model-based analysis of multivariate abundance data: the mvabund R package. *Methods in Ecology and Evolution* **3**(3), 471–474.

Xu R, Wu B, Liang J, He F, Gu W, Li K, Luo Y, Chen J, Gao Y, Wu Z, Wang Y, Zhou W and Wang M (2020) Altered gut microbiota and mucosal immunity in patients with schizophrenia. *Brain, Behavior, and Immunity* **85**, 120–127.

Yogeswara IBA, Maneerat S and Haltrich D (2020) Glutamate decarboxylase from lactic acid bacteria-A key enzyme in GABA synthesis. *Microorganisms* **8**(12), 1923.

Yolken R, Prandovszky E, Severance EG, Hatfield G and Dickerson F (2021) The oropharyngeal microbiome is altered in individuals with schizophrenia and mania. *Schizophrenia Research* **234**, 51–17.

Zheng P, Zeng B, Liu M, Chen J, Pan J, Han Y, Liu Y, Cheng K, Zhou C, Wang H, Zhou X, Gui S, Perry SW, Wong M-L, Licinio J, Wei H and Xie P (2019) The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice. *Science Advances* **5**(2), 8317.

Zhu C, Zheng M, Ali U, Xia Q, Wang Z, Chenlong Yao L, Chen Y, Yan J, Wang K, Chen J and Zhang X (2021) Association between abundance of Haemophilus in the gut microbiota and negative symptoms of schizophrenia. *Frontiers in Psychiatry* **12**, 685910.

Zhu F, Guo R, Wang W, Ju Y, Wang Q, Ma Q, Sun Q, Fan Y, Xie Y, Yang Z, Jie Z, Zhao B, Xiao L, Yang L, Zhang T, Liu B, Guo L, He X, Chen Y, Chen C, Gao C, Xu X, Yang H, Wang J, Dang Y, Madsen L, Brix S, Kristiansen K, Jia H and Ma X (2020a) Transplantation of microbiota from drug-free patients with schizophrenia causes schizophrenia-like abnormal behaviors and dysregulated kynurenine metabolism in mice. *Molecular Psychiatry* **25**(11), 2905–2918.

Zhu F, Ju Y, Wang W, Wang Q, Guo R, Ma Q, Sun Q, Fan Y, Xie Y, Yang Z, Jie Z, Zhao B, Xiao L, Yang L, Zhang T, Feng J, Guo L, He X, Chen Y, Chen C, Gao C, Xu X, Yang H, Wang J, Dang Y, Madsen L, Brix S, Kristiansen K, Jia H and Ma X (2020b) Metagenome-wide association of gut microbiome features for schizophrenia. *Nature Communications* **11**(1), 1612.

https://doi.org/10.1017/neu.2022.38 Published online by Cambridge University Press