

Alteration of Gut Microbiome in Patients With Schizophrenia Indicates Links Between Bacterial Tyrosine Biosynthesis and Cognitive Dysfunction

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ABSTRACT

BACKGROUND: Schizophrenia (SCZ) is a heterogeneous neuropsychiatric disorder for which current treatment has insufficient efficacy and severe adverse effects. The modifiable gut microbiome might be a potential target for intervention to improve neurobiological functions through the gut-microbiome-brain axis.

METHODS: In this case-control study, gut microbiota of 132 patients with SCZ and increased waist circumference were compared with gut microbiota of two age- and sex-matched control groups, composed of 132 healthy individuals and 132 individuals with metabolic syndrome. Shotgun sequencing was used to characterize fecal samples at the taxonomic and functional levels. Cognition of the patients with SCZ was evaluated using the Brief Assessment of Cognition instrument.

RESULTS: SCZ gut microbiota differed significantly from those of healthy control subjects and individuals with metabolic syndrome in terms of richness and global composition. SCZ gut microbiota were notably enriched in *Flavonifractor plautii*, *Collinsella aerofaciens*, *Bilophila wadsworthia*, and *Sellimonas intestinalis*, while depleted in *Faecalibacterium prausnitzii*, *Ruminococcus lactaris*, *Ruminococcus bicirculans*, and *Veillonella rogosae*. Functional potential of the gut microbiota accounted for 11% of cognition variability. In particular, the bacterial functional module for synthesizing tyrosine, a precursor for dopamine, was in SCZ cases positively associated with cognitive score ($p = 0.34, q \leq .1$).

CONCLUSIONS: Overall, this study shows that the gut microbiome of patients with SCZ differs greatly from that of healthy control subjects or individuals with metabolic syndrome. Cognitive function of patients with SCZ is associated with the potential for gut bacterial biosynthesis of tyrosine, a precursor for dopamine, suggesting that gut microbiota might be an intervention target for alleviation of cognitive dysfunction in SCZ.

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Schizophrenia (SCZ) is a heterogeneous neuropsychiatric disorder characterized by hallucinations, delusions, social withdrawal, anhedonia, and cognitive impairment, with the first disease manifestation usually occurring in adolescence (1). SCZ is a highly heritable, polygenic disorder, but at present the molecular genetics of the disorder are only partially explained (2). Recently, there has been increasing interest in how gut microbiota relate to mental health (3), moving focus of apprehension away from the brain. The link is plausible, because psychiatric and gut disorders are epidemiologically linked (4) and antipsychotic drugs have antibacterial properties (5). There are several suggested pathways by which the microbiota harbored by the gut interact with the brain, such as modulation of the immune system, increased permeability of the gut-brain barrier, nervus vagus activation, or metabolization of

neurotransmitters (6,7). The current medical treatment for SCZ consists of antipsychotic medication, which has limited effect on symptom severity but has adverse effects, such as blunting of affect, lethargy, and metabolic dysregulation, leading to obesity and cardiovascular disease. Despite decades of biological optimism, the anticipated breakthrough in understanding SCZ and developing new targets for treatment has been disappointing and calls for radically new thinking.

So far, the field linking microbiota to SCZ has moved extensively in only a few years, from case-control studies of taxonomic and functional alterations to translational studies where candidate microbes are transferred to rodents (8,9). Despite increased evidence of microbiota alterations across all psychiatric disorders (10), replicability of findings is limited. Several features point to limitations in the basic studies,

suggesting gaps in knowledge. Eleven cross-sectional studies (8,11–20) of gut microbiota from patients with SCZ have been published, reporting somewhat contradictory findings, the number of patients ranging from 10 to 90 (21). The poor replicability may be explained by high risks of false positive findings (type 1 errors), false negatives (type 2 errors), and substantial heterogeneity across studies. The high risk of type 1 errors is due to small samples and lack of preregistrations. The heterogeneity concerns methods to collect samples, different clinical inclusion criteria, different techniques to sequence and analyze data, and different research questions, because some aim to identify biomarkers for diagnostic purposes while others search for causal links that can be targets for future treatment of SCZ. While seeming similar, the difference in these two questions concerns controlling for confounders. A specific risk in SCZ studies is the confounding effects of lifestyle factors and psychotropic medication, and proper methods of deconfounding are a prerequisite for casual inference (22).

Another limitation in the field of biological psychiatry is the lack of valid diagnostic categories. While genetic research, acknowledging overlapping genetics, moves toward transdiagnostic designs (23,24), the Research Domain Criteria initiative (25) suggests to focus on symptom domains rather than arbitrary categories.

In this large study, we aim at identifying taxonomic and functional alterations in stool microbiota (as a proxy of gut microbiota) of patients with SCZ using novel methods to adjust for medication and metabolic features and link these to levels of psychotic symptoms, negative symptoms, cognition, and global functioning.

METHODS AND MATERIALS

Study Population

Individuals with SCZ ($n = 132$) and increased waist circumference (which is a common but not guaranteed comorbidity) were recruited from a lifestyle trial, CHANGE (26), conducted at university hospitals in Aarhus and Copenhagen, Denmark from 2012 to 2014. Diagnoses were confirmed by structured diagnostic interviews by trained clinicians. Psychometric measures at study entry included the Scale for the Assessment of Negative Symptoms (SANS) and Scale for the Assessment of Positive Symptoms instruments (27), a measure of cognition (Brief Assessment of Cognition in Schizophrenia [BACS]) (28), and the Global Assessment of Functioning (GAF) (29). The study of SCZ cases was approved by the Ethical Committees of the Capital Region of Denmark (H-4-2012-051).

Healthy control (HC) subjects who reported no acute or chronic medical disorders were selected among age- and sex-matched individuals from the population-based Danish Study of Functional Disorders ($n = 74$) (30). The Danish Study of Functional Disorders was approved by the Ethical Committees of the Capital Region of Denmark (H-3-2012-015). Another sample of healthy individuals by self-report (included in the overall HC group) was phenotyped at Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen (unpublished; $n = 58$). The study was approved by the Ethical Committees of the Capital Region of Denmark (H-3-2012-145). A second, dysmetabolic control group

matched with SCZ cases on body mass index (BMI) and sex was selected among nondiabetic samples from the MetaHIT project ($n = 132$) (31) (Table 1; Table S1 in Supplement 1). This control group is referred to as the metabolic syndrome (MS) group. The MetaHIT subcohort was approved by the Ethical Committees of the Capital Region of Denmark (H-C-2008-017). All study participants gave informed consent, and all examinations were done in accordance with the Helsinki Declaration. Details are provided in Supplement 1.

Metagenomics Profiling

Total DNA extracted from stool samples was sequenced and the sequencing reads used to assess the presence and abundance of 10.4 million genes from the integrated gene catalog 2 (32,33) and 8.4 million genes from the oral gene catalog (34), as detailed in Supplement 1. The genes from both catalogs were organized into metagenomics species (MGSs), the abundance of which was deduced from the cognate gene abundances (see Supplement 1).

Statistical Analysis

All statistical analyses were performed using R version 3.6.0 (35). Contrasts in quantitative variables were performed using the Wilcoxon test to compare two groups or Kruskal-Wallis followed by post hoc Dunn tests to compare more than two groups. Associations between categorical variables were performed using the χ^2 test. Correlations between variables were performed using Spearman's test. The Benjamini-Hochberg method was used for multiple testing correction. Unless stated otherwise, a corrected p value was considered significant when $\leq .1$. Effect size was computed as Cliff's Delta using the package *effsize* version 0.7.4 (36).

Bray-Curtis dissimilarity was computed on the \log_{10} -transformed MGS table with the package *vegan* version 2.5.7 (37). Principal coordinates analysis was performed on the Bray-Curtis dissimilarity with the package *ade4* version 1.7.16 (38). Bray-Curtis dissimilarity variance between groups was then analyzed by permutational multivariate analysis of variance with the function *adonis* from the package *vegan*. Shannon index was computed with the function *diversity* from the package *vegan*.

Covariate Deconfounding

Covariate deconfounding was performed on each metagenomics feature with the R package *metadeconfoundR* version 0.1.5 (39) (see Supplement 1). The pipeline was run on four different partitions of the dataset: 1) SCZ cases versus HC subjects with Anatomical Therapeutic Chemical code drug dosages (i.e., quantitative, encoded as defined daily doses) in addition to age, BMI, sex, and smoking status (these four variables were accounted for in all four runs); 2) SCZ cases versus HC subjects with drug groups as binary trait (medication class taken/not taken) (Figure S1 in Supplement 1); 3) SCZ cases versus dysmetabolic control group (MS) with Anatomical Therapeutic Chemical code dosages as above; 4) SCZ cases versus MS control subjects with drug groups as binary variables, as above. A feature was considered confounded if it was found confounded in at least one of the datasets.

Table 1. Characteristics of the Three Study Samples

Characteristics	SCZ, <i>n</i> = 132	HC, <i>n</i> = 132	MS, <i>n</i> = 132	<i>p</i> _{Healthy}	<i>p</i> _{Dysmetabolic}
BMI, kg/m ² , Mean (SD)	35 (6.2)	24 (3.8)	34 (4.5)	<.001	.21
Age, Years, Mean (SD)	41 (12)	40 (12)	57 (7.7)	.65	<.001
Sex, Female/Male, <i>n</i>	73/59	75/57	71/57	.9	1
Smoking Status, Current/Never/Previous, <i>n</i>	64/38/30	16/70/44	14/61/57	<.001	<.001

For quantitative variables (BMI and age), *p* values are obtained from Wilcoxon tests; for qualitative variables (sex, smoking status), they are obtained from χ^2 tests. *p*_{Healthy} compares SCZ and HC, *p*_{Dysmetabolic} compares SCZ and MS.

BMI, body mass index; HC, healthy control; MS, metabolic syndrome; SCZ, schizophrenia.

Contrasted Metagenomics Features

We performed the Kruskal-Wallis test followed by post hoc Dunn tests on each metagenomics feature to assess microbiome contrast between SCZ cases, HC subjects, and control subjects with MS. Contrasted features were selected as follows: 1) false discovery rate \leq 0.1 for Kruskal-Wallis test; 2) $p \leq .05$ for Dunn test in the SCZ/HC comparison and in the SCZ/MS comparison; 3) same directionality in SCZ/HC and SCZ/MS comparisons regarding status (SCZ or control); and 4) not confounded by covariates.

Status Prediction

We predicted the class of each individual (SCZ vs. HC or SCZ vs. MS) using an elastic net regression with the R package *caret* version 6.0.84 (40). Input was the log₁₀-transformed, scaled, and centered MGS table. MGSs present in <10% of individuals were filtered out. The dataset was split into a training set (75%) and a test set (25%). Models were fit on the training set under cross-validation and then used to predict class of the testing set. The area under the receiver operating characteristic curve was computed on the test set. The entire procedure (splitting, fitting, predicting) was repeated 100 times.

Modeling Cognition Score

Random forests were used to predict cognition score (BACS) using the R packages VSURF version 1.1.0 (41) and randomForest version 4.6.14 (42). Explanatory variables consisted of either functional modules, bioclinical variables (including medication), or the combination of both. Functional modules were log-transformed, centered, and scaled. Those present in <10% of individuals were filtered out. Quantitative bioclinical variables were centered and scaled. The validation scheme consisted of a 5-fold cross-validation repeated 20 times. The variable selection procedure is based on a preliminary ranking of the explanatory variables using the random forests permutation-based score of importance and proceeds using a stepwise forward strategy for variable introduction. The increase of mean square error was the metric used to assess the importance of the variables.

RESULTS

Taxonomic Analyses of Gut Microbiota

Fecal samples were analyzed from 132 SCZ cases and two age- and sex-matched control groups consisting of 132 HC individuals and 132 individuals with MS (Table 1).

Patients with SCZ showed substantially lower MGS richness, which was decreased by 21% to 24% relative to the two control groups (Figure 1A). The decrease in richness was still significant after deconfounding for BMI, age, smoking status, sex, and medication. Results were similar using the Shannon index as alpha-diversity measure (Figure S2 in Supplement 1). Principal coordinates analysis revealed a clear difference in composition of gut microbiota from patients with SCZ as compared with the two control groups ($p = 1 \times 10^{-4}$, permutational multivariate analysis of variance) (Figure 1B). Consistently, we were able to predict the class of each individual based on the MGS abundance using elastic net regression with a mean area under the receiver operating characteristic curve of 0.86 ± 0.04 (SCZ vs. HC) and 0.92 ± 0.03 (SCZ vs. MS) (Figure S3A, B in Supplement 1). Univariate analyses showed that out of 600 MGSs present in at least 10% of individuals, 202 (34%) differed in abundance between SCZ cases and HC individuals or individuals with MS. Among these, 31 (15%) were enriched in SCZ and 171 (85%) were depleted. After deconfounding for medication, BMI, age, and smoking status, 17 MGSs remained enriched in SCZ and 104 MGSs remained depleted in SCZ (referred to as SCZ-MGS and control-MGS, respectively). Most (85%) correlated significantly with MGS richness in the SCZ cohort ($p \leq .05$, Spearman's correlation). SCZ-MGSs correlated inversely with richness, while control-MGSs correlated positively, in line with the decrease in richness observed in the SCZ gut microbiota as compared with HC and MS gut microbiota.

SCZ-MGSs that were also selected by the elastic net regression to predict the individual status included *Flavonifractor plautii*, *Collinsella aerofaciens*, *Bilophila wadsworthia*, and *Sellimonas intestinalis*. In contrast, control-MGSs selected by the model included two subspecies of *Faecalibacterium prausnitzii*, *Turicibacter sanguinis*, *Ruminococcus lactaris*, *Veillonella rogosae*, and *Ruminococcus bicirculans* (Figure 1C). All contrasted MGSs are listed in Table S1 in Supplement 1.

Of note, only two oral bacteria were part of the differentially abundant MGSs before deconfounding: *V. rogosae*, more abundant in control subjects, and *Streptococcus vestibularis*, more abundant in SCZ cases but confounded by smoking and medication status. This shows that there was no increased invasion of oral species in the SCZ gut microbiota in our cohort.

Summing up all MGS abundance according to their taxonomic annotation, we found that, at the phylum level, Actinobacteriota and Desulfobacterota were more abundant in SCZ. At the family level, Tannerellaceae, Lachnospiraceae, Desulfovibrionaceae, Coriobacteriaceae, Actinomycetaceae, and Lactobacillaceae were more abundant in SCZ cases while

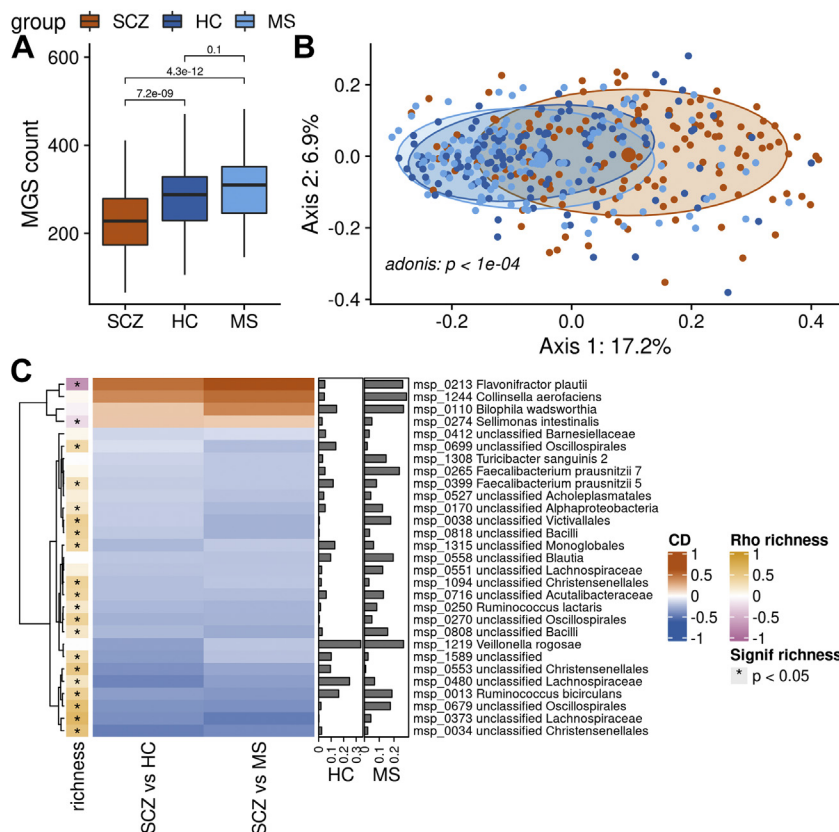


Figure 1. Contrast in MGS richness and taxonomic composition. **(A)** Difference in richness of MGS between patients with SCZ, HC subjects, and individuals with MS. p values from Wilcoxon tests are displayed. Boxes represent the median IQRs between the first and third quartiles; whiskers represent the lowest or highest values within 1.5 times IQR from the first or third quartiles. **(B)** Principal coordinates analysis ordination of the Bray-Curtis dissimilarity matrix computed on the MGS abundance from patients with SCZ and control subjects (HC and MS). **(C)** Heatmap of the effect size (CD) of MGSs significantly contrasted between SCZ and control subjects after covariate deconfounding and selected with elastic net regression to predict status (red and blue denote MGSs enriched in SCZ and control subjects, respectively; scale is shown on the right side of the panel). The right bars show the variable importance of each MGS in the status prediction (for HC vs. SCZ or MS vs. SCZ). The left colored bar shows Spearman's correlations of each MGS with MGS richness (computed in patients with SCZ only). Red = more abundant in patients with SCZ; blue = more abundant in control subjects. CD, Cliff's Delta; HC, healthy control; IQR, interquartile range; MGS, metagenomic species; MS, metabolic syndrome; SCZ, schizophrenia.

Victivallaceae was depleted. At the genus level, *Parabacteroides*, *Lachnoclostridium*, *Lawsonibacter*, *Enterocloster*, *Collinsella*, *Flavonifractor*, *Eveptia*, *Absiella*-*Longicatena*, *Lactobacillus*, *Pauljensenia*, *Sellimonas*, and *Tyzzereella* were more abundant in SCZ cases while *Ruminococcus*, *Eisenbergiella*, *Coprococcus*, *Acetatifactor*, *Angelakisella*, and *Marvinbryantia* were depleted (Figure S4A, C in Supplement 1).

Functional Analyses of Gut Microbiota

To assess potential for bacterial synthesis of various metabolites, we carried out module-level analysis. Of the 44 gut-brain modules present in at least 10% of individuals, 10 were differentially abundant between SCZ cases and control subjects (both HC and MS), and six remained significantly contrasted after drug and other covariates deconfounding (Figure 2, top). Among these, glutamate synthesis, quinolinic acid degradation and synthesis, inositol synthesis, nitric oxide synthesis (nitrite reductase), and *p*-cresol degradation modules were all more abundant in patients with SCZ.

Considering other module types, we found 11 gut-metabolic modules and 17 Kyoto Encyclopedia of Genes and Genomes modules that were enriched in SCZ cases as compared with HC subjects and individuals with MS after deconfounding for covariates. Among Kyoto Encyclopedia of Genes and Genomes modules, 7 of 17 were related to essential amino acid synthesis: lysine (two distinct pathways),

methionine (two distinct pathways), valine/isoleucine, histidine, and leucine (Figure 2, bottom).

Association Between Gut Microbiota, SCZ Diagnosis, and Psychopathology Scores

Most patients with SCZ were diagnosed as ICD-10 F.20 ($n = 120$, 90.9%) (Table S1 in Supplement 1). The second most numerous diagnosis group was ICD-10 F.25 ($n = 10$, 7.5%). There was no significant difference in beta-diversity regarding these two groups (Figure S5 in Supplement 1).

Metrics of GAF, BACS, and SANS correlated to different microbiome taxonomic features (MGS, genus, family, and phylum) (Figure 3A). Features positively associated with GAF and BACS in cases tended to be more abundant in HC subjects, and conversely ($p = -0.34$, and -0.38 , respectively; $p < 2.2 \times 10^{-16}$) (Figure 3B, C), suggesting that the drift of the microbiome composition from that found in HC subjects toward that found in SCZ cases is associated with a decrease of cognition. In contrast, SANS and the Scale for the Assessment of Positive Symptoms showed no or only modest gut associations, respectively (Figure 3D, E).

BACS was positively associated with abundance of the species *Dialister invisus* and inversely associated with the genus *Acutalibacter*, the families Atopobiaceae and Eggerthellaceae, and the phylum Actinobacteriota ($q \leq .1$). GAF metrics were associated with a genus and to a family assigned

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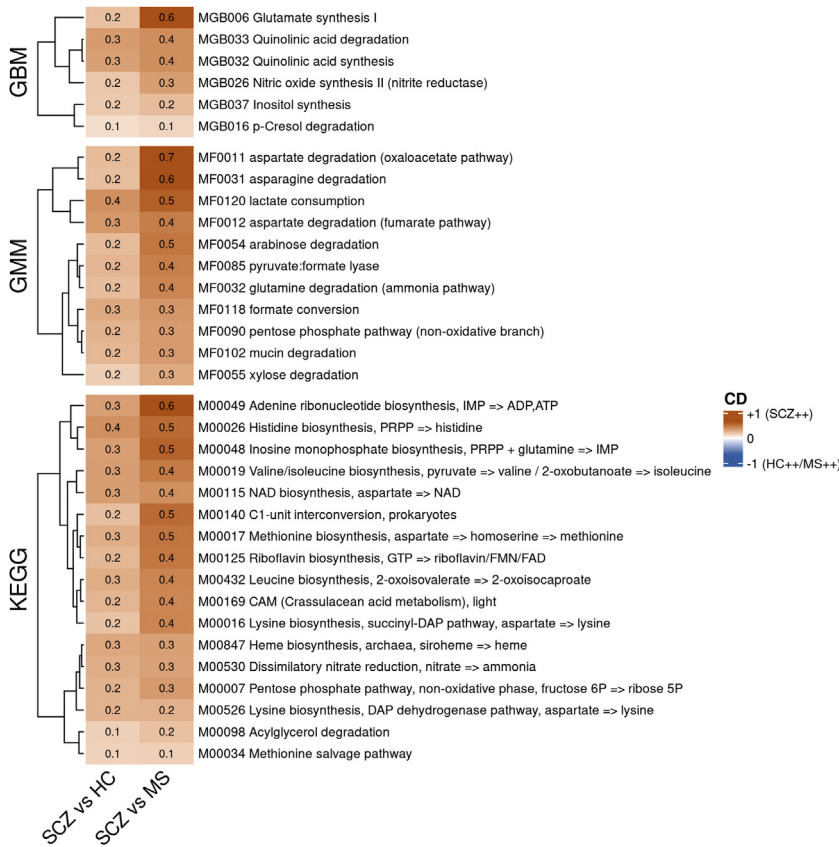


Figure 2. Contrast in functional potential. Heatmaps of the effect size (CD) of the gut metagenome-predicted functional modules (generated from GBMs, GMMs, and KEGG pathway modules) that are contrasted between patients with SCZ and control subjects (both HC and MS) after covariate deconfounding. Figures displayed in the cell give the exact CD. SCZ++ = more abundant in patients with SCZ; HC+/MS++ = more abundant in control subjects. CD, Cliff's Delta; GBM, gut-brain module; GMM, gut-metabolic module; HC, healthy control; KEGG, Kyoto Encyclopedia of Genes and Genomes; MS, metabolic syndrome; SCZ, schizophrenia.

to Alphaproteobacteria. Consistently, the phylum Actinobacteriota and the unclassified family assigned to Alphaproteobacteria remained more abundant in SCZ cases after covariate deconfounding. Having a closer look at the *Bifidobacterium* genus, reported to be more abundant in SCZ (11), we found that it correlated positively with SANS and negatively with BACS ($p \leq .05$), although it was significantly more abundant in SCZ only when considering the MS cohort as control subjects.

At the functional module level, BACS was positively associated with the metagenome-predicted tyrosine biosynthesis potential ($p = 0.34$, $q \leq .1$) (Figure 3A). Bacterial potentials for methionine biosynthesis (from aspartate), more abundant in SCZ after covariate deconfounding, correlated inversely to BACS ($p \leq .05$).

To further analyze correlations with cognition, we examined the proportion of the BACS variance explained by a combination of bioclinical variables (including medication) and functional microbial modules. The functional modules and the bioclinical variables could account on average for 11% and 11% of BACS variability, respectively. The combination of both inputs with bioclinical variables accounted for only a small but significant additional explanation (14% of BACS variability) (Figure S6A in Supplement 1). With functional modules or their combination with bioclinical variables as input, the module that contributed most to explanation of BACS variability was

tyrosine biosynthesis, selected in 100% and 91% of tests, respectively (Figure S6B, C in Supplement 1). Only two other modules were also selected in both cases: aspartate degradation (fumarate pathway) and lysine biosynthesis.

The microbial tyrosine biosynthesis potential from pre-phosphate was the only module significantly associated with BACS after correction for multiple testing and deconfounding for covariates (age, BMI, smoking status, and drugs). Furthermore, when considering only the individuals who carried the module ($n = 54$), the correlation was even stronger than when considering all individuals ($\rho = 0.39$, $p = .0027$ and $\rho = 0.33$, $p = 8.8 \times 10^{-5}$, respectively), suggesting that the relationship is not driven by the zero values (Figure 4A, B). The tyrosine biosynthesis module was not significantly differential between SCZ, HC, and MS groups, neither in presence (43%, 46%, 39%, respectively, $p = .46$) nor in abundance ($p = .5$). The module was encoded in seven species: *D. invisus* and six *Veillonella* (*V. infantium*, *V. parvula*, *V. rogosae*, *V. atypica*, *V. tobetsuensis*, and *V. denticariosi*) (Figure 4C); all but *V. tobetsuensis* and *V. denticariosi* were present in more than 10% of individuals. *V. rogosae* was more abundant in HC individuals and individuals with MS, even after deconfounding; *V. infantium* was more abundant in HC subjects as compared with patients with SCZ but not in individuals with MS, and the remaining MGSs were not contrasted. The most abundant of these MGSs was *D. invisus*, which contributed 98% of the

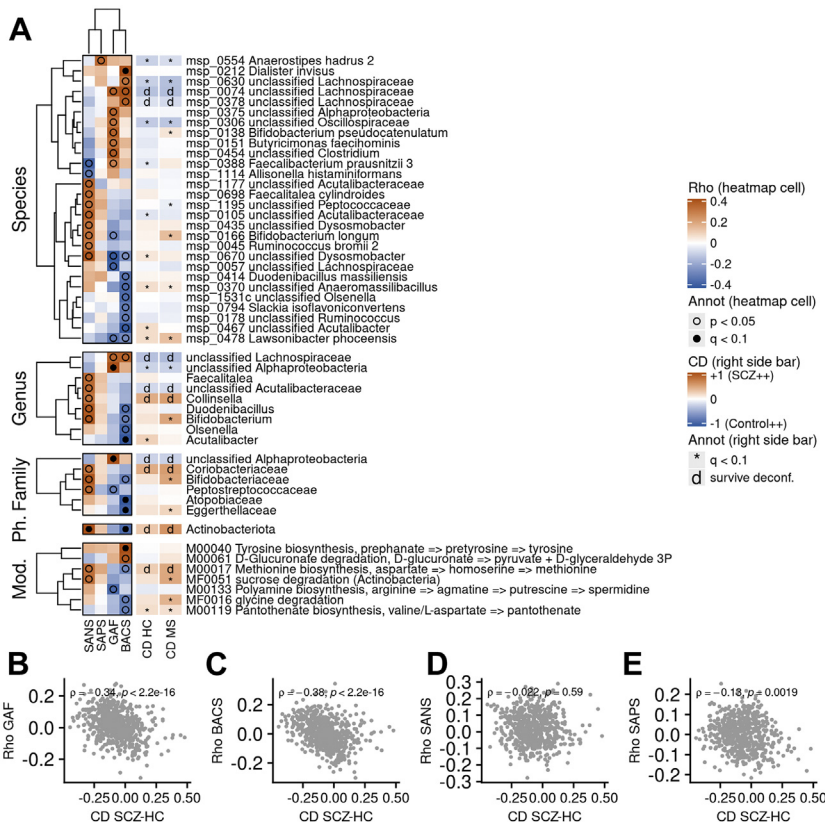


Figure 3. Association between psychopathological scores of SCZ cases and metagenomics features. **(A)** Heatmap of Spearman's correlations between scores for psychopathology and cognitive functions (SAPS, SANS, GAF, BACS) and metagenomics features (from top to bottom: MGS, genus, family, phylum, functional modules) in cases with SCZ. Only features with at least one p value under .01 are displayed. Black dots denote correlations with false discovery rate ≤ 0.1 , while empty circles indicate correlation with $p \leq .05$. The right-side bars indicate CD (effect size) of the feature in the SCZ/HC contrast or in the SCZ/MS contrast (red: more abundant in SCZ; blue: more abundant in HC or MS). Asterisk indicates that the feature was significantly different in the given comparison (either SCZ/HC or SCZ/MS) but was confounded; d means that the feature was still contrasted after covariate deconfounding. **(B-E)** Spearman's correlations of MGSs with **(B)** GAF, **(C)** BACS, **(D)** SANS, and **(E)** SAPS according to CD between SCZ cases and HC subjects. Correlations are computed using the SCZ cases only. The coefficient from Spearman's correlation along with its p value is displayed. Annot, annotation; BACS, Brief Assessment of Cognition in Schizophrenia; CD, Cliff's Delta; GAF, Global Assessment of Functioning; HC, healthy control; MGS, metagenomic species; MS, metabolic syndrome; Mod., modules; Ph., phylum; SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Positive Symptoms; SCZ, schizophrenia.

module abundance (<math><1\%</math> for each of the other MGSs). Consistently, *D. invisus* was also positively correlated to BACS ($\rho = 0.34, p = 5.7 \times 10^{-5}$).

DISCUSSION

To our knowledge, this is the largest study on alteration of the gut microbiota, aiming at identifying potential new targets to treat cognitive deficits, in patients with SCZ.

Very few studies have reported associations between gut microbiota and clinical features in SCZ, which is surprising because these may be more promising than relying on diagnostic criteria outlining groups with high clinical heterogeneity. Cognition is a core feature and cognitive deficits are a currently untreatable symptom that substantially hinders recovery in SCZ. We show that abundances of the tyrosine biosynthesis module and of its main driver, *D. invisus*, are positively associated with measures of cognition in SCZ cases. Tyrosine has

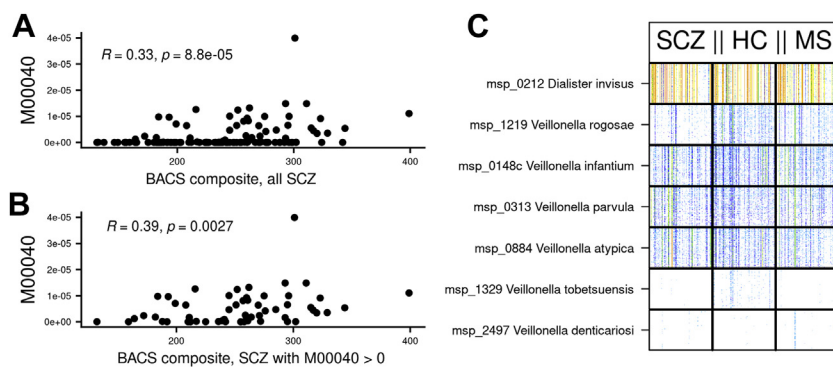


Figure 4. Relationship between tyrosine biosynthesis potential of gut microbiota and BACS in SCZ cases. Spearman's correlation displaying abundance of gut metagenome-predicted tyrosine biosynthesis module (M00040) in function of BACS composite in **(A)** all SCZ cases ($n = 132$) or **(B)** in SCZ cases with a strictly positive abundance only of the tyrosine biosynthesis module ($n = 57$). Coefficients of Spearman's correlation along with the associated p value are shown. **(C)** Bar codes of MGSs involved in the gut metagenome-predicted tyrosine biosynthesis module (M00040). Individuals are in columns and MGSs in rows; each bar indicates the abundance of 50 marker genes of a species in an individual, and the colors indicate the abundance (white, absence; red, relative abundance).

of 1×10^{-4} . Individuals are sorted first by status (patients with SCZ on the left, HC subjects in the middle, individuals with MS on the right) and then by increasing richness (MGS count). BACS, Brief Assessment of Cognition in Schizophrenia; HC, healthy control; MGS, metagenomic species; MS, metabolic syndrome; SCZ, schizophrenia.

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previously been associated with cognition in healthy individuals and in the pathophysiology of SCZ (43,44), in line with what we found. We did not identify any other studies reporting associations to cognition in SCZ.

We found only modest associations between psychotic symptoms and any taxa or functional modules. Li *et al.* (19) reported a positive association between *Succinivibrio* and the Positive and Negative Syndrome Scale, while Nguyen *et al.* (12) found that increased negative symptoms were associated with decreased abundance of family Ruminococcaceae. Schwarz *et al.* (15) investigated first-episode patients and found that the *Lactobacillus* group was associated with positive symptoms and global assessment of functioning, while Lachnospiracae and Ruminococcaceae were negatively correlated with negative symptoms.

As expected, we found gut microbiome diversity and richness to be lower in SCZ cases. Both indices have been suggested to be beneficial to the host, and low diversity has been found across various disorders (45), even if the clinical significance is not well described. Nine of the SCZ studies included gut diversity, of which eight reported significant differences. However, a recent meta-analysis found that diversity across psychiatric disorders was well preserved, while richness was generally low (10).

The relative abundance of microbial taxa is reported at seven levels: kingdom, phylum, class, order, family, genus, and species. Our most important findings are highlighted here and discussed in perspective of earlier findings. At the genus level, *Lachnospiridium*, *Enterocloster*, and *Lawsonibacter* were more abundant in SCZ cases while *Coprococcus*, *Ruminococcus*, and *Eisenbergiella* were depleted. Depletion of *Coprococcus* (14) and *Ruminococcus* (19) replicated earlier studies. At the family level, we found that Lachnospiracae was more abundant in SCZ. This finding opposes three previous reports (8,14,20). The link between Lachnospiracae and SCZ, a short-chain fatty acid-producing family, has been hypothesized to go through an alteration of the gut, triggering proinflammatory immune response (20). The conflicting findings may be explained by different strategies for deconfounding or by differences in geographical origin, because all three studies reporting decreased levels originate from China. At the phylum level, Actinobacteriota and Desulfobacteriota were enriched in SCZ cases. Higher abundance of Actinobacteriota was observed in other studies of severe mental disorders (46–48) and in two previous SCZ studies (11,19).

Identifying contrasting functional modules may be a more promising step forward, because these can be linked to metabolic pathways already hypothesized to be involved in the pathophysiology of SCZ. We identified six functional gut-brain modules more abundant in SCZ, namely glutamate synthesis, quinolinic acid degradation and synthesis, *p*-cresol degradation, inositol synthesis, and nitric oxide synthesis (nitrite reductase). Of these, pathways involved in quinolinic acid may be of special interest in relation to SCZ. There is growing evidence that the immune system plays a major role in SCZ pathology (49) and the pathway involving kynurenine is suggested as a possible link between peripheral immune activation and central neurotransmitters. Kynurenine is metabolized into several neuroactive metabolites, including quinolinic acid, which is neurotoxic (50). This is an indirect replication of

another recent study, where fecal samples from SCZ cases were transplanted to pathogen-free mice, observing elevation of the kynurenine–kynurenic acid pathway of tryptophan metabolism with increased degradation in serum and the central nervous system and an increased dopamine level in the prefrontal cortex (8). Six studies reported findings at the functional level (11,14,16–19). Ma *et al.* (17) found no differences, while Pan *et al.* (18) identified several functional modules that differed, including lipoic acid metabolism, steroid hormone biosynthesis, and alpha-linolenic acid metabolism. Similarly, Li *et al.* (19) reported several differential functional modules. Xu *et al.* (16) especially highlighted the finding of increased glutamate synthase. Shen *et al.* (14) found that pathways including vitamin B12 and fatty acids differed.

The overall inconsistency in reported results should lead to increased attention to cooperation, preregistration, and reporting guidelines as proposed by Knight *et al.* (51), which will likely decrease false discovery rates and increase the comparability of data in future studies.

A major strength of this study is the careful adjustment for two major confounders, metabolic disease and psychotropic medication, and the large sample size. Yet, our study has limitations, including the specificity of our cohort (overweight and obese patients with SCZ mainly with F.20 diagnosis) and the cross-sectional study design, which does not allow for causal inference. In addition, nonreported covariates such as diet, past use of antibiotics, drug or alcohol abuse, poor oral hygiene, and deviation in actual lifestyle from what is reported are likely to contribute to some residual confounding. Furthermore, our study focused only on bacterial genetic content and did not measure or analyze nonbacterial organisms (such as phages) or fecal metabolites, which could have provided new insights into the role of gut microbiota in SCZ. Finally, it should be stressed that our analyses are exploratory because the analyses were not preplanned, and our study may be statistically underpowered to detect important differences. Thus, our findings need confirmation in independent studies.

Conclusions

We show that the gut microbiome of SCZ exhibits reduced species richness compared with both normal weight healthy individuals and metabolically matched control subjects without SCZ. We find further indications that the kynurenine pathway is involved, because two gut-brain modules involving the neurotoxic metabolite quinolinic acid were more abundant in SCZ. With this, we suggest that parts of the gut-microbiome-brain axis involving quinolinic acid should be further explored in future studies, preferentially involving experimental manipulation and longitudinal observations to allow for causal inferences. Similarly, we show that tyrosine biosynthesis may be important for cognition in SCZ and suggest that it should be explored further.

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SDE and FT had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. OP and SDE conceived the research idea. SDE, TH, and OP designed the analytical plan. HS, THH, TN, TJ, and HV recruited and examined study participants. Microbial DNA extraction and sequencing, library preparation, and sequencing quality assurance were supervised by SDE and undertaken by FL and NG. FT conducted bioinformatics analyses supported by SF, ELC, FPO, MB, and NP. FT, TB, LJ, and SKF undertook drug deconfounding of microbiome data. HS and FT wrote the draft manuscript. OP was responsible for overall supervision, edited and revised the manuscript, and is the guarantor of this study. All authors contributed to critically revising the manuscript for important intellectual content and approved the final version of the manuscript.

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Data are available in a public open access repository. Metagenomics reads generated in this study are available (without conditions of reuse) under the accession numbers PRJEB41217, PRJEB41786, and PRJEB41787 at the European Nucleotide Archive in the European Bioinformatics Institute (<https://www.ebi.ac.uk>).

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